J Neurol (2003) 250 [Suppl 2]: II/1–II/224


# Thirteenth Meeting of the European Neurological Society
## *14–18 June 2003, Istanbul, Turkey*
### Symposia and Free Communications

**The abstracts have been reviewed by:**
P. Boon, D. A. S. Compston, A. Czlonkowska, H.-C. Diener, V. Dietz, M. Donaghy, F. Fazekas, J. Ferro, G. Hildebrand, R. Hohlfeld, C. Krarup, M.-H. Marion, E. Melamed, G. Moonen, E. Nobile-Orazio, G. Said, A. Steck, K. Toyka, J. Valls-Solé.

# Contents

## Abstracts of the Symposia



## Abstracts for Oral Sessions



## Abstracts for Poster Sessions

# PRESIDENTIAL SYMPOSIUM
# Axonal degeneration and regeneration
# Chair: Christian Krarup

## Functional recovery after lesions of peripheral nerves

*Christian Krarup*
Dept. of Clinical Neurophysiology, Rigshospitalet, Copenhagen, Denmark

Axonal degeneration is a common pathological response of disorders and lesions of nervous tissue, and recovery of clinical deficits is dependent on regrowth of axons, collateral sprouting from surviving neurons in partial lesions, or functional redundancy. Even though the peripheral nervous system has the capacity for axonal regeneration after Wallerian degeneration, functional recovery is usually not clinically satisfactory due to limitations in growth, aberrant reinnervation of target organs or disturbed functional maturation of regenerated axons. We have been interested in exploring the influence of these factors on the recovery of physiological properties of axons. Elongation of motor, sensory and unmyelinated sensory axons after Wallerian degeneration were found to have similar rates of 3–4 mm/day in experimental animal models; however, unmyelinated sensory fibers showed poorer regeneration after crushing compared with myelinated fibers possibly because they do not have a one-to-one relationship with Schwann cells as do myelinated fibers, and fascicular redistribution of fibers occurred after regeneration. The progression of Wallerian degeneration after nerve crush may be inhibited by destruction of cellular elements in the nerve trunk and this interference was found to severely inhibit axonal growth. When Wallerian degeneration was permitted to recur after revascularization, axonal elongation also increased. Reinnervation of a target muscle in monkeys was highly influenced by the lesion type and repair procedure and showed limited evidence of so-called preferential motor reinnervation. However, functional recovery depends on regrowth of axons occurring within a window of time after the nerve lesion. Thus, time may be a central prerequisite for functional recovery, and factors that speed up reinnervation of target organs may have therapeutic implications. Acceleration by about 20% of axonal elongation and reinnervation of plantar muscles after sciatic nerve section and suture was obtained in rats by daily intraperitoneal injection of FK506 (1 mg/kg), and the number of large myelinated axons was increased. During maturation after regeneration, axons regain 80–90% of their normal conduction velocity. However, in spite of this recovery, longitudinal studies of membrane properties in vivo in the cat indicate that the distribution and function of ion-channels deviate from normal axons for years after reinnervation of target organs, and that the resting membrane potential may be persistently hyperpolarized. Our studies support that a number of integrated processes must occur in an organized fashion within time limits for recovery to occur, and that a number of adverse influences may impair or impede regeneration.

In the central as compared with the peripheral nervous system, axonal sprouting, growth and recovery after lesions are severely limited. The purpose of this symposium is to highlight insights into immune factors involved in Wallerian degeneration that may form the basis for axonal regeneration in the PNS – discussed by Prof. G. Stoll – as well as the CNS: The lack of comparable regeneration in the CNS may be due to the growth cone environment, and inhibition associated with myelin-components has been found to be a major age-related growth limiting factor (Prof. M. Filbin). Inflammatory mechanisms markedly influence both degeneration and regeneration of the CNS, and have in addition been found to infer protection on CNS neurons exposed to adversarial impacts (Prof. M. Schwartz).

## The chemokine/cytokine network of Wallerian degeneration

*Guido Stoll*
Department of Neurology, Julius-Maximilians-Universität, Würzburg, Germany

Wallerian degeneration (WD) is a simple and extremely useful lesion model with implications for our understanding of the pathophysiology of traumatic, inflammatory and neurodegenerative diseases of the nervous system. In 1850, Augustus Waller discovered the disintegration of the frog glossopharyngal and hypoglossal nerves after axotomy. He observed that the axolemma rapidly became disorganized, and later the injured nerves contained fusiform masses at intervals reflecting ovoids of degenerating myelin. What Waller described as a process following sectioning peripheral nerves is now recognized to apply to all kinds of nerve lesions that disrupt the integrity of the axoplasm independent of the underlying cause or disease. In the PNS, WD is followed by a spontaneous regenerative response developing from the proximal nerve segment into the distal stump which is lacking after CNS injury for various reasons. Ramon y Cajal provided a further detailed description of axonal degeneration, ovoid formation, Schwann cell responses and, most importantly, leukocyte infiltration during WD. It is now appreciated that neural-immune interactions are fundamental to the process of WD.

Histological studies employing immunocytochemistry confirmed that macrophages are present in degenerating nerve segments in the PNS and remove myelin containing neurite outgrowth inhibitors. We could recently elucidate in vivo the spatiotemporal dynamics of macrophage migration into injured nerves by use of a novel magnetic resonance imaging technique. Macrophage infiltration started at the lesion site, spread into more distal parts during the first week, and abruptly ceased thereafter. Macrophage infiltration thus corresponded to the local expression of the chemokine monocyte chemoattractant protein-1 (MCP-1). In support of a critical role of MCP-1 in macrophage recruitment, transgenic mice lacking CCR2, the main macrophage receptor for MCP-1, showed impaired macrophage invasion after nerve injury and a significant decrease in myelin clearance.

Macrophage responses are orchestrated by a complex network of pro- and antiinflammatory cytokines that are upregulated during autoimmune and inflammatory disorders. Essentially the same cytokine patterns are expressed in a highly ordered fashion in the distal stump of nerves undergoing WD. Surprisingly, most cytokines are upregulated prior to macrophage entry and expressed by Schwann cells. Interleukin (IL)-1β, IL-6 and IL-10 gene induction reach peak levels within 24 hours after nerve injury, and gradually decrease thereafter. Functionally, IL-1β induced nerve growth factor synthesis and, accordingly, neutralization with IL-1 receptor antagonist impeded nerve regeneration. Mice lacking IL-6 exhibited smaller sensory action potentials and after nerve injury, sensory nerve regeneration was delayed while motor nerve fibres regenerated normally. IL-6 and leukemia inhibitory factor (LIF) furthermore were involved in the induction of MCP-1 attracting macrophages. Transcripts for the proinflammatory cytokines IL-18, interferon (IFN)-γ and TNF-α exhibited a more sustained induction lasting into later stages of WD. IL-18, initially identified as IFN-γ inducing factor, was strongly expressed by infiltrating macrophages during the first week after nerve injury. Schwann cells, fibroblasts, endothelial cells and macrophages expressed TNF-α. TNF-α-activity was promoted by matrix metalloproteinases which control the integrity of the extracellular space. In TNF-α-knock-out mice, the number of macrophages was significantly reduced during WD, while myelin phagocytosis and regeneration was not affected suggesting a major role of TNF-α in macrophage recruitment.

In contrast to the PNS, hematogenous macrophages are virtually excluded from fibre tracts or nerves undergoing WD in the CNS. Microglia, the resident macrophage population of the CNS, could substitute for the lack of macrophage infiltration, but, unexpectedly, microglia only exerted limited phagocytic activity in injured white matter tracts or optic nerves. This microglial behaviour contrasts to their rapid phagocytic transformation in ischemic brain lesions and stab wounds in the CNS, and leads to long persistence of growth-inhibitory myelin debris during WD. Similar to the PNS, proinflammatory cytokines such as IL-18 and TNF-α were expressed in optic nerves undergoing WD, and accordingly microglia exhibited activation markers such as MHC class II molecules. There is suggestive evidence that phagocytic transformation is suppressed by a local inhibitor in the CNS which is absent in the PNS.

## Overcoming inhibitors in myelin to encourage CNS regeneration in vivo

*M. T. Filbin*
City University of New York (New York, USA)

Immediately after injury, before the glial scar has had time to form, the major impediments to regeneration in the adult CNS are inhibitors associated with myelin. To date, 3 inhibitors have been described: myelin-associated glycoprotein (MAG), Nogo and oligodendrocyte, myelin glycoprotein (OMgp). Surprisingly, all 3 of these inhibitors interact with the same receptor complex to exert their effects. The common binding partner was first identified as a receptor for the 66 extracellular amino acids of Nogo (Nogo66) and was termed NgR. All 3 inhibitors bind NgR with similar

affinities (low nM) and it appears that all 3 inhibitors compete for NgR. NgR, then presents an attractive therapeutic target, such that if it is blocked the inhibition of all myelin inhibitors identified to date would be blocked. The NgR, however, is GPI-linked and so cannot transduce the inhibitory signal across the membrane. Instead it associates with the NTRp75 to bring about inhibition. MAG, Nogo and OMgp are each able to precipitate NTRp75 from neurons and neurons from the NTRp75-/- are not inhibited by MAG, OMgp or myelin. Disrupting the interaction between NgR and p75 should also prevent all 3 inhibitors signaling and so encourage regeneration in vivo.

Rather than block the receptor, an alternative approach to overcoming myelin inhibitors is to change the intrinsic growth state of the neuron such that it no longer recognizes the molecules as inhibitory. We have shown that if neuronal cAMP levels are elevated, either artificially with analogues or by priming with a variety of neurotrophins, inhibition by MAG and myelin is overcome and regeneration in vivo is promoted. The cAMP effect is transcription-dependent and involves activation of CREB – a dominant-negative CREB prevents cAMP overcoming inhibition and MAG/myelin does not inhibit neurons expressing a constitutively active CREB. Two genes that are up-regulated in response to cAMP are the enzyme Arginase I and IL-6. Arg I is key in the synthesis of polyamines and either over-expression of Arg I or exogenous addition of polyamines is each sufficient to overcome inhibition by MAG/myelin and influence regeneration in vivo. Likewise, addition of IL-6 to cultures also overcomes inhibition by MAG/myelin. The expansion of knowledge on the myelin inhibitors, their receptor and their signaling pathways, has offered many new potential targets for therapeutic intervention and to encourage regeneration in vivo.

## The role of inflammation during degeneration and regeneration in the central nervous system

*Michal Schwartz*
Department of Neurobiology, The Weizmann Institute of Science. Rehovot, Israël

Acute insults to the central nervous system (brain, spinal cord, and optic nerve) are often followed by delayed degeneration of neurons that were not directly affected by the initial injury. This secondary degeneration causes the damage to spread, resulting in much more extensive functional loss than might have been expected from the severity of the insult. The physiological compounds that mediate the spread of damage are the same as those that are active in chronic neurodegenerative disorders such as Alzheimer's disease, glaucoma, Huntington's disease and ALS. Our group recently discovered that the body attempts to cope with stressful conditions induced by the activity of these destructive self-compounds by recruiting the protective activity of the immune system, in the form of a T cell-mediated autoimmune response directed against self-compounds. The specific target antigens of such autoimmunity differs in different CNS sites. The T cells that mediate protection are identical to the T cells associated with autoimmune disease, except for the way in which they are regulated. Autoimmune protection requires fine timing of both the onset and the shut-off of the autoimmune response. Our experimental data suggest that T cells, via their cross-talk with resident microglia or blood-derived macrophages, control the local inflammatory response in such a way as to derive the benefit of removal of the threat, represented by the presence of the harmful self-compounds, from the lesion site. We have found that the autoimmune response can be boosted using a "safe" synthetic antigen that cross-reacts with a physiological self-antigen, conferring neuroprotection without the accompanying risk of autoimmune disease induction. We further suggest that autoimmune disease and chronic degeneration can be viewed as two extreme manifestations of the same malfunctioning condition. We believe that just as immunity against nonself is the body's defense mechanism against the threat of invading foreign antigens, so immunity against self (autoimmunity) is the body's protective mechanism against the threat posed by the presence of destructive self-compounds. It is proposed that the notion of "bad" or "good" inflammation be replaced by a concept that views inflammation in the CNS as potentially beneficial if well regulated, and supports therapy for spinal cord and other CNS injuries by immunomodulation. In the lecture questions of tolerance against self, the T cell repertoire, and vaccination as a therapy for acute and chronic neurodegenerative conditions will be addressed.

### Selected bibliography

Rapalino O, Lazarov-Spiegler O, Agranov E, Velan GJ, Fraidakis M, Yoles E, Solomon A, Gepstein R, Katz A, Belkin M, Hadani M, Schwartz M (1998) Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. Nat Med 4:814–821

Moalem G, Leibowitz-Amit R, Yoles E, Mor F, Cohen IR, Schwartz M (1999) Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nat Med 5:49–55

Hauben E, Butovsky O, Nevo U, Yoles E, Moalem G, Agranov E, Mor F, Leibowitz-Amit R, Pevsner S, Akselrod S, Neeman M, Cohen IR, Schwartz M (2000) Passive or active immunization with myelin basic protein promotes recovery from spinal cord contusion. J Neurosci 20:6421–6430

Hauben E, Ibarra I, Mizrahi T, Barouch R, Agranov E, Schwartz M (2001) Vaccination with a Nogo-A-derived peptide after incomplete spinal cord injury promotes recovery via a T-cell-mediated neuroprotective response: Comparison with other myelin antigens. Proc Nat Acad Sci USA 98:15173–15178

Hauben E, Agranov E, Gothilf A, Nevo U, Cohen A, Smirnov I, Steinman L, Schwartz M (2001) Posttraumatic therapeutic vaccination with modified myelin self-antigen prevents complete paralysis while avoiding autoimmune disease. J Clin Invest 108:591–599

Kipnis J, Yoles E, Porat Z, Mor F, Sela M, Cohen IR, Schwartz M (2000) T cell immunity to copolymer 1 confers neuroprotection on the damaged optic nerve: possible therapy for optic neuropathies. Proc Natl Acad Sci USA 97:7446–7451

Kipnis J, Yoles E, Schori H, Hauben E, Shaked I, Schwartz M (2001) Neuronal survival after CNS insult is determined by a genetically encoded autoimmune response. J Neurosci 21:4564–4571

Schori H, Kipnis J, Yoles E, Wolde-Mussie E, Ruiz G, Wheeler LA, Schwartz M (2001) Vaccination for protection of retinal ganglion cells against death from glutamate cytotoxicity and ocular hypertension: Implications for glaucoma. Proc Natl Acad Sci USA 98:3398–3403

Schwartz M, Kipnis J (2001) Protective autoimmunity: regulation and prospects for vaccination after brain and spinal cord injuries. Trends Mol Med 7:252–258

# Symposium – 2
# Mood and behavior in neurological disease
# Chairs: J. Bogousslavsky (Lausanne); P. Scheltens (Amsterdam)

## Consequences and mechanisms of processing emotional stimuli

*R. J. Dolan*
Wellcome Department of Imaging Neuroscience, Institute of Neurology, London

Emotion plays a central role in quality of everyday human experience. In addition to indexing occurrences of value it is evident that the effects of emotion extend to influences a wide domain of cognition including memory, attention and reasoning. The neurobiological substrates of human emotion have attracted increasing attention within the neurosciences. A general account will be provided of the architecture of human memory where a key distinction between emotion and feeling will be described.

One of the key questions addressed in this talk is whether emotional stimuli are processed in a manner similar to other sensory stimuli or are subject to privileged processing. I address this question from two perspectives that focus primarily on underlying neuronal mechanisms. Firstly, I will address the degree to which processing of emotional stimuli is influenced by selective attention and awareness. Secondly, I will consider whether there is differential activation in early sensory cortices purely as a function of the emotional value of a stimulus. It will be suggested that privileged processing of emotional stimuli ensures their availability to memory systems. A critical role for the amygdala will be suggested relation to perceptual processing of emotional stimuli.

## Stroke: from depression to fatigue

*Fabienne Staub*
Department of Neurology, Centre Hospitalier Universitaire Vaudois, Lausanne

Depression has been recognized as a common emotional after-effect of stroke for a long time. In 1921, Kraepelin had already noted a link between depression and cerebrovascular diseases. Since then, but essentially during the 20 last years, many scientific works have considered this association, trying to understand the causes and to control the consequences. Nevertheless, although everything virtually has already been studied, the data resulting from the various studies are far from being univocal and clear. Any attempt at synthesizing seems impossible, first because of major methodological differences, second because of the complexity of the studied phenomenon. Post-stroke depression (PSD) has for example been reported between less than 25 % and more than 75 % of patients. The role of the site of stroke also remains controversial. Lesion location in other neurological diseases with a depressive syndrome similar to PSD, low incidence of PSD with vertebro-basilar stroke and the specific neuropsychological PSD profile (executive functions and recall deficits) suggest the role of fronto-subcortical, temporo-limbic and basal ganglia areas. In any case, lesion location may contribute only to a small extent to the risk of developing PSD; psychodynamic, personal and psychosocial dimensions being other complex and dynamic factor risks. Finally, although depression is a frequent consequence of stroke, it is diagnosed and treated in only a minority of cases. Selective serotonin reuptake inhibitors seem to be both safe and effective in the treatment of PSD.

Amongst the manifestations often mistaken for PSD, ongoing research has identified a "new" emotional-behavioral disorder: post-stroke fatigue (PSF). Even if fatigue is commonly present in depression and constitutes one of the criteria for depression in most scales, it can occur in the absence of depressive mood. This dissociation has been particularly studied in Parkinson's disease and multiple sclerosis, but has also been reported in stroke. In this case, it is especially disabling and frustrating in that it typically involves patients with total or near-total neurological recovery, who should have been able to go back to their previous activities but who are becoming severely disabled because of early and persisting exhaustion. Preliminary neuropsychological and MR and PET imaging studies suggest that disruption of subtle mechanisms underlying attention, in the absence of significant cognitive and mood alterations, may be responsible for the development of PSF. Research projects are now being launched to better delineate PSF and its management.

## Mood disorders in multiple sclerosis

*G. Comi*
Department of Neurology, Neurorehabilitation and Neurophysiology, Università Vita-Salute and Scientific Institute H. S. Raffaele, Milan

Multiple sclerosis (MS) has major impacts on emotions: changes of mood and behaviour include anxiety, depression, grief, euphoria and emotional lability. Each patient may suffer from one or more of these phenomena during his life and all these mood changes may have great influences on the quality of life and on the working activity, even more than physical disturbances. The pathophysiology of these disturbances is far from being clarified, with combined influences of nervous damage and reactions to a chronic disabling disease.

Depression is the most frequent emotional disorder. Metanalysis revealed significantly higher scores for depression in MS patients compared to control groups. Depression symptoms may characterize the disease onset in some cases. Interestingly enough, in isolated syndromes depression is not observed, however at follow up patients who developed clinically definite MS resulted significantly depressed compared to patients who did not develop MS and to normal controls. In the early phases of the disease mood changes may be mostly explained by an adaptation to the disease. Depression prevalence tends to increase again in the more advanced phases of the disease as a reaction to the irreversible accumulation of disability. The frequency of suicide is increased by 7 times in MS population compared to the general population.

If the depression is caused by the nervous damage we should expect some association with the measures of brain damage. The amount of lesions in periventricular areas, temporal and frontal lobes, as revealed by magnetic resonance imaging, resulted significantly correlated to the presence of depression in some small studies, The small dimension of the examined samples and some methodological problems limit the value of these observations.

Immunological factors may also play a role in mood changes. An increased risk of depression has been observed in patients undergoing new attacks and it has been explained as a reaction to the increased impairment or to an adverse effect of steroid treatment. However the possibility of psychoneuroimmunologic dysfunction should also be considered (abnormal response to dexametasone suppressor test). Some medications, like steroids, anticonvulsivants, antispastics, etc. may also contribute to the depression. The negative impact of interferon beta treatment on mood reported in the Betaseron North American clinical trials has not been confirmed by subsequent studies with the same drug or other interferons.

Emotional lability is also frequently observed in MS, as in other chronic diseases. Patients may exhibit sudden and unmotivated changes of the mood with periods of anger, irritability, aggressivity lasting a few minutes. Euphoria has been for a long time considered very frequent in MS; more recent controlled studies indicate that it is present in not more than 10 % of the patients. It is frequently associated to executive dysfunctions indicating the key role of frontal lobes. Affective release, emotional crescendo, behavioural abnormalities may also be observed in MS, but probably not more frequent than in the general population.

Pharmacotherapy works in MS as in the general population, however the physician should consider the safety profile of the prescribed drugs because of the possible interference with other problems of the person with MS. Psychotherapy is of the outmost importance in order to help patients to adapt to affective and physical problems; it must be integrated with the involvement of the family and care givers. The correction of affective problems not only increases the quality of life but also allows a better compliance of etiologic treatments.

# Symposium 3
# Therapeutic neuroimmunology
# Chairs: DAS Compston (Cambridge); A. Steck (Basel)

## Past, present and future of humanised monoclonal antibodies

*Alasdair Coles*
Cambridge, UK

Animals produce antibodies of extraordinary specificity which can distinguish, for instance, between proteins differing only by one amino acid. The immune system achieves this with far greater reliability and speed than any chemist synthesizing small molecules. Antibodies have a further advantage that their recognition system (the Fab fragment) is distinct from their effector region (the Fc portion). Natural antibodies may simply bind the target (thus antagonising other ligands), induce signal transduction (acting as an agonist) or induce antibody-mediated cytotoxicity (so killing the cell carrying the target). Therapeutic antibodies can be further engineered to carry toxins or radionuclides, to kill cells in the region of their target. But before antibodies could be used as drugs, two problems had to be overcome: the limited supply of antibodies from one animal and their immunogenicity in man.

In 1975, Kohler and Milstein, working in Cambridge, UK, published their hybridoma technique for generating an immortal supply of monoclonal antibodies. Ten years later, the FDA licensed the first therapeutic murine monoclonal antibody, OKT3 (anti-T cell) which remains in wide use, especially in the transplant setting. In 1991 Weinshenker used OKT3 in the first trial of a monoclonal antibody in multiple sclerosis, with mixed success. A few years later, murine and chimeric (murine/human) anti-CD4 antibodies were trialled in multiple sclerosis; one got so far as a phase II trial which seemed to give a negative result. In 1986, Winter published a technique for "humanising" antibodies, by replacing the entire rodent protein, except for the sites that determine epitope-binding with a human immunoglobulin backbone. This has reduced, but not eliminated, immune responses towards therapeutic monoclonal antibodies. The first humanised monoclonal antibody to be approved by the FDA was daclizumab, in 1997. This anti-CD25 antibody was originally used in prevention of transplant rejection, but has recently been put through phase II trials in autoimmune diseases, including multiple sclerosis. The first humanised antibody to be

used in multiple sclerosis was Campath-1H, whose pilot results were reported in 1994 and is now in phase II. Antagonists to TNF-α, including a blocking monoclonal antibody, were approved in 1998 for the treatment of rheumatoid arthritis and Crohn's disease; paradoxically, their use in multiple sclerosis increases disease activity. In 1999, natalizumab (a humanised monoclonal antibody against an adhesion molecule VLA4) was shown to reduce new MRI lesion formation in multiple sclerosis; it is currently going through phase III trials and may become the first monoclonal antibody to be licensed as a disease-modifying therapy in multiple sclerosis. Currently, antibodies against the CD40 ligand, and the CD20 molecule on B cells are also in phase II.

Monoclonal antibodies are usually given by intravenous infusion, on a monthly or longer basis. The only class-specific adverse effect is immunogenicity; even a humanised monoclonal antibody like natalizumab generated antiglobulin responses in 11 % of patients after only six months of use. This is rarely symptomatic but may well interfere with efficacy. In rheumatoid arthritis, immunogenicity of the anti-TNF-α treatments is reduced by co-treatment with conventional immunosuppressants such as methotrexate. It will be interesting to know if interferon-beta reduces the antiglobulin response to natalizumab.

When antibodies were first taken up by biotechnology companies, their ease of manufacture and specificity of recognition made them ideal tools to explore targets in pre-clinical and phase I/II studies. It was imagined that, once a target had been identified, small molecules would be generated to take forward into phase II trials. The experience of the last decade tells us that this will not happen; instead humanised monoclonal antibodies will increasingly infiltrate the pharmacopoeia. Neurologists, especially those with an interest in multiple sclerosis, need to become familiar with the advantages and disadvantages of their use. The magic bullets have arrived.

## Hematopoietic stem cell transplantation in multiple sclerosis and other neuro-inflammatory disorders

*Francesc Graus, MD*
Service of Neurology, Hospital Clínic, Barcelona

Immune ablation with autologous hematopoietic stem cell transplantation (AHSCT) is presently evaluated as a potential treatment for severe cases of multiple sclerosis (MS) and other systemic autoimmune diseases. The rationale for using AHSCT to treat severe neurological autoimmune disorders is based on isolated case reports who underwent this treatment for a concomitant hematological malignancy and the positive effect of syngeneic or autologous bone marrow transplantation on the prevention or remission of experimental allergic encephalomyelitis. The aim of AHSCT is to produce a profound T-cell depletion and to reconstitute an immune system with a new immune tolerance. Although this objective would be better accomplished using an allogeneic hematopoietic stem cell transplantation, the morbidity and mortality of this type of transplantation prevents its use in MS patients.

How much evidence do we have that AHSCT fulfills the premises described above? First immune ablation is not absolute. Although there is a profound and sustained decrease of CD4 + cells after the procedure, some clones probably persist after the conditioning regimen and others are reinfused with the stem cells. CSF oligoclonal bands do not disappear suggesting that B-cells and, by the same token, T-cells may remain or repopulate the CNS shortly after the AHSCT. On a theoretical basis, the less autoreactive T cells that remain after the AHSCT the better to prevent recurrence of the disease. However, in clinical practice this rule may not be true. In a recent study of AHSCT to treat rheumatoid arthritis, those patients who had the procedure without in vitro selection of stem cells (so a larger number of T-cells were reinfused along with the stem cells) had a better, although not statistically significant, outcome (53 % vs. 28 %). A similar finding was observed in a retrospective study in MS patients. These data would agree with experimental evidence that part of the autoimmune T-cells may exert a neuroprotective effect and in this setting more depletion of autoreactive T-cells does not necessarily mean a better treatment.

A second point of concern is that the autologous nature of the transplant may prevent the raising of a new tolerance. Grafted stem cells mature in a stromal milieu that is not modified by the AHSCT. Consequently, the new T-cell repertoire will be raised in a similar environment and in the same genetic background that may predispose to perpetuate the aggression to the nervous system.

At present, studies of AHSCT for MS have been limited to a few series with small number patients. A recent study reviewed the clinical outcome of 85 MS patients treated with AHSCT and reported to the autoimmune disease working party of the European Group for Blood and Marrow Transplantation. There were 7 deaths, 5 related to transplant complications and 2 due to neurological deterioration. Confirmed progression-free survival was 74 % at 3 years with a trend for a worse outcome for primary progressive MS (66 %) compared with that of secondary progressive MS (78 %). The collected experience from this retrospective series will allow to define better the eligibility criteria for future studies, one of this will compare this treatment with mitoxantrone that has been recently approved for secondary progressive MS.

The present data on the impact of AHSCT in MS and other autoimmune diseases support the idea that this treatment is not a cure for the disease. The questions that remain open to debate are which subset of MS patients, if any, may benefit from a potentially lethal treatment and the most appropriate regimen to use. It has been argued that eradication of memory cells in the host by intensifying the conditioning may be important to achieve a durable response. However, this regimen probably would increase the likelihood of morbidity and mortality.

# Symposium 4
# Headache
# Chairs: H-C Diener (Essen) P. Goasby (London)

## From migraine patients to migraine mice, ... and back

*Professor Michel D. Ferrari, MD, PhD*
Leiden University Medical Centre, the Netherlands

Migraine is a common, episodic, multifactorial, neurovascular disorder with high socio-economic and personal impact. Although much is known about the mechanisms involved in the headache and aura, little is understood how and why attacks begin. Since migraine attacks can occur occasionally in any person, not the attack itself but the *recurrent occurrence* of attacks is abnormal. It has become clear that multiple genetic and environmental non-genetic factors are involved in the susceptibility for migraine attacks, most likely by setting and modulating the trigger threshold for attacks.

The first gene for migraine which has been discovered is the so-called CACNA1A gene on chromosome 19p13 encoding the main $\alpha_{1A}$ subunit of a neuronal P/Q type calcium channel. These channels are primarily involved in modulating the release of neurotransmitters including monoamines, acethylcholine, glutamate, substance P and calcitonin-gene-related peptide. Different CACNA1A mutations have been associated with a wide spectrum of brain disorders. These range from pure episodic disorders without interictal abnormalities such as familial hemiplegic migraine (FHM; a rare subtype of migraine associated with hemiparesis), episodic ataxia type 2 and epilepsy, via combinations of these disorders, to severe progressive ataxia and even uninhibited spontaneous and (mild) headtrauma-triggered fatal cerebral oedema. In addition, spontaneous mice mutants have been recognised displaying similar phenotypes due to mutations in the mouse homologue of the CACNA1A gene. Genetic association and linkage studies support the involvement of the CACNA1A gene in the common types of migraine, although actual mutations in such patients have so far been identified only rarely. Two transgenic knock-in mice models, harbouring human FHM mutations (one associated with pure FHM, the other with FHM and fatal coma) have been recently constructed. Intensive biochemical, pharmacological, neurophysiological, neuro-imaging, behavioural, and genetic studies are underway to unravel the functional *in vivo* consequences of these mutations.

Recently, FHM linked to chromosome 1q23 proved to be caused by mutations in the $Na^+$, $K^+$ ATPase pump gene ATP1A2. Apart from FHM, mutations in this gene can also cause various types of epilepsy. Transgenic knock-in mice models are underway.

Several other loci and genes have been associated with migraine as well. These include loci on chromosome 1q, 3p21 (likely to harbour a neurovascular gene responsible for a combination of vascular retinopathy, Raynaud syndrome, migraine and pseudo-tumour cerebri), 4q24 (linked to migraine with aura in Finnish families), 6p12–21 (migraine with/without aura) and Xq24–28 (in Australian families), as well as the Notch3 CADASIL gene (in migraine with white matter abnormalities) and possibly genes encoding for receptors for insuline receptor, endothelin type A, and dopamine D2.

Unraveling the genetic basis of migraine and studying the functional consequences of the genes involved will improve our understanding of the

mechanisms involved in the onset of migraine attacks and the interaction with migraine trigger factors. This may ultimately lead to specific, more effective and well tolerated migraine prophylactic drugs. Because of the many clinical and pathophysiological similarities, other episodic disorders such as ataxia, epilepsy, and trauma-triggered cerebral oedema may profit as well from these developments.

## What has neuro-imaging told us about the pathophysiology of headache?

*Arne May*
Department of Neurology, University of Regensburg

Cluster headache, like migraine, is still regarded as a *vascular headache* despite the fact that in both conditions a central nervous system cause has been suggested. Functional imaging is increasing our understanding of the pathophysiology of idiopathic headaches, focussing on identifying synaptic changes related to pain transmission.

### Functional imaging in migraine

Early studies using SPECT (single photon emission computed tomography) analysis – a semi-quantitative technique – showed no differences in blood flow between sides or in/out of an attack, in patients with migraine without aura. Positron emission tomography (PET) is the method of choice for quantitative study of metabolic and vascular changes. The question is, are there any PET changes that are specific for the pain of headache? When capsaicin is injected to induce headache in volunteers, there is activation (i. e. increase in regional cerebral blood flow (rCBF)) in the frontal cortex, both insulae, the contralateral thalamus and the cerebellum. These are all non-specific pain responses. Functional imaging is being used currently to look for any differences between migraine attacks and the resting state. Activation has been seen in the auditory and visual association cortex, corresponding to phonophobia and photophobia associated with an attack. Moreover, during the acute migraine attack, but not during the headache free interval, a highly specific activation in brainstem structures has been seen. It has been suggested that this vascular change may reflect a generator as it has not been seen in any studies of experimental headache or cluster headache or in any other models of somatic pain. Furthermore it has been shown that migraine symptoms can occur in patients with no history of migraine if this area of the brainstem (periaquaductal grey matter) is electrically stimulated.

### Functional imaging in cluster headache

Cluster headaches can be studied by using nitroglycerine as a trigger. In PET, during an acute attack, activation occurs in frontal areas, both insulae, contralateral thalamus and cingulate cortex. A specific activation was demonstrated in the ipsilateral hypothalamus. This region is highly intrinsic for cluster headache, as it has not been seen in migraine and experimental induced headache. Furthermore this region controls sleep-wake cycling and circadian rhythms and explains many of the facets of cluster headache.

Thus, functional imaging can identify specific areas of activation in specific diseases. These findings suggest that certain patterns of activation seen in primary headache syndromes are indicative for the specific syndrome.

*Morphology:* Recently MRI T1-weighted scans have been used to look for structural changes in the brains of patients with cluster headache. Cluster headache patients show an increase in grey matter in virtually the same area where the activation pattern using PET has been demonstrated. This was the first report of a structural change in this idiopathic headache syndrome. The data suggest idiopathic headaches to be a primary central nervous system disorder and demand renewed consideration of the neural influences at work in these syndromes.

## From receptor to drug – new therapies in headache

*Volker Limmroth, MD, PhD*
Department of Neurology, University of Essen, Germany

The observation that vasoconstrictive acting agents including serotonin (5-HT) were able to alleviate migraine attacks stimulated the systematic research of cranial vessel pharmacology and its potential role in headache pathophysiology and therapy about 30 years ago. Since serotonin could not be used clinically due to undesirable side effects (e. g. bronchoconstriction) when administered systemically, it appeared promising to develop more selective serotonin-like compounds that would target cranial vascular receptors without or less activity on other serotonin receptor subtypes. Parallel to efforts made in basic pharmacology, molecular cloning of new 5-HT receptors allowed the classification of 5-HT subreceptors within the 5-HT receptor family and paved the way for chemical programs to develop highly specific subreceptor agonists and antagonists. Until now 14 different 5-HT receptors, divided in 7 subfamilies ($5\text{-HT}_{1\text{-}7}$) have been described.

Sumatriptan, firstly designed as a selective $5\text{-HT}_1$ receptor agonist to mediate vasoconstriction in the cranial vasculature, was shown to be a potent selective $5\text{-HT}_{1B/1D/1F}$ agonist and became the very first compound of a new class of anti-migraine drugs, called *The Triptans.* As early as 1987 the first successful treatment of migraine attacks with sumatriptan was reported. Sumatriptan was introduced in 1991 in a subcutaneous and oral formulation and became the 'gold-standard' for the acute treatment of migraine attacks. Soon other 5-HT agonists were developed in the hope to enhance the clinical efficacy by improvement of pharmacokinetic or pharmacodynamic parameter. Hence, all second-generation triptans now exhibit longer half-lives, higher degrees of bioavailability or faster absorption kinetics. Now the triptan family encompasses 7 members (sumatriptan, zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, frovatriptan) in different formulation for different routes of administration (tablet, melting-tablet, subcutaneous injection, nasal spray, suppository) allowing now a custom tailored therapy of acute migraine attacks according to the need of the patient.

## Trigeminal autonomic cephalalgias (TACs): cluster headache and related problems

*Peter J. Goadsby*
Institute of Neurology, The National Hospital for Neurology and Neurosurgery Queen Square London UK

The Trigeminal Autonomic Cephalalgias (TACs) are a group of primary headaches characterised by relatively short-lasting attacks of severe pain in association with prominent activation of the cranial parasympathetic outflow [1]. It is known from animal and human experimental work that the pathophysiology of these headaches involves activation of trigeminal-autonomic reflex with its afferent limb in the trigeminal nerve, most dominantly the ophthalmic division, and its efferent limb in the facial/VIIth cranial nerve vasodilator output through the pterygopalatine/greater superficial petrosal outflow pathway [2].

The recognised TACs in section three of the revised International Headache Society classification are:

3.1 Cluster headache
3.2 Paroxysmal Hemicrania
3.3 Short-lasting Unilateral Neuralgiform headache attacks with Conjunctival injection and Tearing (SUNCT)
3.4 TACs not otherwise classified

Each of Cluster Headache and Paroxysmal Hemicrania has Episodic and Chronic forms. To have the chronic forms one must have breaks no longer than a month from symptoms.

A unifying central localisation for these conditions may be the posterior hypothalamus that is active in both Cluster Headache [3] and SUNCT [4] during attacks.

### References

1. Goadsby PJ, Lipton RB (1997) A review of paroxysmal hemicranias, SUNCT syndrome and other short-lasting headaches with autonomic features, including new cases. Brain 120:193–209
2. May A, Goadsby PJ (1999) The trigeminovascular system in humans: pathophysiological implications for primary headache syndromes of the neural influences on the cerebral circulation. J Cereb Blood Flow Metabol 19:115–127
3. May A, Bahra A, Buchel C, Frackowiak RSJ, Goadsby PJ (1998) Hypothalamic activation in cluster headache attacks. Lancet 351:275–278
4. May A, Bahra A, Buchel C, Turner R, Goadsby PJ (1999) Functional MRI in spontaneous attacks of SUNCT: short-lasting neuralgiform headache with conjunctival injection and tearing. Ann Neurol 46:791–793

# Symposium 5
# Neurostimulation of the nervous system
# Chair: P. Boon, Gent

## Deep brain stimulation for epilepsy

*Paul A. J. M. Boon, Md, PhD*
Reference Center For Refractory Epilepsy, Ghent University Hospital, Belgium

Acute deep brain stimulation (DBS) in various thalamic nuclei and medial temporal lobe structures has recently been shown to be efficacious in small pilot studies of patients with medically refractory epilepsy. Only limited data on chronic thalamic and amygdalo-hippocampal stimulation are available. Chronic DBS in these structures requires resolving many conceptual and technical issues. There is little evidence based information on rational targets and stimulation parameters. Currently available depth EEG recording electrodes are unsuitable for chronic use. Inversely, the use of DBS electrodes for intracranial EEG recording and localization of the ictal onset zone prior to stimulation has only been reported by a single group.

Results from feasibility and pilot studies in the literature will be presented. The experience with DBS in temporal lobe epilepsy using quadripolar DBS electrodes bilaterally implanted in the amygdalo-hippocampal region to identify and subsequently stimulate the ictal onset zone will be described. This work has yielded a significant decrease of seizure counts and interictal EEG abnormalities during long-term follow-up.

Various hypotheses on the possible mechanism(s) of action will also be discussed using EEG, cerebral blood flow and metabolic measures including results from animal experiments.

Data from pilot studies suggests that chronic DBS for epilepsy may be a feasible, effective and safe procedure that reduces interictal EEG abnormalities and seizures. Further trials with larger patient populations, controlled and randomised designs should be initiated.

## Vagus nerve stimulation for epilepsy: clinical experience in Europe

*Christian Elger, M. D., Ph.D.*
Head of the Department of Epileptology, University of Bonn, Germany

Vagus nerve stimulation (VNS) has been introduced as a new treatment of epilepsies and is now also used in depression therapy. Following a series of animal studies (Zabara, 1992), the first stimulation system has been implanted in November 1988 in a 37-years-old epilepsy patient (Penry & Dean, 1990). Since the FDA approval (July 1997) the stimulation system has now been implanted in > 20,000 epilepsy patients from > 20 countries worldwide (Europe: 608 patients from 45 centres in 13 countries). Therefore, VNS is establishing as the 'third way' of epilepsy therapy besides pharmacotherapy and epilepsy surgery. The efficacy of the system was approved in two randomised controlled trials (E03, The Vagus Nerve Stimulation Study Group, 1995; E05, Handforth et al. 1998) in the first of which European centres participated. In this study, only in a patient group under HIGH-stimulation ('treatment') conditions (n = 54) (as compared to a LOW-stimulation 'placebo' group, n = 60) a significant reduction of seizure frequency could be obtained during the 14 weeks of the acute study phase (percent seizure reduction: HIGH 25 % vs. LOW 6 %, responder rate: HIGH 31 % vs. LOW 13 % with response defined as > 50 % seizure frequency reduction). These results were confirmed in the American multicentre E05-study (percent seizure reduction: HIGH 28 % vs. LOW 15 %; responder rate: HIGH 23 % vs. LOW 16 %). Open clinical studies which have been published also from several European centres during the last years report responder rates of about 40–50 % after 1 year of VNS treatment (e. g., Scherrmann et al. 2002). The Cyberonics Patient Registry™ reports higher seizure responder rates (after 3 months: about 50 %; after 1 year: about 60 %; constant cohort, N = 2221). Positive psychological side-effects on emotional well-being have been observed in clinical practice and were confirmed in psychological studies (e. g., Harden et al. 2000; Elger et al. 2000). Cost-benefit analyses of VNS revealed significant total cost reduction in implanted patients (Boon et al. 2002). VNS is a therapy option for severely affected patients and should be considered in case of otherwise intractable epilepsies.

## Vagus nerve stimulation for epilepsy, mechanism of action

*Kristl Vonck*
Reference Center for Refractory Epilepsy, Dpt of Neurology, Ghent University Hospital, Belgium

Vagus nerve stimulation (VNS) is a neurophysiological treatment for patients with medically or surgically refractory epilepsy. Since the first human implant of the NCP device in 1989, over 15.000 patients have been treated with VNS. No clear predictive factors for responders have been identified. To date the precise mechanism of action remains to be elucidated. Better insight in the mechanism of action may identify seizure types or syndromes that respond better to VNS, may guide the search for optimal stimulation parameters and finally improve clinical efficacy. Animal experiments with VNS were initially performed to demonstrate efficacy and safety preceding human trials. In the field of the search for mechanisms of action animal experiments can provide essential clues such as crucial neuranatomical structures that are involved in the seizure-suppressing effect of VNS. The nucleus of the solitary tract as well as the locus coeruleus have been identified to play such a role. However, animal experiments are often labour intensive even in the hands of experienced researchers and the results remain only a reflection of the complicated pathophysiological systems of the human brain. Some discrepancies between animal and human data have emerged. With the development of animal models that mimic human refractory epilepsy more reliably such as the kindling model, the status epilepticus model and genetic models more valuable and usable information for human applicability may become available. Research on the mode of action of VNS in humans is a challenge because of safety concerns, the large number of patients required and the heterogenous nature of various small patient series. In the past ten years some progress has been made through neurophysiological, neuroanatomical, neurochemical and cerebral blood flow studies in patients and animals undergoing VNS. Interesting results have been found in VNS-treated patients that underwent evoked potential measurements, cerebrospinal fluid investigation, neuropsychological testing and PET, SPECT and fMRI testing. Desynchronisation of abnormal synchronous epileptic activity is one of the hypotheses on the mode of action that might primarily be responsible for an anti-seizure effect. There is however increasing evidence from research and clinical observation that VNS might establish a true and long-term anti-epileptic effect. It has been shown that VNS influences neurotransmission in the brain and provokes long-term changes in cerebral blood flow in areas crucial for epileptogenesis such as the thalamus and medial temporal lobe structures. Further elucidation of the mechanism of action may increase the clinical efficacy of VNS and provide inspiration for the development of new therapeutic modalities in the field of neurostimulation for refractory epilepsy. It may also support the use of VNS for other indications such as depression, pain and obesity.

# Satellite symposium on Neurorehabilitation

## Rehabilitation after stroke

*Udo Kischka, M. D.*
Oxford (UK)

Rehabilitation medicine has expanded the traditional medical model of the treatment of illness to a greater extent than most other medical disciplines. Rather than simply focussing on the diagnosis and treatment of a disease, rehabilitation physicians aim to prioritize the patients' wishes and needs, consider their social situation, and involve nurses and therapists as partners.

The WHO proposed an International Classification of Impairment, Disability and Handicap (ICIDH) which has more recently been revised and renamed the International Classification of Functioning, Disability and Health (ICF). It aids understanding the illness and its consequences on physiological, psychological and social levels. The model distinguishes Pathology, Impairment, Activity (called disability in the earlier model), and Participation (called handicap in the earlier model).

Modern stroke rehabilitation has the following characteristics: it comprises a multidisciplinary team of people who work together towards common goals for each patient, and involve and educate the patient and family in the process. Treatments are based on available evidence, and the effect of the rehabilitation is evaluated by standardised assessment methods.

*For the rehabilitation of motor deficits, several different theoretical approaches have been developed:* Proprioceptive Neuromuscular Facilitation *or (PNF);* Neurodevelopmental Treatment *(NDT) developed by Bobath;* Brunnstrom's *approach, and the* Motor Relearning Programme *(MRP).*

*In addition to the active training of movements, passive therapies such as positioning and tonic stretching are used. Adaptive equipment includes ankle-foot orthoses, arm or leg splints, and canes and frames to aid walking.*

*Several additional therapeutic techniques have been developed which can be used in conjunction with the methods described above, such as* treadmill training *with partial body weight support,* constraint-induced movement therapy, *also called forced-use therapy, and* functional electrical stimulation *(FES).*

Recent years have seen a gradual shift in focus of stroke rehabilitation from exercising isolated impairments towards task oriented therapy, which incorporates compensatory techniques and attempts to alter the patient's environment.

#### Evidence regarding effectiveness of stroke rehabilitation

Rehabilitation after stroke undoubtedly "works". A meta-analysis of data from trials of stroke unit rehabilitation has shown that rehabilitation services are effective at dramatically reducing both mortality and morbidity. Furthermore, there is evidence that these benefits can be achieved in actual practice in unselected hospitals, and they may last for many years. The meta-analysis helped characterize the probable important elements of effective rehabilitation: co-ordination, expertise, and education.

The evidence concerning each separate part of rehabilitation is much more difficult to evaluate, but the following elements have been shown to be effective: active therapy, treadmill training with partial body weight support, constraint-induced therapy, functional electrical stimulation, provision of simple equipment, provision of information. Regarding specific treatments for motor deficits (PNF, Bobath, Brunnstrom, MRP), no individual method has been proven more effective than the others.

There is evidence that drugs can have both beneficial (amphetamine, levodopa) and harmful (benzodiazepines, phenytoin, neuroleptics) effects upon a patient's performance.

## Rehabilitation of cognitive disorders

*Armin Schnider, M. D., Prof. Dr. med.*
Genève 14, Switzerland

The rehabilitation of cognitive disorders shares common principles with motor rehabilitation: its effectiveness depends on the specificity and intensity of therapy. Three basic principles can be distinguished:

1. *Re-learning of a lost function:* This is the most straightforward principle. Just like intensive training of a paretic arm promotes recovery of arm functions [Liepert 1998], intensive speech therapy (e. g. Hagen 1973: 18 hours per week) improves language capacity significantly better than scarce therapy (e. g. Lincoln 1984: 2 hours per week). Computer training may enhance such effects in the future.
2. *Pathophysiology driven therapy:* This approach uses knowledge about the precise pathophysiology of a cognitive failure and knowledge about the physiology of a functional system. For example, caloric stimulation [Rubens 1985], neck muscle vibration [Karnath 1993] and prisms [Rossetti 1998] may deviate internal spatial coordinates to the right. These manipulations had long lasting effects, when patients with neglect made an active spatial exploration training during the stimulation [Schindler 2002, Frassinetti 2002].
3. *Compensation of a lost function:* Two forms can be distinguished:
   *a. Internal compensation mechanisms*: Patients may learn new strategies to achieve a goal. For example, severely amnesic patients may acquire new habits and attain some independence in everyday life through their use of preserved memory systems – procedural learning [Ewert 1989], conditioning [Bechara 1995], and habit learning [Knowlton 1996].
   *b. External aids*: Comparable to a plegic patient who attains mobility by using a wheel-chair, patients with cognitive failures may profit from an external aid. For example, patients with amnesia or executive dysfunction may attain independence by using a pager system alerting them at the right time about actions to perform [Wilson 2001]. Such systems will certainly become more efficient and discrete in the future.

**Papers 208–212 will be presented in this session**

# FREE COMMUNICATIONS
# Oral Sessions

## Session 1

### General Neurology – 1

1
**Differential regulation of Toll-like receptor mRNAs in experimental central nervous system infections.** T. Böttcher, M. von Mering, S. Ebert, U. Meyding-Lamadé, U. Kuhnt, J. Gerber, R. Nau, University of Göttingen, University of Heidelberg, Max-Planck-Institute for Biophysical Chemistry (Göttingen, Heidelberg, D)

In central nervous system (CNS) infections, neuronal injury is caused jointly by stimulation of resident microglial cells, direct toxicity of the pathogen or its products on neurons, and the host's systemic inflammatory response leading to leukocyte extravasation into the subarachnoid space, vasculitis, brain edema and secondary ischemia. Toll-like receptors (TLR) play a key role in the recognition of microbial components. We investigated the differential regulation of TLR mRNA expression in bacterial and viral models of CNS infection.

We used reversely transcribed total RNA from frozen brains of experimental murine Streptococcus pneumoniae type 3 meningitis (n = 8), Escherichia coli meningitis (n = 8) and Herpes simplex virus type 1 encephalitis (n = 4) and respective controls. To evaluate the impact of invading immune cells, we also investigated TLR expression in *S. pneumoniae* R6-treated organotypic hippocampal slice (OTC) cultures (n = 180). Expression analysis was performed by quantitative real-time PCR using SYBR(R)-Green I as a fluorescent reporter. The cDNA was amplified by gene-specific, intron-spanning primers for TLR2, TLR3, TLR4, TLR7 and TLR9. For quantification, gene-specific serial standard dilutions were co-amplified and all gene-specific mRNA expression values were normalised against the house-keeping gene GAPDH.

Intracerebral infection with S. pneumoniae type 3 caused an increase in the mRNA expression of TLR2 (6-fold, $p = 0.002$), TLR9 (4.6-fold, $p = 0.002$) and TLR4 (2.2-fold, $p = 0.003$) compared to control animals. The treatment of mouse OTC with R6 pneumococci led to an increased expression of TLR2 mRNA (11.9-fold, $p = 0.0002$) and TLR3 mRNA (5.6-fold, $p = 0.001$) compared to untreated OTC. Intracerebral infection with E. coli caused an increase in the mRNA expression of TLR2 (26-fold, $p = 0.0002$), TLR4 (5.9-fold, $p = 0.001$) and TLR7 (1.9-fold, $p = 0.007$) compared to controls. TLR3 mRNA expression was also 1.9-fold increased in infected animals, yet the difference failed to reach statistical significance ($p = 0.06$). In HSV encephalitis, only TLR4 mRNA expression was increased compared to control mice (2.4-fold, $p = 0.03$).

Our data provide evidence that regulation of TLR mRNA is not fully specific for the molecular patterns of the infectious pathogen. The regulation observed probably represents a combination of specific response to the causative pathogen and non-specific activation of the innate immune system.

2
**Primary coenzyme Q10 (CoQ10) deficiency presenting as encephalopathy and nephropathy: a treatable condition.** S. Sacconi, G. Montini, C. Angelini, A. Naini, S. DiMauro, L. Salviati, University of Nice, University of Padua, Columbia University (Nice, F; Padua, I; New York, USA)

We report the case of a 27-month-old boy, of North African origin, son of first cousins, who presented at 2 months with nystagmus and optic atrophy. Motor development was mildly delayed and the child was hypotonic. At 12 months of age he presented with severe nephrotic syndrome that was treated with steroids, diuretics and cyclosporin and ultimately required peritoneal dialysis. A renal biopsy showed focal and segmental glomerulosclerosis. A cerebral MRI was normal. At 18 months he began to show psychomotor regression, marked hypotonia and tremor. Serum lactate and creatine kinase levels were normal. He developed status epilepticus with focal abnormalities on EEG especially in the left occipital region. MRI showed marked cerebral atrophy, milder cerebellar atrophy and stroke-like lesions in the left cingulated cortex and subcortical area. He had several other episodes of seizures. At 22 months he developed right hemiplegia with myoclonus and swallowing difficulties.

A muscle biopsy was obtained: histology was normal, Cytochrome c ox-

idase histochemistry was normal, but there were some fibers with increased sub-sarcolemmal succinate dehydrogenase staining. Measurement of the respiratory chain enzyme activities showed a reduction in the activities of complexes I + III and II + III, complex I, II and IV were normal. CoQ10 levels in muscle were markedly reduced 12 µg/gram of tissue (normal value: 21.5–28.5). Therapy with oral CoQ10 300 mg/die was started. In the next 5 months the neurological condition showed a dramatic improvement: he has had no more seizures, muscle tone and strength have improved as well as the hemiplegia, he has regained most of his developmental milestones and he is now able to walk. The renal situation remains stable although he is still on dialysis.

Coenzyme Q10 is a small hydrophobic molecule that transfers reducing equivalents from various dehydrogenases to complex III (ubiquinone cytochrome c reductase). Primary CoQ10 deficiency has been reported in several patients with lactic acidosis, cerebellar atrophy and myopathy with ragged-red fibers as the main clinical features and in a family with encephalopathy and renal failure. Our patient can be classified within this second and more rare form of the disease, which appears to be a distinct clinical entity since CoQ10 deficiency is not restricted to neuromuscular tissue but is multisystemic. The excellent response to CoQ10 supplementation underscores the importance of an early diagnosis for this condition. Follow up data will tell if also the kidney will benefit from the therapy.

3

**Prevalence of neuropathic pain and stroke in patients with Fabry disease enrolled in FOS – Fabry Outcome Survey – before and after enzyme replacement therapy with agalsidase alfa.** R. Ricci, M. Beck, F. Dehout, A. Garcia de Lorenzo, A. Linhart, A. Mehta, G. Sunder-Plassmann, U. Widmer, W. Borsini, UCSC, University of Mainz, CHU de Charleroi, Formacion Medica Continuada Hospital Universitario, Charles University, Royal Free Hospital, University of Vienna, University of Zurich, University of Florence (Rome, I; Mainz, D; Charleroi, B; Madrid, E; Prague, CZ; London, UK; Vienna, A; Zurich, CH; Florence, I)

Fabry disease (FD) is a rare metabolic disease caused by a deficiency of the lysosomal enzyme alpha galactosidase A, which is involved in glycosphingolipid catabolism. As a consequence, neutral glycosphingolipids, mainly globotriaosylceramide (Gb3), are stored in several different types of tissue and organs, e. g. nervous system, kidney, heart, eye, blood vessels and gastrointestinal tract. A progressive peripheral neuropathy is usual, but storage of Gb3 is also widespread within the central nervous system of affected individuals, most particularly within selected neurons of the spinal cord, brain stem, amygdala, hypothalamus and entorenal cortex. FD is considered a severe multisystem disease. Males are severely affected by Fabry disease, and typically die in the fourth decade of life as a result of end-stage kidney or heart failure. Females are also affected, but usually later in life.

Neuropathic pain is the major cause of morbidity during the first 2 decades of life in patients with FD. Initial clinical trials show that enzyme replacement therapy (ERT) with agalsidase alfa is effective in reducing neuropathic pain and improving the pain-related quality of life of patients with FD, and clinical experience suggests that it may also be beneficial in preventing hearing loss, headache and early stroke. This analysis aimed to examine the prevalence of neuropathic pain and stroke in patients in FOS – Fabry Outcome Survey – a large European database of patients with FD, and to evaluate the effects of ERT with agalsidase alfa (Replagal™; TKT-5S, Danderyd, Sweden) on neuropathic pain.

Neuropathic pain was found to be present in 76% of males and 66% of females in FOS, with a mean age at onset of 10.95 (standard deviation 9.13) years. Ischemic injury, which may result from occlusion of the vasa nervosa by lipid storage, is also common in patients with FD. Overall analysis of data in FOS shows that the additional risk of stroke is 15% in patients with FD; however, among younger patients (24–64 years) the additional risk is markedly higher than in the normal population, with an odds ratio of 1.45 for males and 1.95 for females.

After 11 months (average) of ERT with agalsidase alfa, the proportion of patients in FOS with no pain increased from 14 to 30%, and the proportion of patients with severe pain decreased from 22% at baseline, to 9% (EuroQoL). Significant improvements (all $p < 0.007$) were observed in mood, walking ability, normal work and social relations as measured by the Brief Pain Inventory (BPI) after 11 months of agalsidase alfa ERT.

In summary the FOS data have confirmed the increased risk for cerebrovascular events in patients with FD. ERT with agalsidase alfa improved several items of the BPI questionnaire, including parameters of pain and emotional function, in patients with FD.

4

**Kynurenine aminotransferases in human cerebro-spinal fluid.** H. Baran, B. Kepplinger, A. Kainz, M. Draxler, H. Ferraz-Leite, J. Wallner, J. Newcombe, H. Erhart, Veterinary University of Vienna, LNK Mauer, Hospital AKH Vienna, University of London (Vienna, Mauer, A; London, UK)

Kynurenic acid (KYNA) is a metabolite of tryptophan degradation and is a well known endogenous antagonist of three glutamate, ionotropic EAAs receptors (Stone, 1993). KYNA's anticonvulsive and neuroprotective activities have been demonstrated in animal models of various neurodegenerative diseases and its neuromodulatory character has been linked to the pathogenesis of neurological disorders and ageing process (Stone, 1993). In the CNS and in the periphery KYNA is synthesised from L-kynurenine by action of kynurenine aminotransferases (KATs). In the brain two enzymes KAT I and KAT II with different enzymatic properties are able to convert L-kynurenine to KYNA. KAT I is working at a high alkali pH and is sensitive to amino acids, whereas KAT II is working under neutral pH and is not sensitive to amino acids. The aim of the present study was to see whether cerebro spinal fluid (CSF) and serum of normal controls are able to convert L-kynurenine to KYNA. A group of ten normal control patients with a mean age of $39.7 \pm 4.1$ years was analysed.

Lumbar puncture was carried out to obtain CSF for routine parameters determination. Blood was carried out for clinical routine investigation. The CSF and serum were coded and made anonymous in accordance with the Medical Research Council guidelines on the ethical use of biological specimen collections in clinical research. For neurochemical analyses samples of CSF and serum were collected in 1 ml fractions and stored at –30°C until analyses. By using radioenzymatical assay (Schmidt et al. 1993) the presence of activities of KAT I and of KAT II in CSF and serum of normal control patients were investigated. By using high performance liquid chromatography (Baran et al. 2000) the levels of KYNA in CSF and in the serum were analysed.

The mean value of KYNA in CSF and serum was $3.35 \pm 0.26$ fmol/µl and $26.8 \pm 2.7$ fmol/µl, respectively. Interestingly, we found that human CSF is able to convert H3L-kynurenine (100 µM, 1.175 µCi/µmol) to KYNA, no synthesis was seen in the serum. Using assay condition for KAT I and for KAT II the conversion of L-kynurenine to KYNA was $155.9 \pm 28.5$ KYNA fmol/µl/h and $16.6 \pm 3.6$ KYNA fmol/µl/h, respectively. Within ten analysed CSF probes in one CSF probe no formation of KYNA using KAT I reaction conditions was seen (negative value). Obtained data indicate an approximately ten times higher activity of KAT I than of KAT II in CSF, and the importance of this finding needs to be clarified.

5

**Detection of intrathecally synthesized antinuclear antibodies in systemic connective tissue diseases with an immunofluorescence assay.** C. Jacobi, H. Rossmann, B. Storch-Hagenlocher, B. Wildemann, University of Heidelberg (Heidelberg, D)

Primary or secondary central nervous system manifestation can occur in various connective tissue disorders. The diagnosis of CNS involvement is based on technical methods such as cerebral angiography or MRI. However, angiography is insensitive if small vessels are affected and MRI lesions are non-specific. Cerebrospinal fluid (CSF) abnormalities are frequently absent or non-specific. Antinuclear antibodies (ANA) in serum are detectable in connective tissue diseases and they are included in the established diagnostic criteria. The gold standard for measurement of ANA is the immunfluorescence assay (IFA). A highly specific diagnostic marker in infections of the central nervous system is the detection of intrathecally synthesized antibodies with specifity for the causative pathogen. This method is established and the intrathecal antibody synthesis is calculated as antibody index (AI). An $AI > 1,4$ is defined as an intrathecal synthesis of the antibody and an $AI < 1,4$ as an absence of intrathecal antibody synthesis.

To assess whether an intrathecal production of ANA is associated with CNS involvement of connective tissue diseases we measured ANA with IFA in the CSF and serum in a group of 10 patients with positive ANA-titers in serum. In all patients CSF specimens were assessed for cell count and total protein and both serum and CSF samples were tested for albumin and IgG concentrations. Three patients had systemic connective tissue diseases including two patients with systemic lupus erythematodes (SLE) and one patient with systemic scleroderma according to the established criteria. In the remaining seven patients there was no evidence of systemic connective tissue diseases. In six patients, with ANA-titers in serum between 1:80 and 1:160, no signal in CSF was detectable. In the remaining four patients ANA in CSF were measurable (CSF-ANA-titers 1:4 up to 1:640) and the antibody-index (AI) was calculated. All patients had high ANA-titers in serum (1: 1280 up to 1:40000). One of these individuals (with a systemic scleroderma) had an intrathecal synthesis of ANA ($AI > 4$) compatible with CNS-involvement.

The calculation of intrathecally synthesized ANA using the AI has not been performed by IFA yet. In one of 10 examined patients included in our study the measurement of the AI > 1,4 confirmed the diagnosis of CNS involvement of systemic scleroderma. IFA seems to be a useful method for detection of intrathecally synthesized ANA and its sensitivity as a diagnostic marker for the neurologic manifestation of connective tissue diseases should be tested in a larger number of patients.

6
**Management of migraine between "state-of-the-art" and "harsh reality": results of the PAMINA study.** C. Wöber, K. Schmidt, K. Aydinkoc, R. Zingerle, J. Holzhammer, E. Geuder, N. Hattinger, K. Hanslik, Ç. Wöber-Bingöl, University of Vienna (Vienna, A)

Among headache specialists, there is a large degree of consent regarding the management of migraine. However, the patients' actual treatment is often inadequate (Brandes, CNS Drugs 2002). The aim of the present study was to compare the state-of-the-art to the actual treatment of migraine in a large sample of population-based volunteers in Austria.

We investigated 392 patients recruited via newspapers. The data are part of a prospective study on precipitating factors of migraine. All patients underwent a semistructured interview covering demographic data, headache characteristics and current migraine therapy. In addition, we asked the patients to assess their current medication as well as the medical management on a five-point scale. In order to quantify the impairment of the quality of life, the patients completed the Headache-Impact-Test (HIT).

Out of 392 patients 19 were excluded, because they did not have migraine. Among the remaining 373 patients, 87.7% were female. The age was 40.5 ± 11.8 years, the duration of the migraine history was 19.2 ± 11.9 years and the number of days with migraine amounted to 5.4 ± 4.4 per month. The HIT-score ranged from 49 to 78 and was 60 + (indicating a very severe impact of migraine on life) in more than 80% of the patients. Despite this marked impairment of the quality of life, only 25.5% of the subjects used triptans. Even though 60% of the patients had 4 or more days with migraine per month, only 6.5% had a prophylactic medication. The percentage of patients with current complimentary treatments was much higher and reached 30%. In the total group of patients, the quality score for the medication was 3.0 ± 1.3 and the score for the medical management was 2.9 ± 1.4. Patients treating their attacks with triptans gave significantly better ratings than patients using other drugs (2.4 ± 1.3 vs 3.1 ± 1.4, $p < 0.001$; 2.6 ± 1.2 vs. 3.1 ± 1.3, $p = 0.001$). Similarly, patients taking prophylactic medication rated their physicians significantly better than those without prophylaxis (2.4 ± 1.1 vs.3.1 ± 1.3, $p = 0.02$). In contrast, there was no difference in the ratings of patients with and without complimentary treatments.

In conclusion, the majority of migraine patients with a high need of effective acute therapy and/or prophylactic medication is undertreated. Educating physicians as well as patients is the most important strategy to improve these dramatic shortcomings in the treatment of migraine.

# Session 2

## General Neurology – 2

7
**Self-treatment of benign paroxysmal positional vertigo: semont manoeuvre versus Epley's procedure.** A. Radtke, K. Tiel-Wilck, M. von Brevern, A. Mainz-Perchalla, H. Neuhauser, T. Lempert, Charité, Virchow-Klinikum, Private practice, private practice, Robert Koch Institute (Berlin, D)

Current treatment approaches of benign paroxysmal positional vertigo of the posterior semicircular canal (PC-BPPV) include therapist-guided manoeuvres such as the Epley's procedure and the Semont-manoeuvre with success rates of about 70–90% after single and nearly 100% after repeated application. Recently, we showed that self-treatment with a modified Epley's procedure (MEP) was more effective in relieving patients from PC-BPPV within a week compared to conventional Brandt-Daroff exercises ($p < 0.01$). Since self-treatment may be a useful supplement for patients who remain symptomatic after single treatment, the aim of this study was to compare the efficacy of a self-applied Semont-manoeuvre and the MEP in 69 patients with PC-BPPV.

Methods: Patients with a history of positional vertigo and torsional nystagmus typical of PC-BPPV on Dix-Hallpike-testing were randomly assigned to self-treatment with a modified Semont manoeuvre (MSM; n = 33) and the MEP (n = 36). Patients performed the manoeuvre three times daily for one week according to an illustrated instruction that was explained to them at their first visit. Positional vertigo during self-treatment and treatment-related side effects were documented in a diary. Outcome measures after one week included absence of positional vertigo and absence of nystagmus on positional testing. Correct performance of the manoeuvre was evaluated by asking the patients to demonstrate self-treatment on their return visit.

Results: Self-treatment with the MEP was more effective in resolving PC-BPPV compared to the MSM: after one week, 35 of 36 patients (97%) who applied the MEP were free of positional vertigo and had no nystagmus on positional testing versus 19 of 33 patients (58%) in the MSM-group ($p < 0.001$). The two manoeuvres did not differ significantly with respect to treatment related side effects or correct performance of the manoeuvre. However, inaccurate performance had a negative effect on treatment outcome in the MSM-group.

Conclusion: Self-treatment is effective in resolving PC-BPPV within one week and should be considered as a supplementary therapy in patients who do not respond immediately to single physician-guided manoeuvres or in patients with frequent recurrences. Since inaccurate performance of the manoeuvre decreases its efficacy, a thorough instruction is essential.

8
**Therapy of neuroborreliosis – Continuous or interval therapy? A multicentre double-blind randomized study (CEFBO-Study).** C. Kayser, D. Feustel, R. Kaiser, H. W. Koelmel, S. Rauer, P. Oschmann, University Hospital of Giessen, University Hospital of Erfurt, University Hospital of Freiburg (Marburg, Pforzheim, Erfurt, Freiburg, D)

Introduction: The prognosis of a Lyme borreliosis is very favorable when an adequate treatment corresponding to stage and clinical syndromes is used. However, the incidence of incomplete response in neuroborreliosis stage II ranges from 6 to 38%. Proposed strategies for an optimized treatment include pulse or interval therapy in order to avoid the development of persistence forms in Borrelia burgdorferi infection.

Methods: A multicentre, prospective, double-blind study was conducted in order to compare a continuous three week cefotaxime-therapy with an interval therapy starting with 7 days cefotaxime followed by intervals of three days without antibiotics and three days with cefotaxime – completing a three week treatment course as well. Only patients with neuroborreliosis stage II were included in this study. In the group receiving continuous therapy 11 patients were evaluated, respectively 14 patients in the group, receiving interval therapy. Follow-up visits appeared 19 days, 3, 6 and 12 months after the treatment start. Clinical symptoms, subjective parameters and laboratory data were assessed at each visit and evaluated as primary and secondary endpoints. Clinical data were analysed in scores using eight items reflecting paresis, sensible deficit, cranial nerve diseases and other organic disorders. Subjective parameters were analysed in a score as well using three items to assess pain, sleeping disorders and corresponding therapy results.

Results: Both groups were comparable regarding demographics, subjective parameters, clinical and laboratory data at onset of treatment.

At inclusion the median clinical score was four in both study groups (SD 4.0 respectivly 3.6). After 19 days this score decreased to1.5 in the group with continuous therapy and 1 in the group with interval therapy. After 12 weeks this score was 0, respectively 1, and 0 in both study groups after 12 months. The subjective parameter score started with values 10 and 9.5, decreasing to 1 and 1.5 after 19 days, after 12 weeks 0 and 0.5 and 0 in both groups after 12 months. Laboratory data as CSF-data investigated at the onset, 19 days and 12 weeks later including cell count, protein, lactat and specific antibody synthesis were similar in both groups. A cell count of in median 200 cells in the group with continuous therapy and 343 cells in the other group was observed at onset. After 19 days the cell count decreased to 51, respectively 48 cells in median. After 12 weeks the median cell count was 2, respectively 6 cells. Wilcoxon-test comparing the clinical and subjective parameter score values as well as the laboratory data showed no difference between the two groups.

Conclusion:In this study the evaluation of the clinical outcome in the two treatment groups was comparable regarding clinical, laboratory data and subjective parameters in patients with neuroborreliosis stage II. We could not show a superiority for one of the treatment regimens.

9

**Migrainous vertigo presenting as episodic positional vertigo.** M. von Brevern, A. Radtke, A. Clarke, T. Lempert, Charité, Klinikum Benjamin-Franklin (Berlin, D)

Migraine is an increasingly recognised cause of recurrent vertigo and can manifest itself with isolated positional vertigo (pseudo-BPPV). Differentiation from benign paroxysmal positional vertigo (BPPV) can be difficult when patients present in the asymptomatic intervall.

In a retrospective chart review the authors identified patients with migrainous vertigo presenting as isolated episodic positional vertigo and analysed them with regard to history and clinical findings.

Five out of 362 patients with positional vertigo fulfilled our criteria for definite migrainous vertigo and presented with pseudo-BPPV. Further five patients had probable migrainous vertigo presenting as episodic positional vertigo. Four patients were examined during the symptomatic episode and showed positional nystagmus of a central type atypical for BPPV. In seven patients episodes with recurring positional vertigo lasted a few hours or days. All patients had at least three episodes and four patients reported 20 or more episodes. In two patients the onset of manifestation was in adolescence.

Migrainous vertigo can present with a syndrome mimicking BPPV. The following factors can contribute to distinguish migrainous positional vertigo from BPPV: short duration of symptomatic episodes and frequent recurrences, manifestation early in life, migrainous symptoms during episodes with positional vertigo, atypical positional nystagmus.

10

**Restless legs syndrome in spinocerebellar ataxia type 1, 2 and 3: functional study of the dopaminergic system by 11C- raclopride PET.** C. Globas, M. Reimold, M. Gleichmann, M. Schulze, C. Gerloff, H.-J. Machulla, R. Bares, J. Dichgans, K. Buerk, University Tubingen (Tubingen, D)

Restless legs syndrome (RLS) and periodic limb movement disorder (PLM) are frequently observed in spinocerebellar ataxia type 1, 2 and 3 (SCA) with a prevalence of up to 28%. In primary RLS and PLM, a putative role of the dopaminergic system has been postulated but functional imaging studies have yielded controversial results. In SCA, neuropathological changes of the basal ganglia have been described in all the three subtypes though clinical basal ganglia symptoms are not observed in most cases. To assess dopaminergic function in SCA1, 2 and 3 patients, dopamine-D2 receptor binding with 11C- raclopride PET was studied in 10 SCA- patients, 4 of whom suffered from RLS and compared to 10 age-matched control subjects. Mean caudate and putamen D2 binding was not significantly impaired in SCA. This finding was neither depending on the precise genotype nor the presence of RLS. These results suggest that RLS is not contingent upon a postsynaptic dopaminergic defect of the nigrostriatal system in various types of SCA.

11

**Prevalence and risk factors of restless legs syndrome (RLS) in Mersin, Turkey.** S. Sevim, O. Dogu, H. Camdeviren, R. Bugdayci, T. Sasmaz, H. Kaleagasi, M. Aral, Mersin University (Mersin, TR)

Background: Up to date, several population-based studies of RLS prevalence have been published. The reported prevalence rates are between 2.5% and 15%. The discrepancy in RLS prevalence and the condition's probable genetic origin suggest that prevalence varies amongst different populations. Further studies in different ethnic populations could help in identifying the underlying pathogenesis.

Objective: To determine the prevalence, risk factors and clinical presentation of RLS in a Turkish population.

Methods: A population-based, cross-sectional and selective study was performed. Multi-step, stratified, cluster and systematic sampling was used. The adult population of Mersin was 962,770 in 2000. The minimum sample size was calculated to be 2658 persons. The target study population consisted of 3500 adults from 20 health centers out of 151 in the province. All the interviews were conducted by 2 neurologists and 2 neurology residents who visited 3500 homes in designated areas in Mersin. Only one respondent per home was interviewed. RLS was diagnosed on the basis of the 4 minimal criteria suggested by the International Restless Leg Syndrome Study Group (IRLSSG). After confirming the diagnosis the interview followed a three-part questionnaire including sociodemographic and biological characteristics of RLS patients, IRLSSG rating scale and Hamilton depression and anxiety scales. An age and gender matched RLS negative individual from the study population was taken into the control group for each person diagnosed as RLS and given the same questionnaire.

Results: The rate of contribution was calculated to be 92.5%. RLS was identified in 103 individuals (3.19%). The mean age of onset of RLS was 36.11 years (SD: 14.08). In 37.9% of patients arms were involved. The prevalence of RLS increased with smoking, anemia kidney disease, but not with age. RLS was higher in females than males ($p = 0.02$). Positive family history, daytime sleepiness, depression and anxiety were more common in the RLS group when compared with the control group.

Conclusion: In comparison with the previously published studies from Europe and North America we found a lower prevalence of RLS suggesting a racial difference. Although most of the risk factors were similar to the previous reports, some incompatible results were also obtained. RLS seems to disturb sleep and mental health and most of the cases still remain undiagnosed.

12

**Adult sleepwalking: new aspects of pathophysiology.** G. Mayer, E. Leonhardt, T. Penzel, Hephata Klinik, University of Marburg (Schwalmstadt-Treysa, Marburg, D)

Introduction: The dynamics of slow wave sleep (SWS) play a pivotal role in pathophysiology of sleepwalking (Sw). Sleepwalkers have increased and more fragmented slow wave sleep (SWS) than controls.

We investigated the neurophysiological correlates of Sw-related and regular movements in NREM 2/SWS and their relation to REM-sleep, since 65% of our sleepwalkers report occasional dream recall prior to Sw.

Methods: PSG of 13 sleepwalkers (9 male; mean age all: 32 years) were compared with PSG from 13 controls (9 male; mean age all: 32.5 years) comprising number of arousals (10 min before and after movement), stage shifts, amount of SWS and slow wave activity (SWA, from O1 electrode), time from REM to Sw- and regular movement, and from the latter to next REM. Movements in NREM2/SWS in both studies were classified by videometry as regular movements or/and movements during Sw. Both types of movements were compared in sleepwalkers and between the latter and controls.

Results: Sleepwalkers had 67 Sw and 66 regular movements, controls had 32 regular movements. Compared to controls number of arousals was 2.5 times increased in sleepwalkers throughout the night and prior to Sw. There was no difference in stage shifts during the PSG and 10 min. prior to any movement. Sw was preceded by significantly longer SWS and more SWA than regular movements in the 10 min. prior to movement in sleepwalkers and controls. SWA in the 10 minutes after Sw was significantly higher than in regular movements of sleepwalkers and controls. Time spent in SWS prior to REM-sleep and duration from Sw to the next REM period was significantly longer than for regular movements. Sigma activity 10 min. prior to Sw was lower than in regular movements. In the latter it decreased within 10 min. after the movement, whereas it increased after Sw.

Conclusion: Our results confirm previous data for electrophysiological hallmarks of Sw: increase of arousal and of SWA. New findings are that Sw-related movements differ from regular movements by higher SWA power prior to movement, delay of REM-sleep in patients with Sw, and increase of sigma power after Sw. These findings suggest that Sw seems to depend on a threshhold of SWA, and maybe arousal. Moreover, lack of decrease of sigma power after Sw implies that increased SWA may overrule REM-sleep, thereby causing a dissociated state between NREM- and REM-sleep, thus explaining vivid dreaming in sleepwalkers. SWA increase after complex and regular movements may indicate a sleep preserving mechanism despite movement and may explain amnesia during overt nocturnal behavior.

13

**Retinal vasospasm.** A. Petzold, N. Islam, G. Plant, Institute of Neurology, Moorfields Eye Hospital, National Hospital of Neurology and Neurosurgery (London, UK)

Objectives: Review of transient monocular blindness to establish the clinical features of attacks due to retinal vasospasm.

Methods: A retrospective case note analysis of 60 patients. Patients with atheromatous or embolic aetiology were excluded. The data were analysed with respect to onset, duration, offset, recovery and frequency of attacks. The pattern of visual field loss was recorded to assess the likely vascular involvement.

Results: A video-reconstruction of the dynamics of retinal vasculature during an attack will be presented. The mean age of the patients was 39.3 [13–77] and the age of onset was 37.6 [12–77] years. 43 (72%) were female. Mean follow-up was 26 months. The median duration of an attack was 10 minutes with a mode of 2 minutes. The mean monthly frequency of attacks was 34 (median 1/month, mode 2/week). The pattern of loss of vision was 'curtain like' in 20 (34%), 'blotchy' in 6 (10%), 'concentric' in 4 (7%), 'coloured' in 3 (5%) and less well defined in 26 (44%). Persistent visual loss

occurred in 3 (5%) patients. Headache was reported during an attack in 8 (15%) and common migraine was reported by a further 6 (11%). Treatment with a Ca-channel blocker was initiated in 12 (24%) patients in all of whom a reduction of attack frequency was achieved.

Conclusion: We report the largest series of non-embolic transient monocular blindness. The response to nifedipine suggests that vasospasm underlies many or all of these attacks. Headache is not a common feature in our series, despite the International Headache Society definition for retinal migraine. The pattern of visual field loss localises the site of vasospasm either to the central retinal artery and its branches ('curtain-like','concentric') or to the choroidal vessels which supply the choroid in a wedge-shaped pattern ('blotchy').

# Session 3

## Higher function disorders and Dementia – 1

### 14

**A prospective Belgian study of neurodegenerative and vascular dementia: APOE genotype effects.** S. Engelborghs, B. Dermaut, J. Goeman, J. Saerens, P. Mariën, B. A. Pickut, M. Van den Broeck, S. Serneels, M. Cruts, C. Van Broeckhoven, P. P. De Deyn, Middelheim General Hospital, University of Antwerp (Antwerp, B)

Objective: We initiated a prospective study of neurodegenerative and vascular dementia in Belgium. Strict diagnostic inclusion criteria were used to include well-defined patients and controls. The results of apolipoprotein E (APOE) genotype effect on risk and clinical characteristics are presented.

Background: Growing interest in possible associations between APOE genotype and clinical features of Alzheimer's disease (AD) lead to numerous studies that yielded conflicting results. Whether or not APOE E4 leads to a more malignant clinical course and faster rate of cognitive decline remains a matter of debate.

Design/Methods: APOE genotyping was performed in patients with probable AD (N = 504), frontotemporal dementia (FTD) (N = 47), vascular dementia (VaD) (N = 152), mixed dementia (N = 132), mild cognitive impairment (MCI) (N = 44), Parkinson's disease (PD) (N = 30), dementia with Lewy bodies (DLB) (N = 17) and multisystem atrophy (MSA)/progressive supranuclear palsy (PSP) (N = 12).

Results: The APOE allele frequencies of our Belgian control population (E2: 6.9%; E3: 76.2%; E4: 16.9%) did not differ from those reported for other Caucasian populations. AD, MCI and mixed dementia patients had higher APOE E4 (32.9%, 38.6% and 28.4% respectively) and lower APOE E3 (62.2%, 53.4% and 66.3%) frequencies compared to controls, whereas only AD and mixed dementia patients had lower APOE E2 frequencies (4.9% and 5.3%). Besides a borderline significant different distribution of APOE allele frequencies in VaD patients compared to controls, no other differences were detected. The influence of APOE E4 on clinical features of dementia was limited to lower age at onset in AD patients and a less pronounced negative correlation between age at onset and number of E4 alleles in MCI and mixed dementia patients.

Conclusions: The present study confirmed the risk association between APOE E4 and AD. The observation that APOE E4 is also associated with mixed dementia reflected the role of AD in the etiopathogenesis of this condition. Although MCI is an etiologically heterogeneous syndrome, the increased APOE E4 frequencies indicated that a large proportion of the MCI patients included in our study might be predisposed to develop AD.

### 15

**The relation between white matter lesions and hippocampal atrophy in Alzheimer's disease patients.** F.-E. de Leeuw, P. Scheltens, University Medical Centre Utrecht, Alzheimer Centre VU Medical Centre (Utrecht, Amsterdam, NL)

Background: White matter lesions are frequently found on MRI scans of both demented and non-demented elderly. However, they cannot account for the characteristic memory dysfunction in Alzheimer's disease, which has been found to be associated with hippocampal atrophy. For a proper function the hippocampus depends on input from cortical association areas by means of projections running through the white matter. Possibly interruptions in these connections, for example by vascular white matter lesions, can lead to hippocampal atrophy.

Aim: We therefore wanted to investigate the relation between the degree and location of white matter lesions and hippocampal atrophy in Alzheimer's disease patients.

Methods: We investigated 179 probable Alzheimer patients according to National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria (NINCDS-ADRDA). From all participants a 1.0T coronal T1 and a Proton Density (PD) or fluid attenuated inversion recovery (FLAIR) cerebral MRI scan was made. White matter lesions were rated using a semi quantitative scale ranging from 0 to 3 with the age-related white matter changes rating scale (ARWMC). Hippocampal atrophy was rated semi quantitatively by visual assessment (range 0–4).

We calculated the mean severity of white matter lesions by category of hippocampal atrophy. All analyses were done by means of age and sex adjusted analysis of covariance.

Results: The mean age of the participants was 68.1 years (SD 8.8) and 53% of them were female. We found a linear relation between the severity of white matter lesions and the degree of hippocampal atrophy (ptrend = 0.001). This was most outspoken for white matter lesions in the frontal and parieto-occipital regions (ptrend = 0.002 and ptrend = 0.001, respectively). There was no relation for white matter lesions in the temporal, basal ganglia and infratentorial region.

Conclusions: We found a linear relation between the degree of white matter lesions, especially in the frontal and parieto-occipital region and the severity of hippocampal atrophy. This is in line with neuro-anatomical studies which found that the hippocampus receives most of its input from frontal and parieto-occipital association areas. It may be that white matter lesions underlie hippocampal atrophy in AD patients with white matter lesions and thereby provide an explanation for the observed memory deficits in those patients.

### 16

**Age-dependent association between interleukin-1A [–889] genetic polymorphism and sporadic Alzheimer's disease: a meta-analysis.** O. Combarros, M. Sanchez-Guerra, J. Infante, J. Berciano, J. Llorca, University Hospital Marques de Valdecilla, School of Medicine (Santander, E)

Background: Genetic risk factors for Alzheimer's disease (AD) may be associated with specific ranges of age at onset. There is controversy as to whether the effect of the interleukin (IL)-1A [–889] polymorphism (IL-1A 2/2 genotype) relates to all cases of AD, or is restricted to the early onset (less than 65 years) form or the late (more than 65 years) form of the disease.

Objective: We performed a meta-analysis using all published findings of case-control studies conducted until December 2002 to evaluate the effect of this polymorphism as a risk factor for early onset as well as late onset AD.

Methods: We identified 15 case-control studies through Medline from which 5 were excluded since data about genotype frequency was lacking or stratification according to age at onset was not performed. In the ten studies included in our analysis the methods were sound and the genotype frequencies of the controls were in Hardy-Weinberg equilibrium.

Results: The combined total of 594 patients and 654 controls from five studies were included in the meta-analysis of early onset AD, and the pooled odds ratio of AD associated with IL-1 A 2/2 genotype was 2.32 (95% CI = 0.99–5.43). The combined total of 1612 patients and 1630 controls from nine studies were included in the meta-analysis of late onset AD, and the pooled odds ratio for AD associated with IL-1 A 2/2 genotype was 1.11 (95% CI = 0.86–1.44).

Conclusions: The risk of AD associated with the IL-1 A 2/2 genotype is significantly modulated by age, in such a manner that the association is limited to early onset AD cases.

### 17

**CC chemokine receptor gene polymorphisms in patients with Alzheimer's disease.** D. Galimberti, C. Fenoglio, C. Lovati, A. Gatti, B. Corrà, I. Guidi, F. Cogiamanian, P. Baron, G. Conti, C. Mariani, N. Bresolin, E. Scarpini, IRCCS Ospedale Maggiore, Ospedale L. Sacco (Milan, I)

Chemokines and their receptors are involved in Alzheimer's disease (AD) pathogenesis. Astrocytes and oligodendrocytes stimulated with amyloid peptides produce Monocyte Chemoattractant Protein-1 (MCP-1) and RANTES, which serve as in vitro potent microglial and macrophage chemoattractants. Genes for their related receptors, CCR2 and CCR5 respectively, are characterized by polymorphisms resulting in a nonfunctional receptor expression. A 32 bp deletion in the coding region of CCR5 (CCR5delta32), present in Northern European populations at a frequency of about 0.2, leads to the expression of a nonfunctional receptor. A polymorphism in the CCR2 gene, causing the substitution of a valine with an

isoleucine (CCR2–64I) is present in various populations at a frequency between 0.1 and 0.2. The importance of this change on the function of the receptor is still debated. The distribution of the CCR5delta32 and CCR2–64I polymorphisms were determined in 140 Northern Italian patients with probable AD, diagnosed according to NINCDS-ADRDA criteria, as well as in 90 healthy subjects. Genomic DNA was isolated from whole blood, polymorphisms were determined by PCR-RFLP assay. Allelic and genotypic frequencies were obtained by direct counting. Hardy Weinberg equilibrium was tested. Fischer's exact test was used for differences in allele distributions between the groups. The frequency of the two polymorphisms in normal Italian population was similar to the one reported for the Caucasian population. No difference in the distribution of the CCR5delta32 allelic frequency between AD patients and controls was shown, while a significant difference in the frequency of the CCR2–64I polymorphism between AD and normal populations was observed (0.9 vs 0.3; $P < 0.05$). None of AD patients was homozygous for the CCR2–64I allele. Stratifying by ApolipoproteinE genotype or gender, no difference in allele frequency was observed. The presence of CCR5delta32 deletion doesnot seem to be a genetic risk factor for AD in Italian population. Conversely, the presence of CCR2–64I polymorphism seems to be a protective factor for the development of the disease. Notably, the genotype 64I/64I is absent in AD population, suggesting a crucial role for the wild type receptor in the pathogenesis of AD. A possible explanation may be that the mutation results in a non functional receptor, unable to bind the main counterligand, MCP-1, and thus limiting the extent of the inflammatory process.

18

**Alzheimer's disease: one clinical syndrome, two radiological expressions.** F.-E. De Leeuw, P. Scheltens, University Medical Centre Utrecht, Alzheimer Centre VU Medical Centre (Utrecht, Amsterdam, NL)

Background: According to clinical criteria, Alzheimer's disease (AD) is diagnosed as a single entity. Radiologically, however, two subtypes can be distinguished; one with and one without white matter lesions. It has been postulated that the type without white matter lesions may be a primary neurodegenerative disorder, while the form with white matter lesions may, at least in part, be caused by vascular risk factors. Vascular factors have been reported to be risk factors for AD, but it may be that this relation is confined to those patients with white matter lesions.

Goal: To investigate the distribution of blood pressure and an indicator of atherosclerosis (pulse pressure) in AD patients with and without white matter lesions.

Methods: We investigated 165 'probable' AD patients according to the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria (NINCDS-ADRDA). All patients underwent magnetic resonance imaging. Hypertension was defined as a systolic blood pressure ≥160 mm Hg and/or a diastolic blood pressure ≥95 mm Hg and/or the use of blood pressure lowering medication. Pulse pressure (PP) was the difference between systolic and diastolic blood pressure. White matter lesions were rated semiquantitatively.

We calculated the mean systolic and diastolic blood pressure, pulse pressure and the prevalence of hypertension in patients with or without white matter lesions by means of age and sex adjusted analysis of covariance.

Results: Patients with white matter lesions had a higher blood pressure, pulse pressure and prevalence of hypertension. There was a strong effect modification by age. Patients below 70 years of age with white matter lesions had a significantly higher systolic blood pressure compared to those without (148.1 mm Hg (SD 20.6) versus 136.2 mm Hg (SD 20.1); $p < 0.05$). They also had a higher, not statistically significant, diastolic blood pressure. They also suffered more from hypertension compared to those without white matter lesions (53.3% versus 28.6%; $p < 0.05$) and had a significantly higher pulse pressure (60.7 mm Hg (SD 15.3) versus 52.9 mm Hg (SD 12.3); $p < 0.05$).

Conclusion: We found evidence for heterogeneity in AD patients with respect to vascular risk factors. AD may include a heterogeneous group of disorders that share a common cognitive profile but with distinct radiological features and possibly also different etiologies.

# Session 4

## Higher function disorders and Dementia – 2

19

**Dopaminergic transport in Lewy body dementia and Alzheimer's disease: use in differential diagnosis.** M. Chatel, J. Darcourt, M. Soret, P. Malik Koulibaly, M. Borg, P. Bédoucha, P. Robert, I. Buvat, CHU Nice, Unité INSERM U494 (Nice, F)

Aim: Clinical misdiagnosis between Lewy Bodies Dementia (LBD) and Alzheimers'Disease (AD) is frequent. It has been recently shown (Walker et al., J Neurol Neurosurg Psychiatry. 2002) that unlike in AD, specific D2 dopaminergic degeneration in DLB could be demonstrated by SPECT. Quantitative DaTSCAN™ (123I-Ioflupane) SPECT and 3D MRI were used to evaluate the respective role of striatal structural and functional changes.

Materials and methods: 14 patients have been prospectively studied. AD diagnosis was made on DSM-IV criteria and LBD on international consensus criteria 96–97 (Mc Keith et al. (1996) Neurology). Six patients were diagnosed as LBD (mean age 70; mean MMSE 20.5) and eight as AD (mean age 76; mean MMSE 21.7). MRI studies consisted in 3D T1-weighted sequences with 2 mm thick slices (GEMS 1.5T scanner). SPECT acquisitions were performed 4 hours after the injection of 185 MBq of 123I-Ioflupane. A 3 headed Prism 3000 XP camera equipped with very high resolution low energy fan beam collimators was used. Images were reconstructed by OSEM (12 iterations and 12 subsets). Caudate (C) and putamen (P) nuclei were segmented manually on the MRI and volumes expressed in mL. The binding potential values (BP) were measured in the corresponding volumes of interest (VOI) after coregistration to SPECT data by mutual information maximization. They were calculated by reference to a posterior non-specific VOI (NS) as BP = [(C or P)-NS]/NS. These values were obtained after correction of attenuation, scatter and partial volume effect.

Results: With a threshold of 4 for the caudate BP value, SPECT has a sensitivity and a specificity of 100%. Considering the putamen BP value and a threshold of 8, the specificity decreases to 88%. There is considerable overlap of volume values that tend to be lower for AD despite higher BP values.

Conclusion: These data confirm that DaTSCAN™ SPECT is able to demonstrate in vivo specific dopaminergic degeneration in LBD. This result is not related to volumetric changes of the striatum. DatSCAN SPECT is a promising approach to differentiate DLB from AD.

20

**Galvanic vestibular stimulation and body posture influence the subjective visual vertical.** A. Saj, J. Honoré, M. Rousseaux, CHRU de Lille, Hopital Swynghedauw (Lille, F)

Background: Patients with spatial neglect present a deviation of the subjective visual vertical (SVV) contralateral to the lesion. However, the influence of vestibular galvanic stimulation or of body posture on this trouble has never been assessed.

Objective: To simultaneously investigate these influences.

Methods: Twelve patients presenting with a right hemispheric lesion, 7 with neglect (N+) and 5 without (N-) were included and compared to 8 control subjects. They had to orient vertically a luminous rod in darkness. There were 2 positions, sitting or lying, and 3 galvanic stimulation conditions, anode right, anode left and no stimulation. Electrodes were fastened onto both mastoids, and the intensity was of 0.8 or 1.5 mA.

Results: Without stimulation, the SVV of N– and especially of N+ patients showed an anticlockwise deviation. Low anodic stimulation (0.8 mA) only influenced N+ patients in left anode condition, with a significant increase in the left SVV error. Conversely, a stronger stimulation (1.5 mA) resulted in a similar significant deviation for left and right anode conditions, in comparison with baseline values. The deviation toward the side of the anode was more severe in the N+ group. The lying position increased the contralesional deviation observed in the sitting position in both patient groups.

Conclusions: This study demonstrated for the first time an influence of vestibular and postural stimulations on the SVV, especially in neglect patients. The asymmetry observed with low stimulation level suggests an asymmetry of the sensitivity of vestibular systems. The influence of vestibular stimulation is likely related to a stimulation of brainstem structures that were not affected by the lesion, and probably to eye cyclotorsion. Results also suggested that peripheral vestibular stimulation can influence the central representation of the peripersonal space. Furthermore, right anodic stimu-

lation can reduce manifestations of spatial neglect, and this could be used in rehabilitation.

21

**Rotation or translation of the spatial egocentric reference in neglect patients?** C. Richard, M. Rousseaux, A. Saj, J. Honoré, CHRU de Lille, Hopital Swynghedauw (Lille, F)

Background: In neglect patients, Jeannerod (1989) proposed that the deviation of the subjective straight-ahead (SSA) corresponds to a rotation of an egocentric reference towards the ipsilesional side (rotation hypothesis). For their part, Vallar (1995) suggested that the deviation would rather result from a global ipsilesional translation (translation hypothesis).

Objective: To test these hypotheses, using a new method of SSA measurement.

Methods: Twelve patients with right cerebral stroke were investigated. Six were considered as neglect (N+) regarding performance in lines bisection, scene drawing and bells cancellation. They were compared to six non-neglect patients (N–) and six control subjects (C). They sat facing (50 cm ahead) a horizontal luminous rod, adjustable in rotation and in translation. Rotation (in degree) and translation (in cm) movements were coded by 2 potentiometers. Subjects had to imagine a line starting from the navel and extending away straight-ahead of them, and had to place the rod on this line. Before each adjustment, the rod was initially translated to −15, 0 or + 15 cm and was rotated to −45°, 0° or + 45°. The translation displacement and the rotation angle were the subject of 2 ANOVAs, including the group as between-subject factor, and the initial translation (iT) and the initial rotation of the rod (iR) as within-subject factors.

Results: The translation significantly differed between groups ($p < 0.0001$). The rod was close to the mid-sagittal plan in C subjects (0.1 cm) and N-patients (0.9 cm) and did not significantly differ. Conversely, N+ patients presented with a right deviation (5.4 cm), compared to C subjects ($p < 0.0001$) and N– patients ($p < 0.001$). Moreover, the rotation angle did not significantly differ between the three groups ($p > 0.913$). The directions indicated by C subjects (−0.7°) and N– (−0.3°) and N+ (−1.3°) patients were close to a sagittal orientation. For both ANOVAs, neither the iT nor the iR nor the interactions were significant.

Conclusions: Healthy subjects and non-neglect patients were accurate. Conversely, the SSA indicated by neglect patients corresponded to a translation towards the right, consistent with what has been demonstrated using SSA pointing. The absence of any rotation suggests that the prevalent interpretation of SSA shift in pointing task is wrong: neglect patients seem to present with a global rightward translation rather than a rotation of their representation of the peripersonal space.

22

**Diagnostic value of CSF markers for classification of dementia.** A. Claus, D. Schöttle, M. Riepe, H. Tumani, University of Ulm (Ulm, D)

Objective: Diagnosis and therapy of cognitive impairment has witnessed growing importance during recent years due to the changing age-structure in the population. Alzheimer disease (AD) with 70% is the most common cause of dementia, followed by vascular form (VD), mixed dementia (MD) and mild cognitive impairment (MCI). In the light of restricted therapeutic options an early classification of the type of dementia is of importance. Measurement of specific proteins in CSF can play a supportive role for the differential diagnosis of dementia. Whereas diagnostic utility of the single tests poses limitations, the combined analysis of multiple variables may be associated with increased predictive value.

Patients and methods: Our cohort consisted of 103 participants, among them were 41 AD patients, 14 MCI patients, 7 patients with VD + MD, and 41 age matched non-neurological controls. All patients had normal basic CSF findings (cell count, blood-CSF barrier function, intrathecal immunoglobulins). In addition following variables were analysed: Tau protein, phospho Tau, S100b, beta-Amyloid, Abeta 40, Abeta 42, ApoE-Genotype. For assessment of the performance of the diagnostic measures the parametric ANOVA test and the non-parametric Kruskall-Wallis-test were performed followed by multiple discriminant analysis.

Results: In single parameter analysis the highest sensitivity and specificity for distinguishing AD patients (sensitivity 76.9%, specificity 76.1%) from the other groups and, respectively, for distinguishing normal controls (sensitivity 53.8%, specificity 96.6%) from others could be obtained by using the tau protein. To distinguish MCI from the other groups the Abeta 42/40 ratio was most useful (sensitivity 63.6%, specificity 73.3%). For VD + MD best distinction from others was possible using beta-Amyloid (sensitivity 66.7%, specificity 71.2%). By implementing discriminant analysis it could be shown that an overall increase of correct classification of the patients to 79.1% could be obtained by using tau, Abeta 40, the Abeta 42/40 ratio, ApoE genotype as well as the patient's age.

Conclusion: The combined analysis of CSF markers enhances the predictive value in differentiating between the various types of dementia and supports exclusion of non-cognitive diseases. The investigated proteins reflect the ongoing changes in the brain during the development of dementia and may be useful in establishing early diagnosis, staging and prognosis in dementia.

23

**Microglial activation in Alzheimer's disease: the role of amyloid beta-associated proteins.** S. Bussini, S. Sanzone, L. Meda, P. Fratta, G. Conti, E. Scarpini, N. Bresolin, G. Scarlato, P. Baron, University of Milan, Centro Dino Ferrari (Milan, I)

Heparan sulphate proteoglycan (HSPG), a1-antichymotrypsin (ACT), Cystatin C (CC) and Apolipoprotein E (Apo E) belong to a set of proteins that are associated with amyloid-beta protein (Ab) in senile plaques of Alzheimer's disease. These molecules are defined as pathological chaperons because they have been shown to bind to Ab, accelerate its fibril formation and maintain its fibril stability. In vitro studies have demonstrated that microglia synthesize proinflammatory cytokines in response to Ab stimulation, suggesting that the inflammatory reaction in senile plaques is triggered by Ab deposition. Whether Ab-associated proteins can also directly induce microglial activation leading to the production of bioactive inflammatory mediators has not been clearly elucidated yet. To test whether the interaction of pathological chaperons with microglia could induce the production of proinflammatory cytokines, we examined mRNA expression and release of TNF-alfa, IL-1beta and IL-6 from cultures of primary murine microglia stimulated with HSPG, ACT, CC, ApoE and, for comparison, with lipopolysaccharide (LPS). RT-PCR and specific immunoassays showed that in resting microglia the production of proinflammatory cytokines was absent or very low. mRNA expression and release of TNF-alfa, IL-1beta and IL-6 were clearly induced after culture with HSPG, CC and ACT, though to a lower extent with the latter. Microglia stimulated with Apo e3 and e4 also released significant amounts of TNF-alfa and IL-6, but did not produce IL-1beta. These results demonstrate that the Ab-associated proteins under study are able to induce the release of proinflammatory molecules by cultured primary murine microglia and that the production of these cytokines is regulated at the level of mRNA accumulation. These data suggest a potential role of HSPG, ACT, CC, ApoE as specific activators of microglial response with production of proinflammatory mediators implicated in neurodegeneration associated with Alzheimer's disease.

24

**Long-term treatment with galantamine for vascular dementia.** T. Erkinjuntti, S. Lilienfeld, University Central Hospital, Janssen Pharmaceutica Products L. P. (Helsinki, FIN; Titusville, USA)

Introduction: Impairment of the cholinergic system appears to be an important mechanism underlying the symptoms of many types of dementia. Galantamine is an anticholinesterase inhibitor that also modulates nicotinic acetylcholine receptors. It has been shown to be effective in the treatment of Alzheimer's disease (AD). This study was designed to gather information about the long-term safety of galantamine and its effects on cognition in patients with vascular dementia.

Methods: This was an open-label, multicentre extension study following 2 placebo-controlled trials. The initial double-blind studies lasted for 6 months with 6-month open-label follow-up. Patients entering the study had vascular dementia (VaD) according to the NINCDS-ADRDA criteria or Alzheimer's disease (AD) with radiological evidence of cerebrovascular disease (CVD). All patients had MMSE scores between 10 and 25 and ADAS-cog scores > 12. Patients who completed both phases were eligible to enter the 12-month open-label study when they received galantamine 24 mg/day. Cognition was assessed using the ADAS-cog/11.

Results: Of the 374 patients who completed the controlled trials, 326 entered the 1-year extension. Of these, 221 had received galantamine for the previous 12 months, and 105 had received placebo for 6 months, followed by galantamine for 6 months. Mean age at the start of the extension was 76 years, 45% of the patients were women, 173 (53%) had AD with CVD and 85 (41%) had VaD. Mean MMSE score at baseline of the original study was 21.

In patients with VaD who received galantamine in the double-blind phase (N = 60), improved ADAS-cog/11 scores were maintained for around 20 months. There was no significant change from baseline to the end of the extension (using last observation carried forward) in ADAS-cog/11 scores for this group (mean change 0.8 ± 7.7) or for patients with VaD who had received galantamine for only 18 months (N = 37, mean change 0.8 ± 8.6).

Galantamine was generally well tolerated. Depression, agitation and insomnia were the most frequent adverse events (reported for 13%, 12% and 11% respectively) as might be expected in this population. The incidence of nausea and vomiting was lower than in the original study and occurred in < 10% of patients.

Conclusion: This study shows that the efficacy of galantamine in vascular dementia is sustained over at least 24 months and that galantamine can safely be prescribed for long-term treatment in this patient population.

25

**Prevalence and incidence of dementia in the elderly in Shanghai urban and rural area.** Z. Hong, B. Zhou, J. Zeng, M. Jin, Institute of Neurology (Shanghai, CHN)

Objective: To estimate the prevalence and incidence of dementia, Alzheimer's disease (AD), and vascular dementia (VaD) in older in Shanghai urban and rural area.

Methods: Using a multistage screening process in 1997 and 1998, and a cohort of 5865 individuals was reexamined in 2001 to identify incidence cases. The Mini-Mental State Examination (cutoff 23/24, 21/22, 18/19 based on education) was employed to screen for dementia. Trained neurologists evaluated the individuals who screened positive. Final diagnoses had to meet Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised criteria for dementia, National Institute of Neurological and Communicative Disorders and Stroke Alzheimer's Disease and Related Disorders Association criteria for AD, and NANDS-AIREN criteria for VaD.

Results: Before the follow-up examination, 335 individuals had died. Of the 5350 survivors, 3331 completed the study. Overall, 109 new dementia (82 with AD) cases were identified among 3331 participants who contributed 11,309 person-years of observation. Average incidence rates per 1,000 person-years were 9.64 for dementia, 7.26 for AD, and 1.95 for VaD. Both AD and VaD showed age-dependent patterns. Women carried a significantly higher risk of developing AD (hazard ratio = 2.12, 95%CI = 1.52–3.75).

Conclusion: Incidence of dementia in Shanghai paralleled that in most industrialized countries.

# Session 5

## Epilepsy – 1

26

**A new gene localized in a family with febrile seizures, absence epilepsy and temporal lobe epilepsy.** R. Nabbout, J. F. Prud'Homme, O. Dulac, E. Leguern, Hôpital St Vincent de Paul, Généthon, INSERM U289 (Paris, Evry, F)

Objective: We report the clinical and genetic study of a large French family with febrile seizures (FS), absence epilepsy (AE) and temporal lobe epilepsy (TLE).

Patients and Methods: This family was identified through a national French campaign for familial epilepsy. It consists of 56 members with 11 affected on 3 generations. The medical history of all members was obtained by personal information and by consulting the medical files of each affected member. All family members gave a written consent to participate and 23 DNA were available for genetic study.

Results: Clinical study: All affected members presented FS in addition to AE in 4 members and TLE in one. All FS stopped before the age of 6 and they recurred less than 4 times. Patients presenting AE had registered absences and characteristic EEG with 3 Hz spike waves. FS were simple except in one patient who had a long lasting complex FS (45 minutes) that occurred at 8 months. He presented later pharmaco-resistant TLE and an abnormal left hippocampus on cerebral MRI. All family members have a normal psychomotor development.

Genetic study: The FS trait segregates as autosomal dominant trait. The genetic study allowed the exclusion of any linkage with reported loci for FS and FS plus (FS+), especially with the locus of GABRG2 gene on chromosome 5q reported in a family presenting FS+ and absences. A genome wide search with 380 markers allowed us to localize a new gene responsible for FS.

Conclusions: This finding emphasizes the heterogeneity of FS and the possible common genetic predisposition of simple FS and absence epilepsy. The TLE as a cause or consequence of FS is discussed. The sequencing of a gene highly candidate by function in this region is in progress.

27

**Risk of seizure recurrence after a first unprovoked seizure in childhood: a prospective study among Jordanian children.** A. Daoud, S. Ajloni, K. El Salem, K. Horani, Jordan University of Science and Technology (Irbid, JOR)

Purpose: There is wide variation in the reported recurrence rate after a first unprovoked seizure in children. We investigated the risk of recurrence after a first unprovoked seizure in Jordanian children and the risk factors associated with increased recurrence rate.

Methods: All consecutive patients aged 3 months to 14 years who presented with their first unprovoked seizures between January 1997 and 2000, were included in a prospective study and followed up for 3 years for possible recurrence. Of the patients studied, there was slight male predominance (56.6%) and 55% of them were 2–9 years of age. Generalized seizures were reported in 75% and the remaining 25% had partial seizures. The duration of seizure was 1–4 minutes in 59%. Family history of epilepsy was positive in 31% and parental consanguinity in 32%. The role of these factors in increasing the risk of recurrence was also investigated.

Results: Two hundred sixty-five patients were included in the study and continued follow up for 3 years. Ninety-eight (37%) of them experienced seizure recurrence. Among the predictor factors for recurrence, partial seizure ($p = 0.003$) and positive family history ($p = 0.000$) were associated with a statistically significant increased risk. Sex, age, duration of seizure and consanguinity were not associated with increased risk of recurrence.

Conclusion: Thirty seven percent of the children studied experienced a second attack after a first unprovoked seizure over the three years follow up period. The risk of recurrence was significantly higher in children with a partial seizure (55%) and among those with a positive family history of epilepsy (59%). Age at first seizure, sex, duration of seizure and consanguinity were not significantly related to the risk of recurrence.

28

**Long-term follow-up of vagus nerve stimulation in patients with refractory epilepsy, the Ghent and Dartmouth experience in a large patient series.** K. Vonck, V. Thadani, K. Gilbert, P. Williamson, P. Boon, Ghent University Hospital, Dartmouth-Hitchcock Medical Center, (Ghent, B, Lebanon, USA)

Objective: Vagus nerve stimulation (VNS) is an alternative treatment for patients with medically or surgically refractory epilepsy. In this study we have evaluated the long-term efficacy and safety in 131 patients treated with VNS at Ghent University Hospital in Belgium and at Dartmouth Medical Center in the USA.

Methods: Between March 1995 and January 2003, 131 patients (71M, 60F) have been implanted with VNS. The implantation, ramping-up and follow-up procedures were similar in both centers. Patients were excluded from the analysis if they had less than 6 months of follow-up (13 patients). We prospectively assessed seizure frequency, seizure type, prescribed antiepileptic drugs and side effects. The mean monthly seizure frequency before implantation and after maximum follow-up were compared.

Results: The patients included in this study had a mean age of 32 years (range: 4–59 years) and a mean duration of refractory epilepsy of 22 years (range: 3–49 years). Mean post-implantation follow-up was 34 months in 118 patients (range:6–94 months). In 95/118 patients with complex partial seizures there was a mean reduction in seizure frequency of 56% (range: 0–100%, SD = 31). In 19/118 patients with generalized epilepsy the mean seizure frequency reduction was 49% (range: 0–95; SD = 32). In 4/118 patients, simple partial seizures were reduced by 57% (range: 0–90; SD = 42). Eight patients (7%) became seizure free. Seventy-two (61%) patients experienced a seizure frequency reduction of > 50%. Twenty-seven patients (23%) were considered non-responders with a reduction of < 30%. Shortness of breath, unpleasant sensation in the throat or hoarseness during the stimulation on-time were the most frequent side-effects. The mean number of antiepileptic drugs was 3 (range: 1–5) before and remained unchanged after implantation.

Discussion: VNS has been successful in reducing seizure frequency in the majority of patients with partial or generalized epilepsy in this series. It is a useful alternative treatment with few side-effects that remains efficacious with longer follow-up.

29
**Indication and timing for pulse generator replacement in epilepsy patients treated with vagus nerve stimulation.** K. Vonck, S. Dedeurwaerdere, L. De Groote, P. Claeys, E. Baert, J. Camaert, J. De Reuck, P. Boon, Ghent University Hospital (Ghent, B)

Objectives: Vagus nerve stimulation (VNS) is an efficacious symptomatic treatment for patients with refractory epilepsy. Stimulation is provided by an implanted pulse generator with a battery that nears depletion after 4–12 years. There is a variable interval (days to months) between end of battery life (EOB) when irregular stimulation may occur and end of service (EOS) when the generator will no longer deliver any stimulation. Presently, no reports or guidelines concerning the indication and optimal timing for generator replacement are available.

Methods: In 9/74 patients, treated with VNS at Ghent University Hospital in Belgium since 1995, generators were replaced at different times following EOB. We retrospectively analysed the different approaches in these patients and correlated them with seizure control before and after replacement.

Results: One patient underwent replacement prior to EOB due to a circuitry failure. In 2/4 seizure-free patients, the time between EOS and replacement was several months. One patient experienced loss of seizure control and one had a recurrence of a major depressive episode. In 2/4 seizure-free patients replacement followed shortly after EOS. Seizure freedom was maintained in one patient and regained in the other one. In two patients with a > 50 % reduction in seizure frequency, immediate replacement following EOS guaranteed continued seizure control. In two patients with a 30–50 % seizure frequency reduction, replacement was performed when a significant increase in seizure frequency following EOS was noticed. In only one patient previous seizure control was regained.

Conclusions: In patients who respond well to VNS, generator replacement should be scheduled before EOS is reached to avoid the risk of seizure recurrence or loss of beneficial VNS side effects. In patients with a < 50 % reduction in seizure frequency, postponing replacement is a reasonable option. This allows to assess seizure frequency evolution and to document the indication for generator replacement.

30
**New developments towards identification of the epileptogenic tuber in patients with tuberous sclerosis.** F. Jansen, K. Braun, J. van der Grond, G. Huiskamp, A. van Huffelen, O. van Nieuwenhuizen, University Medical Center (Utrecht, NL)

Purpose: Patients with tuberous sclerosis complex (TSC) and drug resistant epilepsy may be considered candidates for epilepsy surgery. However, as most patients with TSC have multiple, potentially epileptogenic tubers, this option is often rejected. The surplus value of high resolution (HR) electroencephalography (EEG) and magneto-encephalography (MEG) recordings in combination with advanced MR-imaging is analysed in order to detect the epileptogenic zone. Furthermore, we tested whether diffusion weighted magnetic resonance imaging (DWI) enables differentiation of epileptogenic tubers from inert ones.

Methods: Simultaneous HR EEG and HR MEG recording was performed in 12 patients. Results were integrated in 3 D MRI scans. Integration of results of EEG recording alone was compared with simultaneous EEG-MEG recording. In four patients with clear unifocal interictal spike activity, fluid attenuated inversion recovery (FLAIR) MRI and DWI scans were performed. Apparent diffusion coefficient (ADC) maps were calculated in the identified epileptogenic tuber and compared with non-epileptogenic tubers and regions of normal cortex.

Results: Unifocal epileptiform activity was recorded in 6 of the 12 patients. The unifocal epileptiform activity was related to one tuber in 5 patients. The trace ADC of the four epileptogenic tubers (mean 1099 mm/s, SD 35) was significantly higher ($p < 0.001$) than that of 18 non-epileptogenic tubers (mean 926 mm/s, SD 69). Furthermore, the trace ADC of the non-epileptogenic tubers was significantly higher ($p < 0.001$) than the trace ADC of 16 regions of normal cortex (mean 784 mm/s, SD 62).

Conclusions: It is possible to identify a single epileptogenic zone in close relation to one tuber in patients with TSC. Simultaneous HR EEG and MEG recording has surplus value. Furthermore, DWI is a promising tool in differentiating epileptogenic from inert tubers. The histopathology of the tuber may explain the increase in diffusivity as this reflects an increase in extracellular space and a loss of structural organisation. The increase of ADC in epileptogenic tubers may be explained by an even higher loss of neurons or by oedema, caused by the seizures themselves. Clear identification of the epileptogenic zone may offer opportunities for epilepsy surgery in patients with TSC who were previously considered intractable.

# Session 6

## Epilepsy – 2

32
**How to study the survival of patients with mechanical ventilation for status epilepticus?** P. Liot, A. Delahaye, S. Bastuji-Garin, P. Aegerter, H. D. Outin, Hôpital Henri Mondor, Hôpital Lariboisière, Hôpital Ambroise Paré, Centre Hospitalier Intercommunal (Créteil, Paris, Poissy, F)

Purpose: The medical literature provides a wide range of mortality rate for status epilepticus related to the variety of the population observed and of etiologies. Use of mechanical ventilation is frequent in this situation. Our goal was to determine the risk factors for mortality of patients receiving mechanical ventilation for a status epilepticus.

Methods: This was a retrospective cohort study, drawn from the Cub-Rea database (Intensive Care Units (ICU) of Paris area): we first selected adult patients mechanically ventilated more than 24 hours between January 1, 1997 and December 31, 1998; second, we reviewed medical files of patients where a neurologic distress with seizures was at least one cause of the mechanical ventilation. For each patient the following data were collected: medical history, main cause of admission in ICU, clinical manifestations of the neurologic distress, possible causes of epilepsy. Factors associated with ICU mortality were determined by logistic regression.

Results: 347 patients from 18 ICUs were included in the cohort: 63.1 % men, mean age 55.4 ± 18.5 years, 36 % had a history of epilepsy, out-of-hospital cardiac arrest was the main cause of admission in ICU for 12 %. The crude ICU mortality rate was 30 %.

An electroencephalogram (EEG) was obtained in 275 patients (86.7 %), a mean of 21.7 ± 54.5 hours after ICU admission and within < 2hrs in only 69 patients (21.7 %). More than one cause of epilepsy was found in 204 patients (58.8 %) and more than two causes were found in 98 patients (28.2 %).

By logistic regression, independent factors associated with mortality were:

out-of-hospital cardiac arrest as the main cause of ICU admission (OR = 31.7; 95 % CI [11.8–84.6]); metabolic disturbances (8.3 [3.7–18.5]); relapsing seizures (4.1 [1.9–9.2]); absence of epilepsy history (2.5 [1.1–5.3]); absence of clinical convulsions during the admission episode (2.5 [0.9–7.2]); age (1.03 [1.01–1.05] per year).

Conclusions: The common association with severe neurologic diseases accounts for the difficulties in assessing the risk of death of the status epilepticus itself. Admission for out-of-hospital cardiac arrest and metabolic disturbances are strong predictors of mortality. The variety of other etiologies and lack of EEG during the first hours of the status epilepticus illustrate the practical difficulties in applying the usual classification of epilepsy to study the most critical presentation of status epilepticus.

33
**Visualization of interictal spikes as measured with subdural EEG electrodes using functional magnetic resonance imaging (fMRI).** S. Arnold, L. Jäger, M. Reiser, P. Winkler, S. Noachtar, University of Munich (Munich, D)

Functional Magnetic resonance Imaging (fMRI) using Echo-planar imaging (EPI) and the BOLD effect can detect regional activation during interictal spikes in focal epilepsy. We investigated whether interictal activity as documented by subdural EEG electrodes but not detected by concomitant surface EEG electrodes can elicit regional BOLD activation.

Three patients with temporal lobe epilepsies were investigated prior to resective epilepsy surgery. All patients had subdural electrodes covering the temporal lobe of seizure onset and additional surface electrodes. EEG was recorded using the "EMR" EEG amplifier (Schwarzer GmbH, Munich, Germany) and BrainLab software (OSG, Rumst, Belgium). EEG-artefacts due to MRI aquisition were eliminated based on MATLAB software (Math Works, Inc., Natrick, Massachusetts). MRI was performed using a 1.5 T whole body MR system ('Vision', Siemens, Erlangen, Germany). Images were obtained during "baseline" (without interictal temporal spikes recorded on subdural or surface electrodes) and during interictal spiking as documented by subdural EEG. The interictal activity was not detected at the surface electrodes in all patients. The localization and frequency of interictal spikes before and during MRI aquisition was registered using offline artefact-elimination and analysis. Only episodes without additional spiking during MRI aquisition periods were used for further analysis using the 'Analysis of Functional NeuroImages' (AFNI) software.

Activation maps revealed significant regional hyperperfusion of 8 % corresponding to the localization of the temporal lobe spikes in two of the three patients.

Conclusion: We demonstrate that interictal epileptiform discharges recorded with subdural EEG electrodes but not detected by concomitant surface electrodes are sufficient to elicit regional fMRI activation.

34

**SCN1A mutations in Dravet syndrome.** R. Nabbout, E. Gennaro, B. Dalla Bernardina, O. Bianchi, O. Dulac, F. Zara, Hôpital St Vincent de Paul, Genetica Umana, Ospedali Galliera, Neuropsychiatria Infantile (Paris, F; Genoa, Verona, Arezzo, I)

Objectives: To screen the SCN1A gene in severe myoclonic epilepsy in infancy (Dravet syndrome).

Background: A common genetic predisposition between severe myoclonic epilepsy in infancy (SMEI) and febrile seizures (FS) has been reported. Missense mutations have been identified in the SCN1A gene in families with GEFS+, a familial syndrome associating FS and FS+ to a spectrum of idiopathic epilepsy phenotypes. Two previous studies of SCN1A gene in few patients with SMEI disclosed de novo mutations in all.

Patients and methods: We performed mutation analysis of SCN1A in 93 sporadic patients with SMEI using DHPLC and direct sequencing. SCN1A was analysed also in both parents when the child presented a coding variant.

Results: We identified mutations in only 33 patients (35%). Most mutations were de novo but in 3 patients, mutations were found to have been inherited. Parents carrying the mutation displayed no symptoms of or presented a milder form of epilepsy. There was no significant clinical difference between patients carrying a mutation and those who did not; and between patients with a missense mutation (13 patients) and those with mutation yielding a truncated protein (20 patients).

Conclusion: We conclude that SMEI is genetically heterogeneous and in addition to SCN1A, which plays a major role in a large subset of patients, other genetic factors are involved.

35

**Non-convulsive status epilepticus: semiology, EEG, aetiology.** B. Feddersen, A. Bender, W. Scheuerer, S. Arnold, S. Noachtar, University of Munich (Munich, D)

This study aimed to evaluate the semiology, etiology and course of non-convulsive status epilepticus. We evaluated prospectively 25 patients with non-convulsive status epilepticus established by EEG.

Ictal EEG showed regional status epilepticus patterns in all 25 patients. The localization of status pattern was temporal in 32%, frontal in 32%, occipital in 8%, lateralized right in 16%, lateralized left in 4% and regional non-lateralized in 8%. The status semiology consisted predominantly of automatisms in 52%, of absence in 24%, of aphasia in 24% and of confusion in 12%. Automotor status was associated with temporal EEG localization in 46%, with frontal in 30%, with left hemisphere in 15% and with right central in 8%. Absence status was associated with temporal EEG status patterns in 16%, with frontal EEG status patterns in 33%, with occipital EEG status patterns in 17%, with right hemisphere EEG status patterns in 17% and with focal non lateralized EEG status patterns in 16.7%. Aphasic status showed left hemisphere EEG status patterns in 2 of 3 patients and was frontal non-lateralized in another patient.

Automotor status evolved into generalized tonic-clonic status in 4 patients. Secondary generalization was not observed in the other status semiologies. Etiologies in patients with automotor status included most commonly infarction (n=6), unknown cause (n=2), intracerebral hemorrhage (n=1), hamartoma (n=1), withdrawal of antiepileptic drugs (n=1). Absence status was secondary to CNS infection (n=2), infarction (n=1), unknown etiology (n=1), tumor or metastasis (n=1) and withdrawal of antiepileptic drugs (n=1). Aphasic status was due to withdrawal of antiepileptic drugs (n=1), CNS-Infection (n=1) and MELAS (n=1).

In summary non-convulsive status epilepticus clinically most commonly was characterized by automatisms (52%) and absences (24%) with temporal or frontal EEG localisation. Automotor status tends to evolve into generalized tonic clonic status, thus requiring more aggressive treatment to avoid worse outcome.

36

**Propofol treatment of refractory status epilepticus.** A. O. Rossetti, M. D. Reichhart, M.-D. Schaller, J. Bogousslavsky, P.-A. Despland, CHUV (Lausanne, CH)

Background: Status epilepticus represents a critical medical condition with high mortality. Although propofol (PRO) is considered an alternative treatment to thiopental (THP) for the management of refractory status epilepticus (RSE), only limited data are available. The aim of this study was to assess the PRO doses to induce burst suppression on EEG, efficacy, tolerance, and outcome in patients with RSE.

Methods: We included retrospectively [1997–2002] all patients on PRO for the management of RSE with EEG-controlled burst suppression. Subjects with anoxic encephalopathy showing pathological N20 on somatosensory evoked potentials were excluded. Our data were compared with those of a similar series of patients with RSE treated with THP.

Results: We found 31 RSE cases in 27 adults (16 men, 11 women; mean age 47 years). RSE etiology was idiopathic in 5 and symptomatic in 26. All received PRO, and 6 also THP subsequently. Seven patients died. RSE was successfully treated with PRO in 21 cases, and with THP and PRO in 3. Overall, mean PRO injection rate was 5.8 mg/kg/h (range 2.1–13). In patients treated with PRO alone versus PRO + THP, the data were as follows: days of ventilator treatment, 7.8 vs. 5; days of ICU stay, 10 vs. 7; pneumonia, 71% vs. 68%; hypotension requiring vasopressive treatment, 48% vs. 48%; death, 23% vs. 16%; cognitive deficits, 25% vs. 12% of survivors. Shivering at narcosis-emergence occurred in 10 cases, dystonia and hyperlipemia in 1 case each.

Conclusions: Propofol shows comparable efficacy as thiopental in the management of RSE. Although fatal outcome and hypotension were found in a similar number of patients, advantages of propofol include shorter length of ventilator treatment, less infectious complications, and less cognitive impairment in survivors than after thiopental treatment. Propofol-induced adverse events consisted mainly in benign and non-epileptic abnormal movements (shivering or dystonia).

37

**Reading epilepsy: report of two cases.** N. Bebek, C. Gürses, B. Baykan, A. Gökyigit, Istanbul University (Istanbul, TR)

Reading epilepsy (RE) is a rare and amazing form of reflex epilepsies, mostly classified under the heading of idiopathic partial epilepsies. The pathophysiology and the origin of this unique entity are not well understood and there is still some doubt about its classification. The outstanding feature of RE is involuntary jaw jerking that may progress to generalized tonic-clonic seizures (GTCS) during reading in an otherwise healthy individual, mostly in the second decade.

Two epileptic patients who experienced seizures during reading are evaluated using short-term video-EEG monitoring, MRI and SPECT imaging to find out the relevant characteristics and some clues for the origin of RE.

An 18-year-old male patient with an unremarkable medical history and neurological examination had experienced nearly a dozen of GTCS between the ages of 1.5 and 5 years. Then, he was seizure free with a normal EEG for the last 13 years, and stopped taking the phenobarbital treatment, 5 years ago. He experienced after this long seizure-free interval, an attack with perioral stiffness while talking with excitement and a second attack with jaw contractions when he was reading. Cranial MRI revealed no abnormality. Resting EEG showed slow waves over bilateral centrotemporoparietal regions and spontaneous generalized spike and wave discharges (GSWD) with an increase during the intermittent photic stimulation period. The video-EEG exam disclosed perioral myoclonia and jaw jerking while reading aloud a newspaper. The ictal EEG showed GSWD synchronous with the clinical seizures. SPECT-imaging demonstrated marked left temporal hypoperfusion along with hypoperfusion in biparietal and left frontal regions.

The second patient, a 48-year-old male had experienced only 3 seizures at 14 years of age. They started with jaw jerking, followed by loss of consciousness and GTCS during reading. He used phenytoin and stopped after 3 years of successful treatment. Then, he has been seizure free for 31 years. He was admitted at this stage, with a spontaneous epileptic attack consisting of a period of talking disability followed by GTCS. He had an unremarkable medical history other than epilepsy and a mild congenital asymmetry of the left hand. His EEG showed a prominent epileptic focus on the left frontotemporal region and also spikes over the right temporal region. Cranial MRI revealed no abnormality.

Reading epilepsy has a benign course with long remission periods and associated with rare spontaneous epileptic seizures in our reported cases. The etiology is presumably idiopathic as suggested with normal MRIs and clinical characteristics of our patients. This opinion was further supported by the presence of ictal GSWD in one of our patients, which could be considered a genetic marker for liability of idiopathic epilepsy. However, SPECT data together with the focal temporal EEG findings pointed out a possible left temporal lobe dysfunction as the probable origin of the seizures.

38
**Epileptic nystagmus: two case reports and a literature review.** Y. G. Weber, J. Roesche, A. Jung, M. Riepe, H. Lerche, University of Ulm, Hospital "Die Weissenau" (Ulm, Ravensburg, D)

The epileptic nystagmus is characterized by fast repetitive eye movements and is a rare symptom of epileptic activity. We describe two cases with epileptic nystagmus. Videos of seizures are presented. A 42 year old woman and a 22 year old man were referred to hospital because of attacks of unclassifiable dizziness and sickness. Ictally the female patient developed a horizontal right beating nystagmus accompanied by epileptic discharges in the left temporo-parietal-occipital region. It was the first manifestation of a symptomatic epilepsy based on a cerebral infarction left fronto-temporal 20 years ago. She suffered from an hereditary antithrombin III deficiency. The patient was seizure free under a medication of 800 mg of carbamazepine per day. The male patient showed ictally a horizontal right beating nystagmus and epilectic discharges parieto-occipital on the right side. In the MRI a right sided parietal dysplasia was found. The two cases are compared to a literature review.

# Session 7

## Multiple sclerosis – 1

39
**Chemokine polymorphisms: susceptibility and outcome in multiple sclerosis.** J. M. Partridge, J. A. Woolmore, A. A. Fryer, W. E. R. Ollier, M. D. Boggild, R. C. Strange, C. P. Hawkins, MS Research Group, ARC Epidemiology Unit, Walton Centre (Stoke-on-Trent, Manchester, Liverpool, UK)

Background: Multiple sclerosis (MS) is a T cell dependent inflammatory disease of the central nervous system (CNS). Chemokines are molecules involved in leucocyte recruitment and activation of inflammation in the CNS. Chemokine receptor 5 (CCR5), a major receptor for the chemokine RANTES, is expressed in normal CNS tissue. The receptor is overexpressed in infiltrating lymphocytes, particularly the proinflammatory Th1 type important in MS pathogenesis. RANTES is a chemoattractant for Th1 cells in MS patients due to CCR5 overexpression. This effect can be blocked using anti-CCR5 antibodies. Both RANTES and CCR5 demonstrate functional polymorphisms: CCR5 delta 32 is a truncated allele of the gene that encodes a non-functional receptor while the -403 G-A substitution in the RANTES promoter is associated with an 8-fold increase in transcriptional activity. Objectives: We performed candidate gene association studies to determine whether: (a) CCR5 D32 confers protection from MS or reduced severity, (b) RANTES -403 G-A is associated with MS susceptibility and outcome and, (c) combinations of these genotypes are important.

Methods: 346 patients and 204 controls of Northern European origin were recruited. DNA was extracted from leucocytes and PCR-based assays were used to identify the CCR5 and RANTES polymorphisms. Outcome was assessed with Kurtzke's Expanded Disability Status Scale (EDSS). Cases were stratified into mild/moderate (EDSS 0–5.5) and severe disability (EDSS 6–10) after disease duration of 10 years. Results were analysed using logistic regression to correct for independent covariants of age of onset, gender and disease duration. Significance levels were set at $p < 0.05$. Results: Significant association was found between possession of the -403 A allele of RANTES and MS (OR = 1.54, 95 % CI 1.03–2.29, $p = 0.03$). No association was seen between CCR5 D32 and MS when considered alone. A combined genotype effect was dominated by RANTES. No association was found between genotypes and severity. Conclusions: This is the first report of an association between a RANTES polymorphism and MS indicating a possible role in its onset.

40
**Disease severity in patients with multiple sclerosis may be greater in Northern than Southern Europe.** R. Roxburgh, S. Seaman, T. Masterman, A. Hensiek, S. Sawcer, M. Coustans, E. Le Page, G. Edan, I. Achitti, S. Vukusic, C. Confavreux, G. McDonnell, G. McDonnell, S. Hawkins, M. Liguori, M. Trojano, E. Cocco, M. G. Marrosu, F. Tesser, M. Leone, A. Weber, F. Zipp, A. Oturai, P. Soelberg Sorensen, E. Celius, N. Tellez Lara, X. Montalban, P. Villoslada, A. Silva, M. Marta, I. Leite, B. Dubois, B. Miterski, J. Epplen, J. Rubio, T. Kilpatrick, H. Butzkueven, J. Hillert, A. Compston (Auckland, NZ; Cambridge, UK; Stockholm, S; Rennes, Lyons, F; Belfast; Bari, Cagliari, Novara, I; Berlin, D; Copenhagen, DK; Oslo, N; Barcelona, Pamplona, E; Oporto, P; Leuven, B; Bochum, D; Melbourne, AUS)

The Multiple Sclerosis Severity Score (MSSS) provides a novel approach based on comparing individual scores on the Extended Disability Status Scale with the distribution of scores in patients having equivalent durations of disease. We applied the MSSS method to a dataset of over 10,000 patients from 13 different countries to derive a reference table, the global MSSS, by which to correct EDSS for disease duration.

Rates of disease progression in the 16 different groups who contributed patients to the MSSS were compared using the median global MSSS score. There was a consistent increase in severity of disability with increasing latitude ie. at higher latitudes disability progression was faster.

While this finding may represent differences in recruitment between the centres, examination of the recruitment methods and possible confounding from age, course of disease and use of immunomodulatory medication suggested that these factors did not fully explain the differences.

We conclude that there may be a latitudinal difference in disease severity which mirrors the established difference in disease susceptibility, though this incidental finding will need further study to be verified. If found to be true it may represent different underlying genetic substrates or an unknown environmental factor.

41
**Musiqol: a unique quality of life questionnaire for patients with multiple sclerosis.** P. Auquier, L. Blumhardt, O. Fernandez, P. Flachenecker, J. Pelletier, S. Stecchi, M. C. Simeoni, P. Brasseur, A. Beresniak, Hôpital de Marseille, Hospital Carlos Haya, Bayerische Julius-Maximilians-Universität, Hôpital La Timone Marseille, Villa Mazzacorati, Serono International Sa on behalf of the Musiqol Group

Objective: To establish a health-related quality of life questionnaire that will be used to assess different treatment strategies in multiple sclerosis (MS) and evaluate patients' use of healthcare services.

Background: The Multiple Sclerosis International Quality of Life (MuSIQoL) questionnaire will focus on patient concerns associated with MS. Available in several different western languages, MuSIQoL will be applicable to patients with different forms of MS and varying levels of disease progression.

Design/methods: A total of 107 patients with MS were interviewed in 5 countries: France [23], Germany [20], Italy [21], Spain [20] and the UK [23]. All patients had a diagnosis of MS (relapsing-remitting, secondary progressive or primary progressive) according to the Poser criteria. Patients with clinically isolated events suggestive of MS were also included. A list of patients' common concerns was compiled and a preliminary questionnaire drawn up based upon this. The questionnaire was tested among an additional 178 patients of differing nationalities, to ensure that it was both suitable and comprehensible. This test group comprised patients with a mean age of 42 ± 11 years, of which 60 % were female, 38 % had SPMS and 28 % were in employment.

Results: After careful analysis of the interviews, along with further interviews in Argentina, Brazil, Canada, Greece, South Africa, Turkey and the USA, a definitive list of 73 questions was drawn up. These related to seven aspects of patients' lives: social relationships, psychological health, physical health and activities, autonomy, financial issues and treatment. The questionnaire is currently being validated in a study involving 2000 patients in 21 countries.

Conclusions: MuSIQoL is a unique, patient-centred questionnaire, available in several different languages. Once validation is complete, the final questionnaire will encompass approximately 40 concerns pertinent to patients with MS. MuSIQoL will serve as a valuable assessment tool for different therapeutic strategies in MS, and will enable patients' use of healthcare services to be evaluated.

42
**Brain excitability changes in the relapsing or remitting phases of multiple sclerosis: a study with transcranial magnetic stimulation.** M. G. Palmieri, L. Boffa, D. Centonze, P. L. Galizia, F. Ursini, G. Bernardi, M. D. Caramia, II University of Rome Tor Vergata (Rome, I)

In vivo spectroscopic MRI studies on multiple sclerosis (MS) have shown the importance of measurements that are reliable and responsive to changes in the early stages of the disease; such a new awareness compels a reconsideration of the potential value of identifying neurophysiologically appropriate measures able to monitor changes underlying the clinical manifestations of relapsing-remitting MS. In the present study we explored some characteristics of central motor pathways related to changes of neuronal excitability as measured by using transcranial magnetic stimulation (TMS).

Eighty-five patients affected by relapsing-remitting (RR) MS were examined using single and paired TMS in order to assess excitability changes in the hand motor cortex occurring during relapse and/or remission of the disease. The analysed parameters were: MEP threshold, silent period (SP), intracortical inhibition (ICI) with paired pulses from 1 to 6 msec interstimulus intervals (ISIs) and central motor conduction time (CMCT).

The ANOVA exhibited a strong correlation ($p < 0.001$) between the clinical phase and the type of excitability changes: "relapsing" patients showed increased threshold and reduced SP duration. "Relapsing" patients also displayed a significant lack of normal intracortical inhibition (ICI). By contrast, "remitting" patients showed a significant SP prolongation with normal motor thresholds.

The present findings reveal changes in cortical excitability that can be either explained by excitotoxicity, a mechanism new to the field of inflammatory demyelination, as recently demonstrated in an animal model of multiple sclerosis, and/or by the presence of cortical adaptive changes that might contribute to the maintenance of motor performance despite scattered brain lesions.

43
**New potential target antigens involved in the pathogenesis of multiple sclerosis.** S. Cepok, D. Zhou, S. Stei, S. Nessler, K. Buessow, N. Sommer, B. Hemmer, Philipps-University Marburg, Max-Planck-Institute for Molecular Genetics (Marburg, Berlin, D)

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS) with an as yet unknown aetiology. A hallmark of the disease is the presence of intrathecal antibody synthesis and the occurrence of oligoclonal IgG bands (OCB) in the cerebrospinal fluid (CSF). It is likely that the immune response targets disease relevant antigens expressed in the CNS as found in a variety of other predominately infectious diseases of the CNS (e. g. neuroborreliosis, SSPE). In these disorders the antibodies recognize antigens from the infectious agents. In MS, target antigens – autoantigen or infectious agent – are still unknown. To investigate the antigen specificity of the local antibody response in MS patients we applied a novel protein expression array technology. Protein arrays, comprising 35,000 cDNA inserts from a human fetal brain library, were probed with CSF and serum of 15 MS patients and 5 controls. Immune responses to 25 proteins were identified specifically in MS patients and not in controls. Further analyses disclosed a higher immune reactivity in the CSF of MS patients than controls to 4 of these candidate antigens. Furthermore, we demonstrated specific intrathecal antibody production and binding of the OCB to the proteins. Epitope mapping by substitutional analyses disclosed the peptide sequence of the humoral immune response in two of the proteins. Interestingly, the investigated epitopes matched completely with two proteins derived from the same neurotropic pathogen. Further experiments including CSF and serum of 150 MS patients and 190 controls confirmed a significant higher immunreactivity to these microbial peptides in MS patients. We are currently generating monoclonal antibodies to analyze the expression of these antigens in lesions of MS patients.

In summary, we identified two new antigens targeted by the intrathecal immune response in MS patients. Further studies will focus on the role of these antigens in the pathogenesis of MS.

44
**Cord damage elicits brain functional reorganization after a single episode of myelitis.** M. Rocca, D. Mezzapesa, A. Ghezzi, A. Falini, F. Agosta, V. Martinelli, G. Scotti, G. Comi, M. Filippi, Neuroimaging Research Unit, Ospedale di Gallarate, Department of Neuroradiology, Department of Neurology (Milan, Gallarate, I)

The aims of this study were to assess, using fMRI, the brain pattern of movement-associated cortical activations in patients with a previous remitting episode of acute cervical myelitis of possible demyelinating origin and to investigate whether the extent of cortical reorganization is associated with the extent of cervical cord pathology measured using magnetization transfer (MT) MRI.

From 14 right-handed patients in a chronic and stable phase after an isolated myelitis (M/F = 7/7, mean age = 35.3 years, median disease duration = 21.2 months) involving the cervical cord and 15 sex- and age-matched healthy controls, we obtained: a) fMRI during repetitive flexion-extension of the last four fingers of the right hand; b) brain diffusion tensor (DT) MRI; c) brain and cervical cord conventional and MT MRI. FMRI data were analyzed using SPM99. Brain mean diffusivity (MD), fractional anisotropy (FA) and MT ratio (MTR) histograms of the normal-appearing white (NAWM) and gray (NAGM) matter and cervical cord MTR histograms were produced.

Patients with myelitis had significantly lower average cord MTR ($p < 0.0001$) and cord MTR histogram peak position ($p = 0.002$) than controls. Compared to healthy volunteers, patients with myelitis showed increased recruitment of the ipsilateral hemisphere in the primary sensorimotor cortex ($p < 0.0001$), supplementary motor area ($p = 0.002$) and middle frontal gyrus (MFG) ($p < 0.0001$). Average cervical cord MTR was inversely correlated with relative activations of the ipsilateral MFG ($r = -0.80$), and of the ipsilateral postcentral gyrus ($r = -0.80$). The relative activation of the ipsilateral MFG was also correlated with cervical cord MTR peak position ($r = -0.92$).

This study demonstrates the presence of an abnormal pattern of movement-associated cortical activations in patients with a previous episode of cervical myelitis. These functional cortical changes might have an adaptive role in limiting the clinical outcome of structural cord damage.

# Session 8

## Multiple sclerosis – 2

45
**Multigene DNA vaccination reduces relapse rate and prevents epitope spreading of the B cell response in experimental autoimmune encephalomyelitis.** P. Fontoura, W. Robinson, H. Garren, P. Ho, J. Tom, L. Steinman, Hospital Egas Moniz, Stanford University (Lisbon, P; Stanford, USA)

Introduction: DNA vaccination is an emerging therapeutic option for prevention and treatment of autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS). We have shown previously that DNA vaccines encoding the immunodominant epitope for several susceptible mouse strains can be used to prevent EAE onset, and also to revert established disease. Epitope spreading has been proposed as one of the mechanisms responsible for the occurrence of relapses in EAE and MS. In order to prevent this phenomenon, we proposed to use DNA vaccination against the major myelin proteins implicated in EAE (multigene DNA vaccination).

Methods: DNA vaccines encoding full-length myelin basic protein (MBP), myelin associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), PLPp139–151 and interleukin-4 (IL4) were constructed, purified and mass produced using standard molecular biology techniques. EAE was induced in SJL/J (H-2s) 6–8 week old female mice using PLPp139–151 in complete Freunds adjuvant (CFA), and animals were followed clinically for 3 months using a 6-point locomotor scale. A relapse was defined as an increase of at least 1 point in the EAE scale lasting at least 2 days. Starting on day 17 post-induction, DNA vaccines (PLPp139–151, myelin multigene and myelin multigene plus IL4) were administered intramuscularly on a weekly basis. Serum was collected before disease induction and at several timepoints after disease onset, and the antibody response against myelin peptides was verified using a myelin proteomic array containing 293 distinct myelin proteins and peptides.

Results: Chronic relapsing EAE was induced in SJL mice using a standard induction protocol. Control animals exhibited a mean relapse rate of 2.6 (total of 52 observed relapses). DNA vaccinated animals showed a reduction in relapse rate concurrent with the number of encoded epitopes: PLPp139–151 + IL4 vaccinated animals had a 2.1 relapse rate ($p = 0.225$), multigene ((MBP + MAG + MOG + PLP) vaccinated animals 1.5 relapse rate ($p = 0.015$), and multigene plus IL4 vaccinated animals a 0.9 relapse rate ($p < 0.001$). Proteomic array analysis of the antibody response revealed that mice treated with the efficacious multigene DNA vaccination exhibited a marked reduction of epitope spreading of autoreactive B cell responses. In

contrast, mice receiving control therapies underwent extensive spreading of their B cell response to epitopes on myelin proteins including MBP, PLP, MOG and CNPase.

Conclusion: Multigene DNA vaccination is an effective way of reducing relapse rate in a model of chronic relapsing EAE. There appears to be a correlation between the number of encoded epitopes, relapse rate and reduction of epitope spreading at the antibody level.

46

**Enhancement of experimental autoimmune encephalomyelitis (EAE) by transferred myelin-specific B cells.** T. Ziemssen, J. Bauer, H. Wekerle, A. Iglesias, Neurological Clinic Dresden, Brain Research Institute, MPI of Neurobiology (Dresden, D; Vienna, A; Martinsried, D)

Myelin Oligodendrocytic Glycoprotein (MOG) is one of the candidate antigens in the pathogenesis of Multiple Sclerosis. MOG-specific B cells and antibodies (Abs) are not pathogenic alone but accelerate and exacerbate an ongoing mild EAE. To elucidate the mechanisms behind this phenomenon we adoptively transferred limited numbers of IgMOG-knock in B cells from transgenic TH mice expressing the transgenic heavy chain of the anti-MOG 8.18c5 B cell receptor into recipient animals treated to develop mild EAE. Using green fluorescent protein (GFP)- and TH double transgenic mice, we were able to follow simultaneously clinical disease, anti-MOG serum titer and trafficking of the transferred cells. LPS-activated, but not resting, TH B cells, are efficient in enhancing EAE. TH B cells peak at day 4 after transfer, when they appear predominantly in spleen and peripheral blood, but also in lymph node and bone marrow. 16 days post transfer, only few TH/GFP B-cells are still detectable bearing mostly a plasmacytoid phenotype. MOG-specific IgM is detectable 1 day and peaks 4 days after transfer, while MOG-specific IgG appears at day 4 and its concentration rises continuously through days 16 and 29 after transfer. Thus transgenic TH B-cells undergo isotype switch in recipient animals. However, there is no increase in the number of B-cells in CNS infiltrates of TH transgenic and B-cell transferred mice with exacerbated EAE. Instead, the large inflammatory infiltrates observed in these animals include increased numbers of cells of the myeloid lineage. The injection of MOG-specific monoclonal Abs (8.18-c5) can also exacerbate EAE, especially after intrathecal injection. However, there is no increase in the number of B cells present in CNS infiltrates of TH transgenic and B cell transferred mice with exacerbated EAE. Instead, the large inflammatory infiltrates observed in these animals include increased numbers of cells of the myeloid lineage. We are currently investigating the posssiblity that MOG-specific Abs mediate enhanced recruitment of monocytes and neutrophils into the inflamed site of the CNS.

47

**Effects of brain injury vs. disability on patterns of cortical activation in MS: an fMRI and MRS study.** H. Reddy, S. Narayanan, M. Woolrich, T. Mitsumori, D. Arnold, P. M. Matthews, Montreal Neurological Institute, University of Oxford (Montreal, CAN; Oxford, UK)

Introduction: Previous work has demonstrated cortical plasticity that increases with the extent of brain injury in patients with multiple sclerosis (MS). Animal studies showing use-dependent changes in motor cortex activation suggest that functional changes may occur in response to disability, irrespective of brain injury. We wished to test whether injury and disability produce distinct patterns of cortical re-organisation. Since increasing complexity and effort also affect cortical recruitment of motor areas, we also used a passive as well as an active task to test whether functional changes were independent of task difficulty for impaired patients.

Methods: Magnetic Resonance Spectroscopy was used to assess levels of N-acetyl aspartate, a marker of axonal integrity, thus providing a measure of diffuse central brain injury (DCBI). 14 patients with relapsing remitting MS were assigned to 3 groups. Group 1 had low DCBI and normal hand function. Group 2 had greater DCBI and normal hand function. Group 3 had Greater DCBI than Group1 and impaired hand function. fMRI was used to map brain activation with passive and active finger flexion-extension tasks.

Results: With increasing disability, we found increased activity in ipsilateral premotor and motor cortex (IMC) and ipsilateral inferior parietal lobe. fMRI activation was highly correlated between active and passive tasks for both IMC ($r = 0.87$, $p < 0.001$) and contralateral motor cortex ($r = 0.67$, $p < 0.007$). To separate distinct effects of injury vs. disability we directly contrasted groups of patients differing in one or the other. Patients with greater disability than those with similar DCBI (Group3-Group2) ahowed greater bilateral primary and secondary somatosensory cortex activation as a result of greater disability alone. Contrasting groups matched for disability but differing in DCBI (Group2-Group1) show increases in ipsilateral premotor cortex and bilateral supplementary motor area activation.

Conclusion: We conclude that the pattern of brain activity with finger movements changes both with increasing axonal injury and with clinical disability in MS and these changes are distinct. Those related directly to disability may reflect responses to altered patterns of use. As these activation changes are found even with passive movement tasks, they reflect true re-organisation rather than effort-dependent recruitment of motor areas.

48

**Oligodendrocyte derived signals are necessary to maintain neuronal integrity.** H. Majed, A. Wilkins, S. Chandran, A. Compston, Cambridge Centre for Brain Repair (Cambridge, UK)

Background: Axon loss is a significant cause of progressive disability in patients suffering from multiple sclerosis. In order to investigate whether loss of oligodendrocyte-derived signals in chronically demyelinated lesions may contribute to the chronic axonopathy of multiple sclerosis, an in vitro model was established to examine oligodendrocyte-derived influences on neurons and axons.

Method: Oligodendrocyte conditioned medium (OCM) was generated from highly enriched populations of immature and mature oligodendrocytes. Neuronal survival and axon length of cultured cortical neurons was measured following exposure to OCM.

Results: Factors derived from oligodendrocytes and their precursors increase survival of cultured neurons via activation of PI3kinase/Akt pathways, and that part of this activity is due to insulin-like growth factor type 1 (IGF-1). Furthermore oligodendrocytes, and not their precursors, secrete factors that increase axon length in culture via MAPkinase/Erk pathways, a process mediated, in part, by glial cell line-derived neurotrophic factor (GDNF).

Conclusion: These findings support the hypothesis that continued oligodendrocyte derived signals are necessary to maintain neuronal integrity in chronically demyelinated multiple sclerosis lesions and provide a rationale for the development of future strategies to prevent chronic disability in multiple sclerosis.

49

**Raised cerebrospinal fluid nitric oxide metabolites correlate with disease progression in multiple sclerosis patients: a 3-year follow-up study.** K. Rejdak, M. J. Eikelenboom, A. Petzold, E. J. Thompson, Z. Stelmasiak, R. H. C. Lazeron, F. Barkhof, C. H. Polman, B. Uitdehaag, G. Giovannoni, Medical University, VU Medical Center, Institute of Neurology (Lublin, PL; Amsterdam, NL; London, UK)

This study aimed to investigate the relationship of CSF and serum nitric oxide (NO) metabolites, nitrite and nitrate (NOx) and disease progression and MRI markers of the disease activity.

Methods: Thirty two MS patients [10 relapsing-remitting (RR) and 16 secondary progressive (SP) MS and 6 primary progressive (PP)] and 14 control subjects were included. At baseline, all MS patients underwent lumbar puncture as well as clinical and MRI evaluation. Total CSF and serum NOx was measured using a vanadium-based assay. The clinical status of patients was assessed using the EDSS, AI, 9-HPT scoring. MRI assessment included the number and volume of Gd-enhancing lesions, T1-hypointense and T2-hyperintense lesions. Second set of clinical and MRI evaluation was performed after following 3 years.

Results: NOx was significantly raised in CSF ($9.8 \pm 5.3\,\mu M$ vs. $6.2 \pm 2.3\,\mu M$, $p = 0.003$) but not in serum ($44.2 \pm 12.1\,\mu M$ vs. $36 \pm 15.9\,\mu M$, $p = 0.1$) of MS patients compared to controls. Patients who progressed on EDSS had significantly higher baseline CSF NOx levels ($12.0 \pm 5.7\,\mu M$) compared to those who improved or did not change ($7.8 \pm 4.1\,\mu M$; $p = 0.02$). Similarly, those who got worse on AI had higher baseline CSF NOx ($13.9 \pm 7.1\,\mu M$) compared to patients with stable AI ($8.3 \pm 3.5\,\mu M$; $p = 0.03$).

Conclusion: CSF NOx levels, an indicator of intrathecal NO production, were increased in MS patients and raised CSF NOx predicted progression in MS patients over 3-year follow-up.

50

**Urokinase plasminogen activator (uPA) C57bl/6 mice are partially resistant to MOG35-55-induced experimental autoimmune encephalomyelitis.** R. Furlan, E. Brambilla, V. Basso, L. Zanotti, F. Blasi, A. Mondino, G. Martino, San Raffaele Scientific Institute (Milan, I)

Plasminogen activators (PAs) and matrix metalloproteinases (MMPs) are considered to play an important role in the pathogenesis of multiple sclerosis and experimental autoimmune encephalomyelitis (EAE), the elective animal model of multiple sclerosis. Whereas several studies have addressed the

expression of various MMPs and their inhibitors in the pathogenesis of EAE, the role of PA during EAE is still only partially known. We induced EAE in C57BL/6 mice deleted for the urokinase PA (uPA) gene by immunization with 200 µg of MOG35–55 in CFA and by injection of 500ng of pertussis toxin the same day and 48 hours later. Wild type C57BL/6 mice were used as controls. Disease susceptibility, onset, and severity of EAE were similar in the two groups of mice. We then lowered the amount of the immunogen (50 µg of MOG35–55 in CFA) and of PT (500ng only the day of immunization). Using this protocol, uPA–/– mice showed a significant delay in disease onset (12.8 ± 0.6 vs. 10.7 ± 0.5 in wild type control C57BL/6 mice; $p = 0.0138$ logrank test), and a decrease in EAE severity as measured by mean cumulative clinical score (69.4 ± 14.9 vs. 104.4 ± 7.4 in wild type control C57BL/6 mice; $p = 0.0442$ Mann-Whitney). MOG35–55-specific T cells from uPA–/– mice were partially affected in their ability to proliferate after in vitro re-stimulation with the nominal antigen and produced less IFN-gamma compared to T cells from wild type EAE mice. We conclude that the absence of uPA may affect the differentiation of encephalitogenic T cells resulting in a partial inhibition of EAE development.

# Session 9 & 10

## Motor neuron disease – 1 & 2

51

**The p38 MAP kinase pathway in mutant SOD1 mice, a transgenic model for amyotrophic lateral sclerosis.** M. De Wil, L. Van Den Bosch, W. Robberecht, University Hospital Gasthuisberg (Leuven, B)

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disorder for which there is currently no treatment available. We have previously shown that minocycline effectively slows down disease progression in mutant SOD1 mice, and increases survival of this model for human ALS. The mechanism of action of minocycline remains unknown but in vitro evidence suggests that the p38 mitogen activated protein kinase (MAPK) pathway is a potential pharmacological target for this drug. In the present study we investigated the effect of minocycline on this stress-activated kinase system in vivo and in vitro.

Quantified Western blot analysis demonstrated the p38 MAPK and its activated form, phosphorylated p38 MAPK, to be upregulated in the spinal cord of mutant SOD1 (G93A) SOD1 mice, as compared to wild type SOD1 overexpressing control mice. This phosphorylated p38 was localized in the nuclei of ventral horn neurons and the cytoplasm of glial cells as demonstrated by immunohistochemical double staining experiments of mutant SOD1 mouse spinal cord. Treatment of mutant mice with minocycline, known to increase survival of these animals, significantly attenuated p38 MAPK activation, as demonstrated by immunoblot studies showing inhibition of phosphorylated p38 MAPK upregulation. To elucidate the cell type minocycline acts upon, we studied the effect of minocycline on microglial activation in culture and the effect of the drug on mutant SOD1-dependent motor neuron death in vitro. Minocycline significantly and time dependently inhibited lipopolysaccharide-induced phosphorylated p38 MAPK upregulation in purified microglial cultures. In addition, the drug inhibited cell death of mutant (G93A) SOD1 motor neurons, co-cultured with glial cells.

These results demonstrate that the beneficial clinical effect of minocycline in the mutant SOD1 mouse is accompanied by inhibition of the p38 MAPK system, and that this drug acts on both microglial activation mechanisms and on intrinsic neuronal death pathways. The elucidation of the effect of minocycline on the upstream signalling system of p38 MAPK activation will yield insights into the mechanism of the therapeutic action of this drug and may allow the generation of more active analogues of the drug.

52

**The G93C mutation in the SOD1 gene is an independent prognostic parameter in amyotrophic lateral sclerosis.** G. Cypers, L. Vanopdenbosch, P. Tilkin, G. Matthijs, R. Sciot, V. Thijs, W. Robberecht, University Hospital Gasthuisberg (Leuven, B)

Of all amyotrophic lateral sclerosis (ALS) patients about 10% have a familial form of the disease, which is usually transmitted as an autosomal dominant trait. SOD1 mutations are the cause of ALS in 1 out of 5 in these families. We studied the clinical and demographic characteristics of ALS patients with a G93C mutation in the SOD1 gene (SOD1 (G93C)-FALS) and compared them to patients with FALS caused by other SOD1 mutations, non SOD1-associated FALS (nonSOD1-FALS) and sporadic ALS patients (SALS). In addition we studied the male to female ratio of SALS patients before and after the menopause.

The phenotype of 20 SOD1 (G93C)-FALS patients was characterized by amyotrophy with areflexia, and in some patients mild sensory symptoms. Bulbar involvement was never present, not even in the terminal stage which was characterized by fatal respiratory failure. Pathological examination of the spinal cord of a SOD1 (G93C)-FALS patient showed marked dorsal column involvement, some spinocerebellar abnormalities, and only mild corticospinal tract involvement.

Age of onset in SOD1 (G93C)-FALS (45.9 ± 10.6 years) was significantly younger than that of SALS (58.4 ± 12.0 years). Survival of SOD1 (G93C)-FALS patients, as estimated by Kaplan Meier curves, was 153.0 ± 46.1 months, which was significantly longer than that of SALS (30.0 ± 1.3 months), nonSOD1-FALS (43.0 ± 12.4 months) and SOD1 (L38V) FALS (24.0 ± 0.6 months) patients. The presence of the G93C mutation was an independent prognostic valuable in a multivariate analysis (Cox regression model), which confirmed age of onset, site of onset, vital capacity at onset and diagnostic delay to be independent prognostic variables.

Male to female ratio was 1.43 in the SALS population. In the population younger than 60 years of age at onset, this ratio was 2.08, while it was 1.04 in the population with an onset after the age of 60. Correction for the demographic characteristics of the Belgian population revealed a statistically significant preponderance of male patients in the population with onset below 60; this male preponderance was maintained in the population with onset over 60, although to a lesser degree than in the younger onset population.

Our results suggest that G93C mutation in the SOD1 gene is an independent prognostic valuable in ALS, and that the male preponderance in SALS is maintained, even in the postmenopausal population.

53

**High rate of constitutional chromosomal rearrangements in apparently sporadic ALS.** T. Meyer, B. Alber, T. Martin, V. Kalscheuer, E. Göttert, K. Zang, A. Ludolph, H. Ropers, J. Prudlo, Charite University Hospital, Ulm University Hospital, Homburg University Hospital, Max Planck Institute (Berlin, Ulm, Homburg, D)

Amyotrophic lateral sclerosis (ALS) is clinically and genetically heterogeneous. For the familial form of ALS, which is found in 5–10% of ALS cases, the linkage to several gene loci has been established. However, the cause of the most common sporadic form of ALS is unknown. A number of genes has been reported that may predispose to ALS, acting as a susceptibility or risk factor to the disease. Against the background of genetic heterogeneity in familial ALS and several known genomic risk factors in the sporadic form of the disease we performed a systematic cytogenetic investigation among 85 sporadic ALS patients showing an unexpected high rate of constitutional chromosomal aberrations, all affecting distinct chromosomal loci. G-banded metaphases of three individuals showed a reciprocal translocation t[4, 19](p22;p13.1), t[4, 20](p15.3;q11.2), and t[18, 21](q23;q22), respectively. In two other patients pericentric inversions inv(X)(p11.2q21.3) and inv[12](p11q13) were identified. The apparently balanced chromosomal abnormalities were found in all cells examined, indicating a constitutional form of rearrangement. In three of the five reported patients, the constitutional rearrangement was transmitted from a carrier parent. In these cases, the de novo occurrence of aberrant meiotic recombination was excluded indicating the inheritance of the chromosomal abnormalities. The cytogenetic analysis of family members revealed several carriers of constitutional rearrangements, including individuals of advanced age (64, 72, and 73 years), being clinically asymptomatic. The family studies showed that the clinical phenotype did not segregate with the chromosomal rearrangement. An incomplete penetrance of a heritable condition has to be considered. Given the negative family history on ALS the results suggest that more than one factor may be required to produce the clinical phenotype. This observation contributes to the current concept that sporadic ALS is a multi-factorial disease in which modifying genes and environmental agents affect its clinical expression. From the increased rate of reciprocal abnormalities we conclude that ALS is in part associated with recombination-based rearrangements of genomic sequences. We propose that the chromosome rearrangements may represent a previously unknown genomic risk factor for apparently sporadic ALS.

54

**Characteristics of ALS-CSF toxicity in primary cell cultures.** J. M. H. Anneser, C. Chahli, G. D. Borasio, University of Munich (Munich, D)

In vitro toxicity of cerebrospinal fluid from ALS-patients (ALS-CSF) has been described previously. However, the noxious factor and cell death mechanisms are unknown. We observed the following characteristics of ALS-CSF toxicity in vitro:

1. ALS-CSF is toxic in chick motoneuron-enriched spinal cord cultures. Compared to CSF from control patients, addition of 10% ALS-CSF resulted in significant differences of survival rates after 24hrs. (72 ± 11 vs. 43 ± 12%) and 60 hrs. (43 ± 15 vs. 15% ± 8).
2. ALS-CSF may have toxic as well as trophic properties: In serum supplemented cultures, survival promoting effects of muscle extract (MEX) were abolished by toxic properties of ALS-CSF, while in serum free cultures, addition of MEX and ALS-CSF had a significant survival promoting net effect.
3. Motoneuronal death induced by ALS-CSF is apoptotic in our culture system as verified by TUNEL-staining.
4. The mediator of toxicity seems to be a rather small molecule, since centrifugation through a 5.000 kDa mesh did not abolish toxic properties.
5. Addition of the group I metabotropic glutamate receptor antagonist 1-aminoindan-1,5-dicarboxylic acid (AIDA) had a protective effect against ALS-CSF toxicity (60% ± 10 vs. 43% ± 8 after 24 hrs).
6. Addition of ALS-CSF to glial spinal cultures resulted in higher proliferation rates compared to control-CSF, as evidenced by the number of BrdU-positive cells (37.3 ± 8.1 vs. 20.4 ± 7.4) and immunoblotting with vimentin. The proliferation promoting effects of both treatment groups could be reduced by addition of AIDA.

Although the exact mechanism of ALS-CSF toxicity is unknown, motoneuron death and glial proliferation – crucial histopathologic hallmarks of ALS – can be modelled in these in vitro systems. Furthermore, the toxic and proliferation promoting effects may -at least partially- mediated by glutamatergic mechanisms.

55

**ALS2/alsin involvement in early, pure form of primary motor degeneration.** E. Eymard-Pierre, G. Lesca, M. di Capua, J. Attia-Sobol, H. Plauchu, V. Leuzzi, A. Ponsone, F. Santorelli, O. Boespflug-Tanguy, E. Bertini, INSERM U384, Université de Lyon, Bambino Gesu Children's Hospital, La Sapienza, Ospidale infantile Regina Margarita (Clermont-Ferrand, Lyon, F; Rome, Turin, I)

The ALS2/Alsin gene has been recently found mutated in 4 Arabic consanguineous families, two with a juvenile amyotrophic lateral sclerosis (ALS2) phenotype and two with a childhood-onset primary lateral sclerosis (PLS). We report on 16 patients from 11 families (1 from Algeria, 1 from lybia, 2 from France and 7 from Italy) who presented severe progressive ascending spastic paralysis with an autosomal recessive mode of inheritance. They were considered normal at birth and spastic paraplegia initiated during the two first years of life. Weakness and spasticity extended to upper limbs around the age of 7–8 years. The patients were all wheel-chair bound by the age of 10 years and during the second decade the disease progressed to tetraplegia, anarthria, dysphagia and slow eye movements with a long survival (> 30 years). No signs of lower motor neuron involvement were observed whereas motor evoked potentials showed abolition of corticospinal response in contrast with normal somatosensory evoked potentials. MRI was normal in young patients but showed brain cortical atrophy in the oldest predominant in the motor areas and T2 weighted hyperintense signal of the pyramidal tracts in the internal capsule and brainstem. In 4 families, we found ALS2/Alsin mutations (3 deletions and 1 splice site mutation) leading to truncation of short as well as long form of Alsin protein. In the remaining 7 families, haplotype analysis with 18 microsatellites located in and around the ALS2 locus, demonstrated a recombinant event in only one family. No clinical differences were observed within patients with or without Alsin mutations suggesting a genetic heterogeneity in this infantile onset but long survival form of primary motor degeneration.

56

**SMN genes are a susceptibility factor in adult motor neuron disorders.** P. Corcia, J. Khoris, V. Mayeux-Portas, C. Andres, W. Camu, Inserm U316, Department of Neurology, Inserm U336 – French ALS Study Group

Motor neuron diseases (MND) are a heterogeneous group of affections with lower and/or upper lower motor involvement in spinal and/or bulbar territories. The origin of adult MND remains largely unknown but seems to be multifactorial and probably multigenic.

Spinal muscular atrophy (SMA) is an infantile lower MND linked to the homozygous deletion of exon 7 of the SMN1 gene, the telomeric copy of SMN genes. Moreover, the copy number of centromeric copy, namely SMN2, may influence the prognosis.

This strong association between SMA and SMN genes led us to study during the last 6 years these genes in several adult MND (Sporadic LMND, Sporadic ALS and Familial ALS).

We found that SMN gene deletions may have an influence in adult MND genetic susceptibility. These data can be summarized as the following: 1) abnormal SMN1 copy number (1 and 3) is a susceptibility factor in sporadic ALS; 2) homozygous SMN2 deletions are a susceptibility factor both in sporadic adult LMND and non monogenic familial ALS.

Despite the apparent controversial aspect of these data, molecular genetics of SMN genes and mRNA expression studies from the literature show that they are consistent and warrant further insights into the genetics of SMN in MND. In particular, mRNAs expression should be further studied in the families in order to identify truncations as it has been recently shown in children with MND. Moreover, other SMN gene abnormalities, on different exons, should be also searched for in ALS.

Our data led us to consider that both SMN genes (and not only SMN1 gene) are involved in MND onset, and our future molecular genetics strategy will be presented.

57

**Quality of life in patients with amyotrophic lateral sclerosis: the QuaC-ALS study database.** G. Filippini, V. Bonito, A. Chiò, G. Mora, R. D'Alessandro, F. Salvi, R. Schoenhuber, G. Cavaletti, A. Galli, G. Savettieri, D. Testa, E. Beghi, V. Silani, National Neurological Institute C. Besta on behalf of the QuaC-ALS Study Group

Background: Under the auspices of the Italian Health Ministry, the QuaC-ALS Database has been collecting information on patients with ALS, their caregivers, and their management since February 2001 with the aim of prospectively monitoring the management of patients with ALS over time and outcomes. Only recently have health related quality-of-life (HRQOL) and Individual QOL inventories been recognised as meaningful outcomes in clinical research on ALS.

Objective: To determine the acceptability and clinical validity of measures of QOL in patients with ALS, how functional status and QOL change over time, and what relationships these changes have to one another.

Methods: The study group consists in a consecutive series of 130 patients with ALS – inpatients and outpatients – and their caregivers enrolled between February 2001 and January 2002 and prospectively followed at 11 Centers in Italy. The follow-up of the patients is ongoing; data are collected at 4, 8 and 12 months after inclusion. The primary outcomes are survival, QOL measured with the SF36 and the SEIQoL-DW and the functional status measured on the basis of the ALS-FRS.

Results: At baseline, clinical characteristics of the 130 patients were mean age 60.1 ± 10.6 years, males 57%, mean ALS-FRS score 24.5 ± 9.7. The extent to which the SF-36 questionnaire was completed by the patients was satisfactory. The SF-36 physical and mental health composite scores were 30.5 ± 10.3 and 43.3 ± 12.3 respectively. Compared with the Italian norms of the SF-36, ALS patients had greater dysfunction in all domains, with most marked differences for physical function. The patients were stratified by ALSFRS scores into two severity groups. As expected, the domain which most clearly distinguished the groups was physical function, but mental health and emotional role limitations did not differ between the groups. Of a possible score of 100, the mean SEIQoL-DW score was 58.1 ± 21.2. At 31 December 2002, 26 (20%) patients had died and 17 (13%) were lost to follow-up. Eighty-seven subjects are actually in follow-up.

Conclusion: The ability to adapt to severe invalidity enables patients with ALS to improve their QOL and even though their physical function continues to get worse, they frequently consider their QOL acceptable, right up to the most advanced stages of the illness.

58

**Botulinum toxin B treatment of hip adductor muscles in hereditary spastic paraplegia.** L. Schöls, T. Schulte, S. Otto, H. Przuntek, Ruhr-University (Bochum, D)

The potential of botulinum toxin in the management of spasticity has been approved for the treatment of arm spasticity due to stroke and in spastic pes equinus due to perinatal hypoxic brain damage/cerebral palsy. Several reports encourage botulinum toxin treatment of other types of spasticity e. g. elbow flexor spasticity or pronounced knee flexor spasticity. Botulinum toxin treatment of severe hip adductor spasticity can improve personal hygiene in the genital region in patients with multiple sclerosis. To our knowl-

edge the potential of botulinum toxin in the management of leg spasticity in hereditary spastic paraplegia has never been determined systematically.

Ten patients with hereditary spastic paraplegia received bilateral EMG guided injections into the hip adductor muscles at a total dose of 20.000 IU botulinum toxin-B (Neurobloc®). Spasticity was measured by the expanded Ashworth scale. Gait function was evaluated by videography documenting dependence on walking aids for a 10 m distance, gait speed, "scissoring" and stair climbing. Additionally pain related to spasticity, functional ability and caregiver dependency were scored. All parameters were recorded before treatment and after one month of therapy. Global assessment from baseline in signs and symptoms of spasticity in the legs has been rated by the examiner and the patients.

Following injections improvement of gait speed, scissoring, stability of gait and pain was observed. Time for the 10m distance was reduced by 20% from 37 ± 28 to 31 ± 23 s. Stair climbing improved in 3 patients. On the global assessment scale signs and symptoms of spasticity improved by + 1.5 points in rating of patients and physicians. Painful spasms decreased in all patients [3] who presented with this problem. No side effects of botulimun toxin B therapy have been reported by the patients.

The findings of this study showed botulinum toxin-B to be a safe and effective treatment for reducing hip adductor spasticity and improving gait function in patients with hereditary spastic paraplegia.

59

**Increased frequency of the tau gene A0/A0 genotype in amyotrophic lateral sclerosis.** C. Münch, R. Xu, P. Linke, S. Winter, A. C. Ludolph, T. Meyer, University of Ulm, Humboldt University (Ulm, Berlin, D)

Amyotrophic lateral sclerosis (ALS) is clinically and genetically heterogeneous. The Guam variant of ALS (ALS-G) is accompanied by neurofibrillary tangles containing aggregates of the microtubule-associated protein tau. Among ALS patients, about 5% of all cases arise in conjunction with frontal-type dementia, although in these patients tau pathology is only sparse or absent. Pathological hallmarks of tauopathy are mainly found in patients with classical frontotemporal dementia (FTD). In the light of the clinical overlap of ALS-dementia with FTD and the presence of tau deposits in ALS-G we raised the question whether genetic variants in the tau gene constitute a risk factor for ALS. We investigated the susceptibility of the dinucleotide repeat polymorphism A0 in the tau gene to ALS. In a sample of 170 unrelated ALS patients and the same number of unrelated control subjects the A0/A0 genotype was significantly overrepresented in ALS. We found no association of the A0 polymorphism with the age and site of disease onset or the presence of dementia. The studied tau genotype may behave as a risk factor for the development of the disease and contribute to the multifactorial genetic background of ALS.

# Session 11

## Peripheral neuropathy – 1

64

**In vivo monitoring of macrophage infiltration during autoimmunity by magnetic resonance imaging in rats.** G. Stoll, C. Wesemeier, R. Gold, L. Solymosi, K. Toyka, M. Bendszus, Julius-Maximilians-Universität (Würzburg, D)

We report on the in vivo assessment of macrophage infiltration in autoimmunity by magnetic resonance imaging (MRI). Adoptive transfer experimental autoimmune neuritis (AT-EAN) served as prototype of a Th-1 mediated autoimmune disorder. Lewis rats received intracardial injections of superparamagnetic iron oxide particles (SPIO) at days 2, 3, 4, 5 or 9 after injection of P2-specific T-cells, and were scanned by MRI always 24 hrs later. MRI revealed focal signal loss of the cauda equina indicating iron accumulation in spinal nerves already at the preclinical stage (day 3). Signal loss peaked at day 4, when first clinical signs developed. At the maximum of clinical disease signal loss declined already, and had disappeared on days 6 and 10. Perl's stain of spinal nerve sections showed focal cellular accumulation of SPIO at days 3, 4, and 5 in ED1-positive macrophages. Spinal nerves at day 6 and 10 exhibited massive macrophage infiltrates, but no more iron deposition.

Thus, signal loss on MRI and cellular iron deposition in AT-EAN indicate periods of active monocyte entry into the nervous system. SPIO-enhanced MRI provides a dynamic view on a fundamental process in autoimmune disorders, macrophage infiltration.

65

**Reactive nitrogen species: potential target for the therapy of inflammatory demyelinating neuropathies.** S. Kastenbauer, S. Jander, H.-P. Hartung, B. C. Kieseier, Ludwig-Maximilian University, Heinrich-Heine University (Munich, Dusseldorf, D)

Background: An emerging body of evidence suggests that oxidative stress is involved in the pathogenesis of inflammatory demyelinating disorders of the peripheral nervous system. Treatment with superoxide dismutase, for example, was shown to attenuate the clinical course of experimental autoimmune neuritis (EAN). The role of nitric oxide (NO) is less clear, since conflicting results were obtained with different inhibitors of NO synthases in EAN. Many beneficial effects of NO are currently being recognized. By contrast, the reaction product of superoxide anion and NO, peroxynitrite (ONNO), seems to exert mostly detrimental effects.

Methods: The formation of reactive nitrogen species, such as peroxynitrite, was studied by immunohistochemical detection of tyrosine nitration in sural nerve biopsies from patients with Guillain-Barré-Syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), or degenerative neuropathies (controls). The effect of the antioxidants and peroxynitrite scavengers uric acid (500 mg/kg i.p. tid) or N-acetylcysteine (NAC, 300 mg/kg i.p. tid) was evaluated in myelin-induced EAN in Lewis rats (treatment from day 10 to day 21 after disease induction).

Results: Nitrotyrosine immunoreactivity was markedly increased in the inflamed PNS compared with control patients. Axons, Schwann cells, and particularly macrophages were detected as cellular sources. Antioxidant treatment significantly ($p < 0.05$) reduced the maximum disease severity (clinical score ± SD on day 17 after disease induction: sham 5.9 ± 2.0, uric acid 3.2 ± 0.4, and NAC 3.8 ± 1.5).

Conclusion: Our findings demonstrate that reactive nitrogen species, such as peroxynitrite, are expressed in the inflamed PNS and as such might be involved in the pathogenesis of inflammatory demyelinating neuropathies. Our observations in the animal model suggest that the application of the natural peroxynitrite scavenger uric acid and the widely clinically used antioxidant N-acetylcysteine might represent interesting new therapeutic strategies in inflammatory demyelinating diseases of the PNS.

Acknowledgement: This work was supported by an ENS fellowship stipend to S. Kastenbauer.

66

**Long-term prognosis of multifocal motor neuropathy in relation to IVIg therapy.** F. Terenghi, N. Meucci, E. Nobile-Orazio, Milan University (Milan, I)

Multifocal motor neuropathy (MMN) is characterised by a slowly progressive distal asymmetric limb weakness, mostly affecting upper limbs. The majority of MMN patients improves with high-dose intravenous immunoglobulins (IVIg) even if their effect on the long-term disability is still unclear. We report on the long-term outcome of 22 patients with MMN (mean age of onset 40 years; range 21–62) first examined by us between 1990 and 2000. In all patients the severity of neuropathy was assessed using a modified Rankin disability scale (score 0–5) and a functional impairment scale for upper (UL) and lower (LL) limbs (score 0–5, each). At entry, after a mean duration of symptoms of 9.3 years (range 0.25–25 years) the mean Rankin and UL + LL scores were 2.2 and 3.7, respectively. Fourteen patients (63%) were unable to perform any manual activity (UL score > 2) including 7 (31%) with a Rankin score > 2 (symptoms significantly interfering with lifestyle). The mean duration of symptoms was higher in disabled (12.2 years) than in not disabled patients (5.3 years). None had LL score > 2. Of the 22 patients initially examined, 19 (86%) were followed until January 2003. All but one patient were treated during follow-up with periodic IVIg infusion for a mean of 5.6 years (range 3–12). By the end of follow-up, after a mean duration of symptoms of 15.2 years (range 4–35, at least 5 years in 21 patients and 10 years in 17), the mean Rankin and UL + LL scores were 1.8 and 2.7, respectively. Six patients (27%) had an UL score > 2 and 4 (18%) a Rankin score > 2. The disability rates at 5, 10, 15 and 20 years from neuropathy onset were 5, 18, 33 and 42%, respectively with a considerable difference between treated and untreated patients at each interval (0 vs. 6%, 0 vs. 25%, 14 vs. 60% and 25 vs. 66%).

Our findings indicate that MMN induces a progressive disability in the majority of patients after several years and that IVIg therapy is effective in preventing the long-term disability in most of them.

67
**Increase in proinflammatory cytokines and T-cell immunophenotyping in peripheral blood of patients with anti-MAG polyneuropathy.** L. Sanvito, R. Nemni, L. Speciale, E. Calabrese, G. Santuccio, M. Sgandurra, P. Ferrante, N. Canal, Don C. Gnocchi Foundation – University of Milan (Milan, I)

Background: Anti-myelin-associated glycoprotein (MAG) antibodies have been associated with a chronic demyelinating neuropathy and their pathogenic role has been demonstrated. The disease course and response to therapy have been previously related to antibody titre and M-protein level in the serum. Few data are available concerning the extent of the involvement of T-cell immunity and the imbalance of the cytokine network.

Objective: We studied peripheral T and B cell immunity as well as cytokine network in patients with anti-MAG antibodies to detect immunological imbalance potentially informative on disease course and response to therapy.

Methods: We studied 7 untreated patients with IgMk anti-MAG neuropathy and we compared the results with 7 age-matched healthy controls (HC). Anti-MAG antibody titre ranged from 1/9,000 to 1/200,000. We analysed, by flow cytometry, the CD3+, CD3+DRII+, CD4+, CD8+, CD16+ and CD19+ cells subset distribution. We also evaluated intracellular IL2, INF-g, IL1, IL6, IL10 and TNF-a production by CD4+, CD8+ T lymphocytes and CD14+ cells after stimulation with Staphylococcal enterotoxin B plus anti CD28. Statistical analysis was performed by Student's T test.

Results: Anti-MAG patients had a significant increase in T cells total number ($p = 0.04$) and CD4+ ($p = 0.02$) while B cells total number was decreased ($p = 0.01$). TNF-a was significantly increased in CD4+ ($p = 0.01$) and CD14+ ($p = 0.01$); IL6 was increased in CD14+ only ($p = 0.04$). IL10 was significantly increased in CD4+ ($p = 0.05$) and CD8+ ($p = 0.04$). IL1 was increased in CD8+ ($p = 0.05$). No significant difference was found in IL2 and INF-g levels between patients and HC.

Conclusion: The finding of a significant increase in total number of T cells and in proinflammatory cytokines in patients with anti-MAG M-protein suggests an imbalance of cellular immunity. The M-protein has an inhibitory effect towards B-cells either direct either mediated by the inhibitory cytokine IL10. We think that further study of lymphocytes subset distribution and intracellular cytokine expression may help to understand the immunological mechanisms regulating M-protein secretion. This could have clinical implications in order to define a better treatment strategy.

68
**Recovery of excitability of peripheral motor axons during long-term regeneration – 'in vivo' electrophysiological study in cat.** M. Moldovan, J. Sørensen, C. Krarup, Rigshospitalet (Copenhagen, DK)

Following degeneration, peripheral axons can regenerate and, given a proper pathway, reconnect with targets. In spite of this capacity, the recovery of function after a nerve lesion is clinically unsatisfactory possibly in part due to the abnormal membrane properties caused by differences in channel distribution and function in regenerated axons. The purpose of our study was therefore to assess the internodal and nodal membrane maturation process of regenerated nerve fibers, with particular emphasis on ion channel function, for extended periods after target reinnervation. The investigations were carried out in cats with experimental tibial nerve crush by means of a non-invasive electrophysiological technique (threshold tracking). Regenerated nerve function was further tested under various transient conditions such as cooling and ischemia. The data indicate that the maturation of membrane properties of regenerated motor nerve fibers takes place over several years after target reinnervation and some functional abnormalities may be permanent. The most striking abnormality is the higher than normal threshold increase during hyperpolarization, and this may be reduced by cooling and increased following release from ischemia. The rectification to depolarization is also increased. We speculate that regenerated nerves maintain a resting membrane hyperpolarization and an abnormally increased K+ conductance activated by depolarization.

The noninvasive nature of our investigations allows direct application to studies in the clinical situation and may be particularly relevant in the evaluation of new treatment procedures.

69
**Peripheral neuropathy due to necrotizing arteritis (NA) in non-connective tissue disorders (NCTD): a clinicopathological retrospective study.** C. Lacroix, P. Lozeron, A. Ferreira, G. Said, Hopital de Bicêtre (Le Kremlin-Bicêtre, F)

NA of the type observed in polyarteritis nodosa (PAN) can induce ischemic nerve lesions in NCTD. To learn more on the subject, we reviewed the clinical and nerve biopsy findings of 400 patients with necrotizing arteritis demonstrated in the nerve and/or the muscle biopsy specimens investigated in our service for peripheral neuropathy. Patients with PAN, rheumatoid arthritis, lupus, and those with isolated NA associated neuropathy were excluded, as well as those with asymptomatic seropositivity to B or C hepatitis virus.

Seventy-four patients were thus included, 47 males and 27 females, age range 23–88 years.

Twenty-nine patients had active viral infection including HIV, CMV, HTLV-1 infections and chronic B and C viral hepatitis; some patients had simultaneous viral infections (HIV and HCV, CMV or HTLV-1). Fourteen patients had multifocal neuropathy associated with type 2 diabetes mellitus. Monoclonal gammapathy was present in 13 patients, including one patient with Waldenström's disease and one with myeloma. Eleven patients had a malignant hemopathy, including the two with malignant monoclonal gammopathy, 2 had familial amyloidosis and 8 had sarcoid neuropathy.

General manifestations were present in most patients. In some patients the neuropathy was the first symptom of their illness. Seven patients had a mononeuritis, 33 mononeuritis multiplex; 44 had a distal sensorimotor neuropathy, along with MM especially in diabetic patients. NA was detected in 70/73 nerve specimens and in 27/73 muscle specimens (19 in both specimens). NA was visualised only on serial sections in 64/74 nerve samples. Endoneurial inflammatory infiltrates were present in 57% of the nerve specimens in NCTD versus in less than 20% of nerve specimens of patients with PAN. In addition to medium-sized arteries, smaller endoneurial vessels had wall necrosis associated with red cell bleeding, especially in diabetic multifocal neuropathy and in CMV lesions.

DSMN was more common in NCTD than in PAN (58%/38%), and NA more frequently found in nerve specimens (95%) than in classical PAN (66%); muscles were affected in only 35% of the patients versus 66% in PAN. Simultaneous lesions of the background disease including amyloid deposits (2 patients) and sarcoid granulomas were present on the same nerve sections.

We found that NA with subsequent ischemic nerve lesions occurs in NCTD, presumably as result of local destruction of small arteries mostly by an inflammatory infiltrate. These lesions which can induce focal nerve lesions and modify the neuropathic pattern have important therapeutic consequences.

# Session 12

## Peripheral neuropathy – 2

70
**IgM deposits in skin nerve fibres in anti-MAG neuropathy.** R. Lombardi, B. Erne, D. Pareyson, M. Morbin, A. Arnold, A. Czablinski, G. Lauria, N. Schaeren-Wiemers, A. J. Steck, C. Besta National Neurological Institute, Basel University Hospital (Milan, I; Basel, CH)

Monoclonal IgM antibodies against myelin-associated glycoprotein (MAG) are associated with a chronic demyelinating neuropathy characterised by predominant involvement of large sensory fibres, although nerve conduction study reveals preferential demyelination of distal motor fibres. In sural nerve, deposits of monoclonal IgM are found at sites of MAG localisation such as Schmidt-Lanterman incisures and paranodal loops, but also on the surface of myelinated nerve fibres (mesaxon). Involvement of cutaneous distal sensory nerve fibres has never been assessed. We performed skin biopsies to quantify the density of intra-epidermal nerve fibres (IENF) and investigate the presence of IgM deposits in dermal myelinated fibres in 13 patients with anti-MAG neuropathy. Patients with CIDP ($n = 8$) and IgM anti-MAG negative paraproteinemic neuropathy ($n = 3$) served as diseased controls. Skin biopsies (3-mm punch) were performed at the proximal thigh ($n = 20$), at the distal leg ($n = 21$), at the hand or arm or fingertip ($n = 23$). Specimens were processed to quantify the density of IENF using the anti-protein gene product 9.5 (PGP 9.5). Both anti-MAG and CIDP patients showed a proximal-distal gradient in IENF density decrease, indicating a length-dependent fibre loss, as typically seen in other neuropathies. Double staining confocal microscope studies were performed with polyclonal anti-IgM antibodies, and with monoclonal anti-MAG or anti-myelin basic protein (MBP) antibodies in both patients and controls. IgM deposits were detected on the surface of dermal MAG-positive and MBP-positive nerve fibres in 11 anti-MAG neuropathy patients (85%). IgM deposits were located also

at the paranodal loops. Although a similar number of fibres were evaluated, CIDP and paraproteinemic neuropathy patients did not show IgM deposits in MAG-positive and MBP-positive dermal fibres. Our study shows that unmyelinated IENF are involved in anti-MAG, CIPD, and paraproteinemic neuropathies. Most interestingly, we demonstrated that in anti-MAG neuropathy specific IgM deposits are found in cutaneous myelinated sensory fibres. Skin biopsy provides a useful, minimally traumatic method for early diagnosis and, possibly, follow-up study in anti-MAG neuropathy.

This work was supported by a Fellowship of the European Neurological Society.

71

**Flow cytometric analysis of B-cell lymphocytes in blood or bone marrow aspirates as a promising tool for the management of neuropathy associated with monoclonal gammopathy of undetermined significance (MGUS).** D. Adams, K. Abbed, P. Lozeron, C. Theodore, C. Leonard, G. Tchernia, Y. Taoufik, G. Said, CHU de Bicêtre (Le Kremlin Bicêtre, F)

Background: Most of monoclonal gammopathies associated with neuropathy are considered to be of undetermined significance (MGUS). The link between MGUS and the neuropathy is usually unclear and the treatment of these neuropathies is still controversial.

Objectives: Aim of the study was to identify underlying B cell clonal proliferation by immunophenotyping B cells in blood and/or bone marrow aspirates in patients with neuropathy associated with MGUS.

Methods: In this prospective monocentric study, we performed 1) a 4-colour flow cytometry analysis of surface phenotype on circulating lymphocytes and/or bone marrow aspirates looking for light chain restriction, 2) molecular studies looking for IgH rearrangement (FR3/JH), 3) cytogenetic studies if necessary.

Material: in a prospective study, we investigated patients with neuropathy and IgM or IgG monoclonal gammopathy which has been considered as MGUS after appropriate haematological investigations including complete clinical examination, white blood cell count, bone marrow aspiration, bone marrow biopsy, body CT scan, skeletal X-rays.

Results: Between September 2002 and January 2003, fifteen patients with neuropathy and MGUS were investigated including 13 pts with IgM and 2 pts with IgG-MG. Seven of them have been previously treated. The mean age was 65 years (range 33–81), 11 pts were male and 4 female. The median duration of the disease was: 3.5 y. (range from 0.5 to 15). Neuropathy was demyelinating in 13 pts and axonal in 2 pts. The neuropathy was sensory in 10 patients and sensorymotor in 5. Ten of these patients had functional impairment including 5 with severe walking disability.

The mean value of total IgM level in serum was 4.93 g/L (range from 1.54 to 20; N < 1,48). Among IgM Mc gammopathy, 5/11 had anti-MAG antibodies.

All patients underwent immunophenotyping of the blood and 5 also in the bone marrow aspirates. Clonal expansion of B cells was identified in 7 pts (47%) with combined flow cytometric analysis of B cell and IgH rearrangement. All B cell clonal expansions expressed CD20+cell surface marker. Their immunophenotype was heterogeneous and suggested plasmacytic lymphoma in one pt with IgM-MGUS and follicular lymphoma in one pt with IgG-MGUS which was confirmed by cytogenetic studies.

Conclusion: Flow cytometry analysis of surface phenotype of circulating and/or bone marrow B lymphocytes is a useful method for detecting B cell clonal proliferation in patients with neuropathy associated with probable MGUS. It should be added to classical investigations and opens future ways of immunointervention.

72

**Inhibitory effect of therapeutic immunoglobulins on presynaptic blockade by Lambert Eaton myasthenic syndrome autoantibodies.** B. Buchwald, R. Ahangari, K. Toyka, University Wurzburg (Wurzburg, D)

Background: Intravenous immunoglobulin treatment (IVIg) ameliorates muscle strength in LEMS. Objective: To delineate the mode of action of IVIg on VGCC autoantibodies.

Methods: Effects of sera and purified IgG from patients with LEMS on evoked quantal release were investigated by a perfused macro-patch clamp electrode in mouse hemidiaphragms. Purified IgG from LEMS-patients' plasma were prepared by affinity chromatography and coincubated with different concentrations of IVIg. A commercial therapeutic IVIG was used for the coincubation experiments.

Results: All LEMS sera and purified LEMS-IgG fractions blocked evoked quantal release irreversibly by up to 90% dose-dependently. Amplitudes of postsynaptic currents remained unaffected by LEMS IgG. IVIg coincubated with LEMS IgG diminished the presynaptic blocking activity of LEMS-IgG dose-dependently. Monovalent Fab fragments prepared from IVIg were equally effective as whole IVIg in neutralizing the blocking activity of LEMS-IgG.

Conclusion: LEMS IgG blocks evoked quantal release. Therapeutic IVIg is capable of inhibiting the action of presynaptic blocking antibodies in LEMS by an antibody (Fab) mediated mechanism. This direct neutralizing effect is similar to that observed in Guillain-Barré syndrome (Buchwald et al., Ann Neurol 2002;51:673–680) and may, amongst other mechanisms, contribute to the beneficial effect of IVIg in LEMS.

73

**Capsaicin receptor (VR1) expression in human peripheral sensory system.** G. Lauria, M. Morbin, M. Borgna, R. Lombardi, D. Pareyson, M. Manconi, J. Davis, P. Geppetti, National Neurological Institute Besta, S.Raffaele Scientific Institute, Neurology-CEDD GlaxoSmithKline, Department of Experimental and Clinical Medicine (Milan, I; Harlow, UK; Ferrara, I)

The vanilloid receptor VR1 is a non-selective ligand-gated channel, structurally related to the transient receptor potential (TRP) family of ion channels. It responds to noxious stimuli including capsaicin, heating in the noxious range, and extracellular acidification. It is able to integrate simultaneous exposure to these stimuli and is involved in different forms of tissue hypersensitivity. The precise localization of VR1-immunoreactivity in the human peripheral nervous system has not been investigated yet. We assessed VR1 immunoreactivity, using a polyclonal anti-human VR1 antibody, either on formalin fixed, paraffin-embedded, thoracic and lumbar spinal cord, dorsal root ganglia (DRG), and sural nerve sections from patients without sensory PNS diseases or on hairy and glabrous skin biopsies from healthy subjects. Double staining confocal microscope studies using polyclonal anti-PGP 9.5 (PGP), monoclonal anti-unique b-tubulin (TuJ1), and polyclonal anti-human VR1 antibodies were also performed. VR1 was intensely expressed in laminae I and II of spinal cord dorsal horns and in dorsal roots. Most small and medium-size sensory neurons of DRG showed VR1 immunoreactivity, which was notably absent in large-size cells. Almost 2/3 of unmyelinated sural nerve fibers were intensely labeled by VR-1 antibody. Occasionally, VR1-immunoreactivity was found also in smooth muscle cells of perineural and epineural arterioles. VR1 immunolabelling was absent in myelinated fibers and in part of unmyelinated fibers, which possibly represented autonomic fibers. In both glabrous and hairy skin, intra-epidermal nerve fibers (IENF) widely expressed VR1. Double staining studies disclosed co-expression of PGP and TuJ1 and, most interestingly, of TuJ1 and VR1 in IENF, thus indirectly demonstrating the co-localization between VR1 and the panaxonal marker PGP. In conclusion, we demonstrated that VR1 is widely expressed in human peripheral sensory nerve fibers involved in nociception. Moreover, the diffuse VR1 immunoreactivity in IENF, unmyelinated terminals without Schwann cell ensheathment, conjoined to the recent evidence that also keratinocytes express a heat-sensitive TRP channel, suggest the existence of functional interactions in pain transduction between epidermal nerves and resident cells.

74

**Pregabalin is effective in patients with painful diabetic neuropathy: a pooled analysis of three clinical trials.** R. W. Richter, R. H. Dworkin, U. Sharma, J. P. Young Jr., L. K. LaMoreaux, A. E. Corbin, L. Knapp, R. M. Poole, University of Oklahoma, University of Rochester, Pfizer Global R&D (Tulsa, Rochester, Ann Arbor, New London, USA)

Aim of Investigation: To evaluate the efficacy and safety of pregabalin administered three times a day (TID) in three clinical trials in patients with painful diabetic peripheral neuropathy.

Methods: Pregabalin was studied in three randomized, double-blind, placebo-controlled, fixed-dose, parallel-group trials with a 1 week baseline phase followed by a double-blind treatment phase ranging from 5–8 weeks in duration: 1008-14 (150 and 600 mg/day; 6 week duration), 1008-29 (75, 300 and 600 mg/day; 5 week duration), and 1008-131 (300 mg/day; 8 week duration). The primary efficacy parameter was the endpoint mean pain score from the daily pain diaries (recorded daily using 11-point numerical rating scale). Secondary efficacy measures included 50% and 30% responder analyses, Short Form McGill Pain Questionnaire (SF-MPQ), sleep interference diary, and Patient and Clinical Global Impressions of Change (PGIC, CGIC).

Results: The ITT population comprised 729 patients (164 received 600 mg/day pregabalin, 157 received 300 mg/day, 79 received 150 mg/day, 77 received 75 mg/day, and 252 received placebo). Both the 300 and 600 mg/day pregabalin doses were effective in reducing endpoint mean pain scores (each p-value ≤ 0.0001). Forty-three percent of patients in the 300 mg/day dose group and 44% in the 600 mg/day dose group were 50% responders compared to 16% of placebo patients (both comparisons p = 0.001). Similarly,

significantly more patients showed a 30% reduction in pain in the 300 and 600 mg/day doses compared to placebo. Statistically significant improvements were also seen in sleep interference scores, SF-MPQ scores, PGIC and CGIC. The efficacy of the 300 and 600 mg/day pregabalin doses was evident by week 1 on the weekly mean pain and sleep scores and SF-MPQ scores, and efficacy was maintained through study end. Pregabalin was generally well tolerated. Dizziness and somnolence were the most commonly reported adverse events. Eighty-nine percent of patients completed the studies and 88% entered the follow on open-label studies.

Conclusions: Pregabalin at doses of 300 and 600 mg/day produced statistically and clinically significant improvements in patients with painful diabetic peripheral neuropathy.

75

**Pain in Guillain-Barré syndrome: incidence and response to methylprednisolone.** L. Ruts, B. Jacobs, R. Van Koningsveld, P. Van Doorn, Erasmus Medical Center Rotterdam (Rotterdam, NL)

Objective: Pain is a common and severe symptom in patients with Guillain-Barré Syndrome (GBS). A variety of pain syndromes may occur at different stages of the illness. Case reports suggest that corticosteroids may relieve severe pain in GBS. The objectives of this study were: 1) to evaluate the incidence, characteristics, severity and course of pain; 2) to assess the efficacy of methylprednisolone (MP) in treatment of pain.

Methods: Patients were recruited from a randomised placebo-controlled trial (RCT) comparing intravenous immunoglobulin (IVIg) + MP (500 mg for five days) versus IVIg + placebo. Presence and severity of pain were collected at randomisation and after four weeks.

In addition, medical records of 39 GBS patients treated in the EMC were screened for different pain symptoms, course and level of severity: 1) pain needing paracetamol; 2) more than paracetamol; 3) extreme pain despite treatment. Pain was scored at different time intervals: 0–4 weeks before randomisation, 0–2 weeks, 2–4 weeks, 1–6 months and > 6 months after randomisation.

Patients were stratified for CMAP of the extensor digitorum brevis (EDB) < or ≥ 3.3 mV (median value). Efficacy of MP was evaluated using the endpoint: percentage of patients improving in level of pain-severity.

Results: 55% of the 223 patients enrolled in the RCT described pain at randomisation.

Of the 39 retrospectively analysed patients, 67% described pain 0–4 weeks before randomisation. Painful par/dysaesthesiae (18%), backache (31%), radicular (18%), interscapular (28%) and muscle pain (23%) most frequently occurred. Most pain symptoms decreased within two weeks. However, painful par/dysaesthesiae and muscle pain remained rather constantly present during at least 6 months.

The median CMAP of the EDB for patients without radicular pain was 4.6 (2.5–19.5), for patients with radicular pain 1.91 (2.5–4.9) ($p = 0.075$). All patients with severe radicular pain (severity 2–3) had a CMAP < 3.3 mV ($p = 0.01$).

The efficacy endpoint showed similar trends for MP compared to placebo.

Conclusions: Pain frequently occurs and causes severe complaints. Especially painful par/dysaesthesiae and muscle pain may persist for months. Severe radicular pain is related to a low CMAP, suggesting a relation with axonal damage. Methylprednisolone has no significant effect on the decrease of pain.

76

**Training intervention and activity registration in patients with Guillain-Barré syndrome or chronic inflammatory demyelinating polyneuropathy, a pilot study.** M. P. J. Garssen, J. B. J. Bussmann, A. Zandbergen, M. Scheerder, P. I. M. Schmitz, T. Stijnen, H. J. Stam, P. A. van Doorn, Erasmus MC (Rotterdam, NL)

Background: Severe fatigue and endurance intolerance are major complaints in 80% of patients with immune-mediated polyneuropathies. We started this feasibility study to examine the effect of physical training on fatigue, endurance intolerance and activity pattern in patients after Guillain-Barré Syndrome (GBS) or during the stable phase of Chronic Inflammatory Demyelinating Polyneuropathy (CIDP).

Methods: 20 GBS or CIDP patients and 10 healthy controls were included. Primary endpoints were improvement of at least one point on the fatigue severity scale (FSS, range 1–7) and increase in daily physical activity as measured with the Rotterdam Activity Monitor (RAM). The RAM measures mobility-related activities (e. g. standing, sitting, walking, cycling) during normal daily life. Secondary endpoints were chosen at the level of impairment (muscle strength, maximal physical capacity, anxiety and depression), disability (secondary RAM measures, fatigue impact scale, functional disability scales) and handicap/quality of life (including SF-36). All patients received a 12-weeks physical training program of cycling, 3 times a week: 5 minutes warming-up, 30 minutes cycling on 85–90% of maximal heart rate, 10 minutes cooling down. Patients were measured pre-training (visit 1), after 6 weeks (visit 2) and post-training (visit 3).

Results: 20 patients started training, 2 patients dropped out for other reasons. Baseline: 16 GBS and 4 CIDP, mean age 49 years (range 22–66), mean 4.1 years after diagnosis, 30% male patients. Primary endpoint: mean FSS visit 1: 6.1, visit 2: 5.1 and visit 3: 4.8. Change in fatigue on FSS from visit 1 to 2: $p = 0.002$ and from visit 1 to 3: $p = 0.0014$. Change in fatigue on FIS from visit 1 to 3: $p = 0.0002$. No difference in FSS and FIS changes between GBS- and CIDP-patients. No major side effects, although muscle cramps and pain in lower extremities were reported in the first weeks. Follow-up visits after finishing this study are scheduled.

Conclusion: This is the first training study in severely fatigued, apparently recovered GBS- and stable CIDP-patients, in which also the activity pattern in normal daily life is measured. A significant decrease of fatigue on FSS was reached, 80% were motivated to continue training after this study. Results of the activity registration are currently being analyzed. Physical training is well tolerated and effective for treatment of fatigue in GBS and CIDP patients.

# Session 13

## Cerebrovascular disorders – 1

77

**A multicentre, randomised controlled trial of an outreach nursing care programme for recently discharged stroke patients.** H. Boter, G. J. E. Rinkel, R. de Haan, University Medical Centre Utrecht, Academic Medical Centre Amsterdam for the HESTIA study group

Background: Many patients who are discharged home after being admitted for a first-ever stroke experience a decreased quality of life and are dissatisfied with the care received. We studied the effectiveness of an outreach nursing care program for such patients and their informal carers.

Methods: In a multi-centre trial, 536 patients were randomised at discharge to [1] standard care plus outreach care or [2] standard care only. The outreach care consisted of three telephone calls and one home visit within five months after discharge by one of 13 stroke nurses. Patients were kept masked and assessed six months after discharge the primary outcomes quality of life (SF-36) and satisfaction with care. Secondary outcomes were disability, handicap, depression, anxiety, information about stroke, and use of health care services and secondary prevention drugs. Informal carers assessed strain, social support, and information about stroke.

Results: Twelve patients died during follow-up, 38 declined follow up interviews. Overall, 234 of 484 (50%) patients were dissatisfied with care received at home, 123 of 461 (27%) had high scores for depression and 126 of 461 (28%) high scores for anxiety. Except for an improvement in the outreach care group on the SF-36 Role Emotional domain (mean difference 7.9; 95% CL 0.1–15.7), no differences were found for quality of life or satisfaction with care. The outreach care group had decreased anxiety scores (median difference 1; 95% CL 0.38–3.01) and less use of rehabilitation services (relative risk 0.66; 95% CL 0.44–1.00). No other differences were found.

Conclusion: The results do not warrant implementation of outreach care by stroke nurses. Strategies to discern and manage depression and anxiety might be beneficial.

78

**Diagnostic accuracy of combined clinical and color duplex sonography assessment of spontaneous cervical carotid artery dissection.** D. H. Benninger, R. W. Baumgartner, University Hospital of Zurich (Zurich, CH)

Objective: To determine the diagnostic accuracy of combined color duplex sonography (CDS) and clinical assessment of spontaneous dissection of the cervical internal carotid artery (ICAD).

Methods: 430 consecutive patients (173 women, 257 men; mean age 50 ± 11 years) presenting the following combinations of clinical and extra- and transcranial CDS findings were investigated prospectively: A) normal CDS or with ipsilateral cervical ICA obstruction ± unilateral local symp-

toms/signs, both consistent with ICAD, and ± ipsilateral cerebral or retinal ischemia; B) cerebral or retinal ischemia, age < 65 years, and normal CDS. ICAD was diagnosed in group A using cervical MR imaging (MRI), catheter angiography (CA), or both; excluded, respectively, in group B by detection of an embolic or other determined etiology, if need be by cervical MRI, CA, or both.

Results: Combined CDS and clinical assessment correctly diagnosed 67 ICADs in 64 patients and excluded ICAD in 357 patients but identified nine false positives, showing a sensitivity of 100%, specificity of 98%, positive and negative predictive values of 88% and 100%, respectively.

Conclusion: Combined clinical and CDS provide a sufficient diagnostic accuracy for assessing spontaneous ICAD. The possibility of false positive and negative CDS findings indicates that additional investigations such as cervical MRI are mandatory for a reliable assessment of ICAD.

79

**Death after cerebral venous thrombosis – the results of the ISCVT.** P. Canhao, J. M. Ferro, M.-G. Bousser, J. Stam, F. Barinagarrementeria, Stroke Unit and the ISCVT Collaborators

Background: Accordingly to available evidence, 4 to 33% of patients with cerebral venous thrombosis (CVT) die during the acute phase. The causes of death were not addressed in previous studies. The aim of the present study was to analyse death in the International Study on Cerebral Venous Dural Sinus Thrombosis (ISCVT) and to identify its causes.

Methods: ISCVT is a multinational (21 countries) prospective observational study that included 624 patients with proven CVT between 5.1998 and 5.2001. A questionnaire inquiring the mechanisms of death was send, to the investigators, listing different possible causes of death.

Results: 27 patients died as a direct consequence of CVT (4.3%). Median time between symptoms onset and death was 13 d (3–106 d) and between diagnosis and death was 5 d (1 to 63 d). At admission 15 cases had decreased consciousness (GCS less than 14); none had isolated intracranial hypertension syndrome; CT or MRI showed intracranial lesions in 25 cases and haemorrhage in 14 cases. All but 2 patients were anticoagulated, 6 were treated with thrombolytics and 13 received antiosmotic therapy. Multivariables predictors of acute death were coma (GCS less than 9) (OR = 8.3, 95% CI 1.4–9.8), mental disturbance (OR = 2.7, 95% CI 1.1–6.8), deep venous thrombosis (OR = 3.6, 95% CI 2.9–22.7) and right-sided haemorrhage on CT or MRI (OR = 4.6, 95% CI 1.8–11.5). The mechanisms of death were: transtentorial herniation due to focal mass effect (10 cases), due to diffuse oedema and focal mass effect (4 cases), due to diffuse oedema and multiple lesions (2 cases) or due to multiple haematomas (1 case); diffuse oedema and pulmonary embolism (1 case); diffuse oedema, multiple lesions and sepsis (2 cases), and underlying disease (1 case).

Conclusions: A hemispheric lesion with cerebral herniation was the direct responsible cause of death in the great majority of cases. These results may have important implications as regards treatment.

80

**Risk of recurrence and stroke after a first event of cervical artery dissection: a multicentre study.** E. Touzé, J. Y. Gauvrit, T. Moulin, S. Bracard, J. F. Meder, J. L. Mas, Hôpital Sainte-Anne, Hôpital R Salengro, CHRU for the investigators of the Multicenter Survey on Natural History of Cervical Artery Dissection

Background: Although the prognosis of cervical artery dissection (CAD) is commonly considered as good, few studies have been devoted to their natural history.

Objective: To assess the risk of CAD recurrence, stroke and transient ischaemic attack (TIA) in a large population of patients with a first event of CAD.

Methods: We undertook a historical cohort study of consecutive patients who presented with a first event of CAD between January 1995 and September 2001 and who were admitted in 24 departments of Neurology. In each center, consecutive patients with CAD who were alive after the first month were retrospectively selected from a stroke data bank or from the local administrative data base using the 10th revision of the ICD. A neurologist and a radiologist reviewed all charts. The diagnosis of CAD was based on classic angiographic signs. Occlusive forms should have been confirmed by the presence of a mural hematoma. In 2002, patients were interviewed by phone or during a visit by the local investigators.

Results: Four hundred fifty-nine patients (mean age 44.0 ± 9.7 years) were included in the study. During the study period, 2 patients died. Among the 457 survivors, 24 (5.3%) could not be contacted in 2002 because they had moved. After a mean follow-up of 30.9 months, 4 (0.9%) patients presented a recurrence of CAD (delay: 13–39 months), 4 (0.9%) an ischemic stroke including 2 patients with CAD recurrence and 8 (1.8%) a TIA without CAD recurrence (delay: 2.4–51.9 months). Seven patients received antithrombotic therapy (aspirin: 6, oral anticoagulants: 1) at the time of event. Only 1 CAD recurrence affected the previously dissected vessel. Strokes without CAD recurrence occurred in the territory of the previously dissected vessel and arterial lesions persisted in all. Five TIA occurred in the territory of a previously affected vessel but only 2 patients had apparent persistent arterial abnormalities.

Conclusion: Our results confirm the very low risk of CAD recurrence in patients with a first ever CAD.

81

**In-vivo clinico-topographical correlation of corticospinal tract stroke using colour coded diffusion-tensor-imaging.** C. Lie, J. G. Hirsch, C. Roßmanith, M. G. Hennerici, A. Gass, Universitaetsklinikum Mannheim (Mannheim, D)

Background: Assessment of the pyramidal tract is limited with conventional brain imaging. The extent of pyramidal tract damage is difficult to assess in small strokes of the internal capsule or pericapsular area. CDTI provides a quantitative means to visualize the course of the corticospinal tract and its topographical relationship to adjacent structures. We used CDTI to analyse the topographical patterns of small lacunar corticospinal tract strokes and compared clinical and morphological findings.

Patients and Methods: A series of 15 consecutive patients with acute strokes of the internal capsule were investigated using both conventional MRI and CDTI (1.5T Siemens Sonata and Vision systems). Both CDTI sequences and postprocessing are in-house developments. Conventional and CDTI images were coregistered and the quantitative change in regard to tissue integrity (Mean diffusivity < MD > and Lattice-anisotropy-index < LAI >) and its exact position in regard to corticospinal tract fibers were visualised. Clinical manifestations were compared to CDTI findings.

Results: We identified 5 different anatomical patterns of corticospinal tract involvement. Patients fell into 2 groups: 1. With marked deficits and relatively minor improvement (6/15) and 2. Those with good recovery or minor motor dysfunction (9/15). Group 1 was characterised by long lesions centered in the pyramidal tract at the level of the internal capsule also involving the thalamus at lower levels and globus pallidus at higher levels (Ant. choroidal a.). Group 2 lesions were either very small lesions and/or located anteriorly and medially (Thalamogeniculate branches, periventricular ant. choroidal territory, lateral striate branches, tuberothalamic branches). Infarcted tissue showed an increased MD (mean 1.58 ± 0.23) and decreased LAI (mean 0.22 ± 0.03) as compared to healthy homologue brain areas (MD mean 0.9 ± 0.08; LAI mean 0.36 ± 0.07).

Conclusion: CDTI allows to differentiate specific subcortical stroke patterns of pyramidal tract damage and their extent in great detail correlating closely to the clinical syndrome and outcome. The lesion patterns identified largely meet a traditional clinical syndromal categorisation based on small penetrating artery territories. CDTI is a useful tool for an improved in-vivo demonstration of the exact location of the underlying pathology in patients with subcortical stroke. New generation MRI systems may well allow this technique to become an additional diagnostic tool in selected patients.

82

**Cerebellar speech representation: lesion topography in dysarthria as derived from cerebellar ischaemia and functional MRI.** P. Urban, J. Marx, S. Hunsche, J. Gawehn, G. Vucurevic, S. Wicht, P. Stoeter, University Hospital (Mainz, D)

Background: Lesion topography and pathophysiology of dysarthria due to focal cerebellar lesions have not yet been fully clarified. This is due to the fact that dysarthria may result not only from a cerebellar lesion but also from frequently associated brainstem ischemia. Previous studies of individuals with cerebellar lesions and associated brainstem damage have, however, not explored the possibility of brainstem involvement as a contributing factor in dysarthria.

Objectives: In the present study we investigated the lesion topography of dysarthria due to cerebellar ischemia in 18 patients, and evaluated brainstem functions using multimodal electrophysiological techniques, including brainstem auditory evoked potentials (BAEP), blink reflex (BlinkR), masseter reflex (MassR), somatosensory evoked potentials (SEP), and transcranial magnetic evoked potentials of the orofacial muscles and tongue (TMS). Functional magnetic resonance imaging (fMRI) was performed in 19 normal subjects. Activation tasks consisted of repetitive vertical silent movements of the tongue and lips at a self-paced rhythm.

Results: Cerebellar lesions and additional signs of brainstem involvement were observed in 11 subjects with PICA, AICA and SCA infarctions, re-

spectively. In all other patients with isolated cerebellar infarction (n = 7) only the SCA-territory (6 right-, 1 left-sided) was affected, and the common lesion site was the rostral paravermal region of the anterior lobe. FMRI in normal subjects indicated that the cerebellar representation of the tongue and orofacial muscles corresponds to that of the area involved in patients with cerebellar dysarthria. During silent tongue and lip movements 14 and 12 patients, respectively, showed bilateral activation of the primary motor cortex. A bilateral task-related increase in MRI signal intensity within the rostral paravermal region of the anterior lobe was observed in 11 (tongue) and 8 (orofacial muscles) subjects.

Conclusions: The results of this study demonstrate that articulatory movements of the tongue and orofacial muscles are involved in the activation of the rostral paravermal area of the anterior lobe. This location corresponds to the area involved in cerebellar ischemia in patients with dysarthria. We conclude that lesions in the upper paravermal area of the right cerebellar hemisphere, the site of coordination of articulatory movements of the tongue and orofacial muscles, may lead to the development of dysarthria which is unrelated to (often concomitant) brainstem infarctions.

# Session 14

## Cerebrovascular disorders – 2

### 83

**Evolution of secondary prevention after stroke between the periods 1988–1994 and 1996–2002 in practice.** M. Girot, D. Deplanque, M. Mackowiak-Cordoliani, C. Lucas, H. Henon, D. Leys, Hopital Roger Salengro (Lille, F)

Background: an optimal management of vascular risk factors associated with antithrombotic therapies reduces the risk of new vascular events in stroke patients. Although several studies have shown that the secondary prevention measures are not appropriate in many patients, the question of whether there is an improvement over time remains unanswered.

Objective: to test the hypothesis that secondary prevention measures were more appropriate in patients who had a stroke between 1996 and 2002 than in patients who had a stroke between 1988 and 1994.

Method: we have enrolled 123 consecutive patients in 1994, and 125 consecutive patients in 2002, who were admitted in a neurological department for any reason and had had a stroke less than 6 years before. We compared both groups for the presence and management of arterial hypertension, hypercholesterolemia, diabetes mellitus and smoking. We recorded the blood pressure and biological parameters during the hospital stay, presence of antithrombotic therapy, lipid-lowering and anti-hypertensive drugs. Whether patients were properly treated or not, was determined by a comparison between their current treatments and available guidelines.

Results: the secondary prevention measures were inappropriate in 96/123 patients in 1994 and in 77/125 hospitalized in 2002. The identification and the management of risk factors were better in 2002 than in 1994, especially for the screening of hypercholesterolemia. Aspirin or other antithrombotic therapy, statins and anti-hypertensive drugs, except calcium channel antagonists, were more often used in 2002. The proportion of patients in whom arterial hypertension and hypercholesterolemia were identified was higher in 2002, but the proportion of patients identified as diabetics remained stable. However, the proportion of patients with blood pressure > 140/90 mmHg, or glycemia > 126 mg/dl, or total cholesterol level > 250 mg/dl, or being current smokers, was lower in 2002 than in 1994.

Conclusion: although most of patients with previous stroke still receive an inappropriate management of their risk factors, there was an improvement between 1994 and 2002.

### 84

**Acute physiology and chronic health evaluation score predicts early death or dependency after acute ischaemic stroke.** Y. Krespi, Y. Kaplan, M. E. Gürol, O. Çoban, R. Tuncay, S. Bahar, Istanbul University (Istanbul, TR)

Background: Scales reflecting both acute physiology and neurological impairment may refine our short-term prognostic modeling in acute stroke patients. Acute Physiology and Chronic Health Evaluation II (APACHE II) score is the most widely used outcome evaluation tool in intensive care medicine. No prospective study assessed its efficacy in acute ischemic stroke patients.

Objective: To prospectively assess the accuracy of APACHE II score in predicting short-term outcome in acute ischemic stroke patients in a stroke unit setting and to compare its performance to Glasgow Coma Score (GCS) and National Institute of Health and Stroke Scale (NIHSS).

Methods: We prospectively evaluated 84 consecutive ischemic stroke patients admitted in the first 24 hours after stroke onset. A single operator obtained the APACHE II, NIHSS and GCS scores. The main outcome measures were death and death or dependency at 1 month. Functional status was determined according to modified Rankin Score (mRS) and scores of 3–5 were defined as dependent. The receiver operating characteristic curves of the three scales were compared using Z statistics and the level of significance was chosen to be $p < 0.05$ ("T [–2] or "d 2). Using a logistic regression analysis, the components of APACHE II score were assessed for predicting subsequent death.

Results: The mean APACHE II, NIHSS and GCS were 15.7 (SD 7.9), 12.2 (SD 8.4) and 13.1 (SD 2.7) respectively. Of 84 patients, 16 (19 %) died and 46 (55 %) were either dead or dependent at the end of the first month. All the scores were significantly associated with the main outcomes ($p = 0.0001$) and no scoring system performed significantly better than the other ($z > [-2]$ or $z < 2$). The partial oxygen pressure ($p = 0.007$), chronic health evaluation ($p = 0.005$) and GCS ($p = 0.016$) scores were the individual components of the APACHE II system showing an independent correlation with death.

Conclusions: The APACHE II score is a powerful tool in predicting early fatality and overall bad outcome after ischemic stroke and it may be more informative than the neurological scales on the associated critical physiologic factors and co-morbidities contributing to outcome.

This work was supported by the Research Fund of The University of Istanbul. Project number T-1192/01112001

### 85

**Statins intake and non-lacunar ischaemic stroke outcome.** R. Merino, E. Díez-Tejedor, B. Fuentes, J. Gracia, V. Mejías, S. Monteagudo, S. Escalante, La Paz University Hospital (Madrid, E)

Background: A recent study suggests that previous treatment with statins could be associated with better clinical outcome in ischemic stroke. Our goal is to evaluate the possible influence of previous treatment with statins in the non lacunar infarction (NLI) outcome.

Methods: Observational and sequential study from Stroke Unit Registry [1998–2001]. Patient with NLI (etiological subtype: Atherothrombotic infarction (AI), cardioembolic infarction (CI) and undetermined etiology (UE) were included. We classified in two groups depending with/without previous treatment with statins. Outcome was evaluated by means of modified Rankin Scale (mRS) at discharge (poor outcome: mRS 3–6). Statistic test: Fisher test, Chi square, t-student, multivariate logistic regression analysis.

Results: Of 663 patients with NLI (313 women and 320 men, age 70 ± 11); 289 (45.7 %) AI, 243 (38.4 %) CI and 131 (15.9 %) UE. 7 % were on treatment with statins. In univariate analyses, treatment with statins was associated with a better outcome at discharge AI ($p = 0.011$), CI ($p > 0.05$) In multivariate logistic regression model we demonstrated that previous treatment with statins was an independent predictor of outcome ($p = 0.023$); OR = 2.627 (CI 95 %: 1.143–6.038).

Conclusions: Previous treatment with statins was associated with a better outcome at discharge in NLI (subgroup AI) and also has been shown to be a good outcome independent predictor factor in this group. In addition to known properties, could a neuroprotective mechanism be implicated? Further prospective studies are needed to analyse the significance of this association.

### 86

**Acute ischaemic stroke and sleep apnea: evolution of clinical findings, diffusion-weighted MRI, and blood pressure in the first 3 days after stroke onset.** M. Siccoli, D. Hermann, D. Schmid, E. Werth, P. Summers, T. Järmann, S. Kollias, C. Bassetti, University Hospital (Zurich, CH)

Background: Sleep apnea (SA) is present in about 50 % of patients (pts) with acute ischemic stroke. Hypoxia and hemodynamic changes accompany SA. Diffusion-weighted magnetic resonance imaging (DWI) detects ischemic injury early after stroke onset. The extent of DWI changes correlates with stroke severity and predicts stroke outcome.

Objectives: To test the hypothesis that in the acute phase of stroke moderate-severe SA leads to an enlargement of the ischemic volume on DWI that is more pronounced than in pts without SA.

Design/methods: We include pts with neuroradiologically proven ischemic stroke and hospital admission within 12 hours after stroke onset.

Stroke severity is estimated by NIH and Scandinavian stroke scale (NIHSS, SSS) at admission (day 1), day 2 and day 3. Clinical stroke progression is defined as a decrease ≥2 points in consciousness or motor power or ≥3 points in speech scores in the SSS. Sleep breathing is assessed by an intelligent CPAP device (Autoset® Portable II plus, ResMed) the first night after admission. Moderate-severe SA is defined by an apnea hypopnea index > 25. Blood pressure (BP) monitoring is performed at intervals of 30 minutes with an ambulatory device (bp one, Cardiette) from 7 p. m. of day 1 until 7 a. m. of day 3. Mean systolic and diastolic BP values are calculated for daytime and nighttime. Nighttime BP dipping is defined by a ratio of nighttime/daytime mean systolic and/or diastolic BP values of < 0.9. MR imaging is performed on a 1.5 T MR system at 7 p. m. of day 1 and again 7 a. m. of day 3. Stroke volumes are measured on DWI.

Results: We included so far 8 pts with a mean age of 64 years (range 44–83). Sleep breathing and blood pressure recordings were performed in all pts, acute and follow-up MRI studies were completed in 4 pts. At admission the mean NIHSS was 12 (range 2–17) and the mean SSS was 35 (range 24–56). Moderate-severe SA was present in 5 pts. Three of these 5 pts had a clinical stroke progression which was accompanied in two pts by a clear-cut increase of stroke volume on DWI (from a mean value of 18 $cm^3$ to 39 $cm^3$). In three pts SA was mild or absent. None of these pts had a clinical stroke progression and only one of them had an increase of stroke volume on DWI. Mean values of blood pressure were not statistically different in pts with and without moderate-severe SA (daytime values: 159/102 vs 148/94; nighttime values: 147/91 vs 150/94). A nighttime BP dipping was absent in 2 of 5 pts with, and in all 3 pts without moderate-severe SA.

Conclusions: Preliminary results of this ongoing project suggest that in pts with acute ischemic stroke moderate-severe SA may lead to 1) clinical stroke progression, and 2) increase of stroke volume on DWI within the first 3 days after stroke onset. These detrimental effects may not be related to blood pressure changes.

87

**Turkish ischaemic stroke genetics collaboration: factor V Leiden mutation is a risk factor in young adults with cryptogenic ischaemic stroke.** Y. Krespi, A. Ozturk, D. B. Guney, N. E. Unaltuna, O. Coban, R. Tuncay, S. Bahar, Istanbul University (Istanbul, TR)

Background: The causes of ischemic stroke of undetermined etiology in young adults ("T 45 years) are poorly understood. Few studies looked for a role for factor V (FV) Leiden mutation in this setting and gave contradictory results.

Design/Methods: We made a case-control study to assess the prevalence and significance of FV Leiden mutation in a hospital cohort of young Turkish patients with cryptogenic ischemic stroke. To this purpose we prospectively evaluated 95 consecutive patients recorded to Istanbul Stroke Registry between January 1994 and February 2003. Twenty seven patients did not give an informed consent and a total of 68 cases (31 males, 37 females; mean ± SD age at index event 33 ± 8 years, min 15, max 45) were included in the study. Cryptogenic ischemic stroke was defined as the absence of major cardiac source of embolism, atherosclerotic or nonatherosclerotic vasculopathy and clear-cut small vessel disease. Patients who may have had some risk factors for stroke but in whom the mechanism of stroke was uncertain were also included in the study. This latter group included patients with a right-to-left interatrial shunt, an atrial septal aneurysm, elevated anticardiolipin antibodies or lupus anticoagulant, low level of protein S, protein C, or antithrombin III, oral contraceptive use and migraine. Eighty apparently healthy subjects (39 males, 41 females; mean ± SD age, 30 ± 8 years, min 15, max 46) served as controls.

Results: Heterozygosity or homozygosity for the FV Leiden mutation was found in 15 of 68 patients (22%) and in 5 of 80 controls (6.2%); this difference was statistically significant (p = 0.005, 2 tests). Thirteen patients had a heterozygous and 2 patients had a homozygous FV Leiden mutation. No individual in the control group had a homozygous mutation.

Conclusions: Our findings support the hypothesis that some genes associated with a prothrombotic state may be risk factors among a subgroup of young people with stroke of undetermined cause. In the present cohort of patients of Turkish ancestry, FV Leiden mutation revealed as a potential risk factor for ischemic stroke.

Acknowledgements: This work was supported by the Research Fund of Istanbul University. Project number 1454/05052000

88

**Echo-enhanced transcranial colour-coded duplex sonography in the diagnosis of cerebrovascular events. A validation study.** A. Kunz, G. Gahn, G. Hahn, A. Mueller, R. von Kummer, H. Reichmann, University Hospital Carl Gustav Carus (Dresden, D)

Objective: This study was designed to assess the diagnostic efficacy of echo-enhanced transcranial color coded duplexsonography (eTCCD) for evaluation of the intracranial vasculature in patients with cerebrovascular symptoms.

Background: eTCCD is a minimally invasive and fast diagnostic method that considerably improves ultrasound evaluation of the intracranial arteries by permitting direct visualisation of the large vessels. To date a systematic evaluation of the diagnostic efficacy of eTCCD in comparison to digital subtraction angiography (DSA) has not been investigated yet.

Methods: From 01/00 to 12/01, we prospectively examined 132 consecutive patients (40 women, 92 men, mean age 58 ± 14 years) for evaluation of cerebrovascular symptoms with DSA and eTCCD. 121/132 patients had evaluation of the anterior circulation (AC), 99 of the posterior circulation (PC) by eTCCD. 67 patients underwent DSA due to a symptomatic internal carotid artery (ICA) stenosis. For eTCCD we applied an intravenous galactose palmitic acid based echo-enhancing agent (Levovist®, Schering, Germany). We used the transtemporal bone window for evaluation of the AC and the suboccipital access for the PC. All ETCCD- and DSA-findings were reviewed by a blinded and experienced examiner.

Results: AC: eTCCD showed all major arteries of the circle of Willis in 110/121 patients. 66/80 patients with pathological findings in DSA were correctly depicted by eTCCD [sensitivity 83% (95% CI 72–90), specificity 93% (95% CI 81–99)]. In the subgroup of patients with symptomatic ICA stenosis eTCCD correctly identified collateral flow patterns in 40/49 patients [sensitivity 82% (95% CI 68–91), specificity 85% (95% CI 55–98)]. PC: eTCCD visualized the distal portions of the V4-segments of both vertebral arteries and the proximal 2/3 of the basilar artery to a mean depth of 97 ± 15 mm in 98/99 patients. 13/16 patients with pathological findings by DSA had abnormal flow patterns in eTCCD [sensitivity 81% (95% CI 54–96), specificity 100%].

Discussion: eTCCD is a minimally invasive and efficient diagnostic bedside tool for evaluation of the large cerebral arteries. The study demonstrates good validation data for the AC, for the PC a larger number of abnormalities has to be evaluated. eTCCD evaluation is limited due to insufficient bone windows in only a small percentage of patients. eTCCD might be an efficient alternative imaging procedure especially for patients not eligible e. g. for DSA or Angio-CT.

89

**Local thrombolysis for CVST in the Netherlands.** H. P. Bienfait, J. T. J. Tans, P. H. Hoogland, Gelre Hospitals, Medical Centre Haaglanden for the Dutch CVST study group

Cerebral venous and sinus thrombosis (CVST) is a rare disease. It is often treated with anticoagulants. However it has never been demonstrated in a prospective placebo controlled trial that this therapy improves the outcome of CVST, but it seems to be a safe therapy, even in the case of CVST related haemorrhages. It is known that CVST patients with a severe or deteriorating neurological condition have an unfavourable prognosis, even with anticoagulant therapy. Local thrombolysis with urokinase or recombinant tissue plasminogen activator (rtPA), directly administered in the obliterated sinus by the Seldinger method, seems to be a promising new therapy. Currently there is no extensive evidence concerning the safety of this recent treatment, though approximately 150 cases have been reported in literature. Most of these reports showed extremely good results. Therefore in 1999 a national prospective multi-centre pilot study was started in the Netherlands. This pilot study is conducted to investigate the safety and practical use of local urokinase or rtPA for CVST patients in a critical neurological condition. Patients are included when CVST is identified with MRI/MRA or cerebral angiography. The patients in a severe neurological condition must be in a critical condition as defined by a GCS less than 10 or a NIHSS of more than 18. The principle measure of outcome is the Rankin score (or death) at six months. Local thrombolysis is defined as administering urokinase or rtPA locally in the obliterated sinus or cerebral vein.

Results at this moment: Six patients were treated with local thrombolysis in four different medical centres. Three patients died of the complications due to CVST. None of the six patients had serious hemorrhagic complications. Two patients recovered completely and one almost completely. Unfortunately another three young patients recently died from CVST even before they could be enrolled in the pilot study.

Conclusion: Our modest experience in The Netherlands with local thrombolysis for severe CVST confirms the technical feasibility to recanalise

the obliterated sinuses. In three patients with a critical neurological condition due to CVST, local thrombolysis could not reverse the fatal course. When one considers local thrombolysis for CVST we recommend not to hesitate and to perform the procedure with minimal delay.

# Session 15

## Parkinson's disease – 1

90
**Parkinson's disease progresses in patients treated by STN DBS.** F. Vingerhoets, H. Russmann, C. Wider, P. Burkhard, J.-G. Villemure, J. Ghika, CHUV, HUG - Vaud-Geneva programme of functional neurology and neurosurgery: stereotaxy & movement disorders, Lausanne, Switzerland

Objective: Subthalamic (STN) deep brain stimulation (DBS) is a recognized treatment of advanced Parkinson's disease (PD) with motor fluctuations. It has been suggested that DBS may alter PD progression. We studied the clinical evolution of PD Off medication and Off STN DBS between 1 and 3 years in order to estimate disease progression.

Methods: Nineteen PD patients (age: 62 ± 15y; disease duration: 14 ± 5y; UPDRS mot off 46.5 ± 16.2) treated by STN DBS were examined. Practically off medication UPDRS III were performed, before and three hours after turning STN DBS off: once 6.5 ± 3.7 months after implantation and a second time 22.4 ± 10.6 months after the first evaluation.

Results: Compared to preoperative results, UPDRS III at first follow-up worsened to 52.5 ± 20.1 ($p < 0.05$, paired t-test). This worsening continued over the following period up to 59.9 ± 21.8 ($p < 0.01$). Similar results were observed for bradykinesia (25.7 ± 8.5; 30.0 ± 10.5; 33.6 ± 11.6; $p < 0.01$) and rigidity subscales (9.1 ± 3.4; 9.2 ± 4.8; 12.8 ± 6.0; $p < 0.01$), while tremor (5.4 ± 4.5; 6.3 ± 5.2; 5.7 ± 4.0; $p > 0.45$) and axial signs (6.4 ± 3.1; 7.1 ± 4.3; 7.7 ± 5.0) were not affected. At the same time points on stimulation UPDRS were stable (25.1 ± 11.5; 24.0 ± 12.8; $p = 0.56$) and improved in comparison with preoperative results ($p < 0.001$). There was no significant difference in stimulation parameters or treatments between follow-ups.

Discussion: We found that UPDRS motor scores in practically off medication and STN DBS turned off worsened significantly over 2 years. This worsening was mainly related to bradykinesia and rigidity subscales that best reflect the nigrostriatal lesion progression. In contrast, on stimulation UPDRS, stimulation parameters and medication did not change significantly. These results suggest that Parkinson's disease's progression is not significantly affected by STN DBS, but that this progression is masked by STN DBS.

91
**H-reflex modulation by stimulation through electrodes implanted in the subthalamic nucleus of Parkinson's disease patients.** M. Gómez-Choco, M. M. Mascia, F. Valldeoriola, J. Valls-Solé, Hospital Clinic, Hospital San Giovanni di Dio (Barcelona, E; Cagliari, I)

Introduction: Transcranial magnetic stimulation (TMS) induces two phases of facilitation of the soleus H reflex in normal subjects: an early phase between 5 and 30 ms, and a later phase between 60 and 100 ms. In patients with Parkinson's disease (PD) the late phase is often reduced. However, the origin of those facilitatory phases is not well understood. In an attempt to gain further knowledge on the physiology of those findings, we have examined the effect on the H reflex of deep brain stimulation through the electrode implanted in the subthalamic nucleus (STN) in PD patients.

Methods: This study was carried out in four PD patients who had stimulators implanted bilaterally on the STN, before the subcutaneous tunnelization of the electrodes. In all patients electrical STN stimulation was followed by stimulation of the posterior tibial nerve at an intensity adequate to elicit the soleus H-reflex. Inter-stimulus time intervals (ISI) were 0 to 110 ms. The stimulation of the tibial nerve was applied at different times on the sides ipsilateral and contralateral to the STN stimulation.

Results: STN stimulation induced an early phase of H reflex facilitation beetwen 5 and 20 ms in the side contralateral to the stimulus, and a late phase of H-reflex facilitation, between 70 and 100 ms, in both sides. The peak of the early facilitation was 270 %. That of the late facilitation was 261 % in the contralateral side, and of 172 % in the ipsilateral side.

Discussion: The fact that the early facilitation was only present in the side contralateral to the STN stimulation suggests that it is due to activation of the crossed corticospinal tract. The late bilateral facilitatory phases should be due to activation of tracts with bilateral projection to spinal motoneurons, which is characteristic of the reticulospinal tract. This tract can be activated through stimulation of cortico-reticular connections.

92
**Abnormal sensory modulation on transcranial magnetic stimulation is correlated with symptoms severity in patients with Parkinson's disease.** S. Tamburin, A. Fiaschi, D. Idone, P. Lochner, P. Manganotti, G. Zanette, University of Verona, Hospital Pederzoli (Verona, I)

Background: Hyperexcitability of the motor system has been reported in PD.

Objective: To evaluate how cutaneous afferents modulate motor excitability in PD patients and whether abnormal modulation plays a role in the genesis of parkinsonian symptoms.

Methods: The effect of electrical stimulation of the second (D2) and fifth finger (D5) on motor evoked potentials (MEPs) in response to transcranial magnetic stimulation (TMS) in abductor digiti minimi muscle was evaluated in 10 unilateral (u-PD) and 9 bilateral (b-PD) PD patients. Digital stimuli preceded TMS at interstimulus intervals (ISIs) of 10–100 msec.

Results: Digital stimulation caused an MEP reduction in normal controls (up to 38 % of test MEP) and on the non-affected sides in u-PD patients (up to 60 % of test MEP) and MEP potentiation on the affected sides in u-PD (up to 136 % of test MEP) and b-PD patients (up to 186 % of test MEP) at ISIs of 20–50 msec. The difference between patients and controls was significant (ANOVA: D5, $P < 0.001$; D2, $P < 0.001$). The amplitude of conditioned MEPs at interstimulus intervals of 20–50 msec (MEP20–50) correlated significantly with UPDRS ($P < 0.05$), UPDRS III ($P < 0.02$), Hoehn and Yahr score ($P < 0.02$) and limb bradykinesia ($P < 0.02$). No correlations were found between MEP20–50 and Schwab and England score, resting tremor or rigidity.

Conclusions: Digital stimulation causes abnormal enhancement of motor responses in patients. This effect may be one of the features of motor hyperexcitability in PD, probably related to the patients' inability to generate appropriate muscular activation. Cutaneomotor hyperexcitability correlates with clinical scores, suggesting that abnormal processing of cutaneous inputs plays a role in the pathogenesis of parkinsonian symptoms, particularly bradykinesia.

93
**Changes in activity of abnormal metabolic brain networks in Parkinson's disease patients treated with Vim DBS.** M. Trost, A. Barnes, E. Simon, V. Dhawan, D. Eidelberg, H. Fodstad, University Medical Center Ljubljana, North Shore-Long Island Jewish Research Institute, Tel-Aviv Sourasky Medical Center, VA Medical Center (Ljubljana, SI; New York, USA; Tel Aviv, IL)

Eight Parkinson's disease (PD) patients, seven men and one woman, mean age 65.1 ± 9.9 years and disease duration 8.6 ± 4.5 years (mean ± SD) with intractable tremor were treated with unilateral deep brain stimulation (DBS) of the ventral intermediate (Vim) thalamic nucleus. Changes in brain metabolism caused by DBS were studied with [18F]-FDG and PET.

Patients were clinically evaluated during Vim DBS ON and OFF. SPM99 (Wellcome Department of Cognitive Neurology, Queen's Sq, London, UK) analysis was performed, to study mean changes in brain glucose metabolism with Vim DBS ON versus OFF. Additionally, network analysis (Functional Imaging laboratory, Northshore University Hospital, Manhasset, New York, USA) was performed to determine whether the activity of pathological metabolic brain networks, associated with PD, change with Vim DBS.

In previous PET studies of brain metabolism with (18F)-FDG, we identified a specific PD-related pattern (PDRP) characterized by pallidal, ventral thalamic, and pontine hypermetabolism associated with decrements in motor cortical regions. The expression of this pattern correlates with disease severity and duration. However the expression of PDRP did not differ in PD patients with and without tremor. A different, tremor related pattern (TP), was identified in PD patients with tremor comprising the thalamus, pons and premotor cortical regions. The expression of both networks was calculated in each subject for Vim DBS ON and OFF state.

During Vim DBS the mean tremor score of the contralateral limbs improved from 9 ± 3.9 to 1.2 ± 2.6 (mean UPDRS score for rest + action tremor score ± SD), $p < 0.001$. Motor UPDRS declined from 38.7 ± 11.7 to 24.5 ± 9.1 (mean motor score ± SD), $p < 0.01$. With Vim DBS ON, SPM analysis ($p = 0.01$ peak uncorrected) showed increased metabolism in the ventral posterior lateral thalamic nucleus [-20, -18, 10], T max = 3.84. Decreased metabolism was found in cerebellum, lobule III of the contralateral side [12, -50, -16], T max = 18.3, of the stimulated side. TP activity decreased significantly with DBS ON in the stimulated hemisphere ($p < 0.01$, students paired t-test, 2

tailed) and did not change in the non-stimulated side ($p = 0.4$). The PDRP activity however did not change significantly with DBS ON in either of the hemispheres.

Our findings show that Vim DBS is a suitable procedure to treat parkinsonian tremor. With DBS ON tremor improved clinically and the expression of a tremor related pattern declined. VIM DBS for PD affects the expression of the tremor related pattern, but has no consistent effect on other parkinsonian network abnormalities like PDRP, which relate mainly to akinetic-rigid disease manifestations.

94

**Objective ambulatory quantification of the effect of STN-DBS on bradykinesia and tremor.** H. Russmann, A. Salarian, P. Burkhard, K. Aminian, J.-G. Villemure, Y. Blanc, C. Dehollain, F. Vingerhoets, CHUV, EPFL, HUG (Lausanne, Geneva, CH)

Objective: To objectively quantify bradykinesia and tremor in PD patients with STN-DBS switched ON and OFF and compare them to healthy subjects with the help of a new portable system.

Patients and Method: Ten PD patients (5 male and 5 female, age: 61.5 ± 7.8 y, min: 48.7, max: 75.1), 20 ± 3 months after STN DBS implantation and ten age-matched controls were studied with two 3D miniature gyroscopes attached above the wrist providing measurement of angular velocity (Mh: degree/sec) in the three directions and the mean range of hand rotations (Rh:degree). Signals were recorded by a portable datalogger (Physilog®, BioAGM, CH). These measurements were performed during a 30 minutes protocol of activities mimicking daily living: once with DBS ON, once 180 minutes after turning DBS OFF. UPDRS III was performed in the same conditions for comparisons. Tremor was evaluated by spectral analysis the angular velocity of hands. Statistics were performed with rank-sum test, Wilcoxon test and Pearson's Correlation coefficient.

Results: Mobility parameters in roll axis had the best correlation with UPDRS bradykinesia subscore (Mh: = −0.837; $p < 0.0001$; Rh: = −0.827, $p < 0.0001$). For the three axes there was a significant difference in Mh between healthy subjects and PD patients with STN-DBS switched OFF (roll:$p = 0.002$; yaw:$p = 0.0025$; pitch:$p = 0.0019$; Whitney-Mann = $p < 0.01$) with STN-DBS switched ON there was a significant difference only for the roll ($p = 0.05$) and yaw ($p = 0.0025$). Similarly there was an excellent correlation between tremor evaluation and tremor subscore (UPDRS parts 20 + 21, $r = 0.87$).

Conclusions: Our portable system provides objective quantification of bradykinesia and tremor in PD patients. The results obtained on an ambulatory mode: a) are in high correlation with the UPDRS III, b) allow differentiation between ON, OFF state in PD patients and normal values. Developping such portable systems will allow future longterm objective monitoring of treated PD patients.

95

**Analysis of motor cortex oscillatory activity under internal globus pallidus or subthalamic nucleus stimulation in Parkinson's disease.** D. Devos, L. Defebvre, E. Labyt, P. Drambure, F. Cassim, J. Bourriez, C. Ozsancak, S. Blond, J. Guieu, A. Destée, Hopital Salengro (Lille, F)

Background: In Parkinson's disease (PD), the motor preparation impairment is revealed by a delay in the movement-related desynchronization appearance over the contralateral primary sensorimotor cortex (PSM). Bilateral chronic high frequency stimulation of basal ganglia (internal globus pallidus GPI or subthalamic nucleus stimulation STN) can be proposed in parkinsonians with severe motor fluctuations to control the parkinsonian triad and motor complications.

Objective: We aimed to assess the cortical activation improvement before and during a movement induced by GPI or STN stimulation, compared to L-Dopa, using pre-movement and movement desynchronization.

Methods: Ten PD patients with STN stimulation and 6 PD patients with GPI stimulation performed self-paced wrist flexion movements in 4 conditions: without stimulation and L-Dopa (worst off), with stimulation and without L-Dopa (on stim), with L-Dopa and without stimulation (on drug), with stimulation and L-Dopa (best on). Desynchronization, based on EEG activity average was analysed from 14 source derivations covering the motor cortical structures.

Results: With STN stimulation, ERD before and during the movement significantly increased on central contralateral derivations in the 3 conditions compared to worst off; the same result was obtained during the movement on the central ipsilateral derivations. Pre-movement desynchronization significantly decreased over frontocentral regions in the three conditions compared to Worst Off. With GPI stimulation, ERD significantly increased only during the movement on central contralateral derivations in on stim and best on conditions compared to worst off. For both stimulations, we observed a significantly higher increase in Best On (the benefit was correlated with the bradykinesia improvement).

Conclusions: STN stimulation, as L-Dopa could improve contralateral PSM cortex activation during the movement planning and execution and decrease the pre-movement frontocentral activation which possibly corresponded to compensatory mechanism. Internal globus pallidus high frequency stimulation could also improve cortical activation during the movement but failed to improve the delay of desynchronization appearance corresponding to the movement preparation phase. STN stimulation could induce dopamimetic effect on PSM cortex oscillatory activity which may explain bradykinesia improvement.

# Session 16

## Parkinson's disease – 2

96

**The impact of dyskinesias in Parkinson's disease: development of a patient-based outcome measure.** R. Katzenschlager, A. Schrag, J. Hobart, A. Evans, A. Lees, National Hospital for Neurology and Neurosurgery, Institute of Neurology, Plymouth Hospitals (London, Plymouth, UK)

Background: Drug-induced dyskinesias are common in the long-term management of Parkinson's disease (PD). They are the specific focus of treatments. To evaluate the effectiveness of strategies aimed at reducing dyskinesias, investigators require rating scales to measure the impact of dyskinesias as rigorously as possible. Currently, patient-based outcome measures used in PD, such as the PDQ-39 and SF-36, do not specifically address the problem of dyskinesias. Therefore, the aim of this study was to develop a patient-based measure of the impact of dyskinesias on activities of daily living.

Method and results: A literature review of published scales identified 13 activities considered important to patients. Five activities were added which, in the authors' experience, were often affected by dyskinesias. A sample of 35 patients were asked to rate the impact of their dyskinesias on each activity on a 5 point scale (high score = greater impact). Patients were also asked to add other activities they considered important. This process generated a 42-item questionnaire that was administered to a sample of 72 patients known to have problematic dyskinesias, from the National Hospital for Neurology and Neurosurgery in London. Standard psychometric methods were used to reduce the items, and to develop a rating scale that satisfied published criteria for reliable (Cronbach's alpha, corrected item-total correlations, test-retest reliability) and valid (scaling assumptions, group differences, factor analysis) measurement of the impact of dyskinesias from the patients' perspective.

Conclusions: Preliminary results support the reliability and validity of this patient-based scale. Further validation studies are in progress and responsiveness is being examined.

97

**Ambulatory measurement of the effects of STN DBS on gait parameters in Parkinson's disease and comparison to normal control subjects.** H. Russmann, A. Salarian, F. Vingerhoets, K. Aminian, J.-G. Villemure, Y. Blanc, C. Dehollain, P. Burkhard, CHUV, EPFL, HUG (Lausanne, Geneva, CH)

Objective: To quantify different gait parameters in Parkinson's disease (PD) patients with and without subthalamic deep brain stimulation (STN DBS) compared to healthy subjects by using an ambulatory measurement system based on gyroscopes.

Patients and methods: Ten PD patients (5 men, 5 women, age: 61.5 ± 7.8), 20 ± 3 months after STN-DBS implantation, were asked to walk 20 meters twice with STN DBS switched ON and once 120 minutes after STN DBS has been switched OFF. Spatial and temporal parameters for each gait cycle were estimated using 4 uniaxial miniature gyroscopes attached to the lower limbs and two 3D gyroscopes for the upper limbs. The same protocol was used to measure ten normal age matched subjects (5 men, 5 women, age 63 ± 10.5). UPDRS III was obtained for each patient in the ON and OFF states. Data recording was performed with a portable data-logger (Physilog®, BioAGM, CH) carried by the patient. Statistics were performed using two-sided t-test and rank sum test.

Results: Compared to controls, PD patients (OFF state) had significantly

shorter normalized stride length and velocity (SL = 0.40 vs. 0.79, $p < 0.004$ and SP = 0.34 vs. 0.80, $p < 0.002$), longer gait cycle time (GC = 1.39s vs. 1.01s, $p < 0.03$) and double-support phase (DS = 33 % vs. 19 %, $p < 0.001$) as well as significantly shorter range of rotations of shanks (SR = 41° vs. 75°, $p < 0.001$), thighs (TR = 26° vs. 46°, $p < 0.001$), knees (KR = 36° vs. 52°, $p < 0.01$) and hands (HR = 11° vs. 48°, $p < 0.001$) and significantly reduced peak angular velocity of shank (SV = 203°/s vs. 383°/s, $p < 0.001$). With STN-DBS most gait parameters improved significantly ($p < 0.005$) but were significantly different from controls ($p < 0.05$). STN-DBS significantly decreased stride-stride variability of SV ($p < 0.002$). HR and SR showed significant correlation ($r = -0.76$ and $r = -0.85$ with $p < 0.001$) with UPDRS (sub scores 22.b + 23 + 24 + 25 + 31 for HR and 29 + 22.c for SR).

Conclusions: STN-DBS significantly improved gait parameters but not up to normal values with a good correlation between range of rotation of the limbs and UPDRS scores. Physilog® system offered a simple way to evaluate spatio-temporal gait parameters as well as the range of limbs' movement and provides an efficient way to estimate stride-stride variability.

98

**Efficacy of an intensive speech treatment (LSVT) in Parkinson's disease: a pilot study of 22 French patients.** C. Ozsancak, L. Cabrejo, M. Jan, P. Auzou, D. Hannequin, CHRU de Lille, CHU de Rouen, Serv d'Explorations Neurologiques Fonctionnelles (Lille, Rouen, Berck sur Mer, F)

Background: Dysarthria is a common manifestation of Parkinson's disease (PD) which increases in frequency and severity with the progress of the disease. Its response to drug therapy is often disappointing. Speech therapy, when proposed, is generally done only once or twice a week without a standardized approach.

Objective: To assess short and mid term (6 months) effects of the Lee Silverman voice treatment (LSVT), a standardized and intensive method designed to improve speech function in patients with PD.

Subjects and methods: We studied twenty four consecutive patients with Parkinson's disease (6 women, 18 men; age 66 ± 9 years [mean ± SD]; disease duration 12 ± 7 years; Hoehn & Yahr "on" stage 3.0 ± 0.7; treatment: 917 ± 526 mg/day of levodopa equivalent). The Unified Parkinson's disease rating scale (UPDRS) motor part was 35 ± 15. Two patients did not complete the study. All patients received the LSVT, which emphasizes intensive phonatory-respiratory effort: 4 sessions of an hour every week for four weeks. Treatment was undertaken by outdoor speech clinicians who had been specifically trained to the method. Four evaluations were done: twice before LSVT (EV1 and EV2), one in the week following the end of the treatment (EV3) and one 6 months later (EV4). All evaluations were done under identical conditions. Treatment was kept constant between EV1 and EV3. An intelligibility score (IS) was obtained based on reading material and conversational speech as well as acoustic and aerodynamic parameters: mean intensity of a sustained vowel (in dB), variation in voice fundamental frequency (Fo) in a sentence (variation coefficient of Fo), maximum phonation time (MPT), jitter and shimmer. These parameters assess respectively vocal intensity, voice stability, the respiratory capacity and vocal quality (jitter and shimmer). Statistics were done using repeated-measure (EV1, EV2, EV3, EV4) analyses of variance with post hoc analyses using Bonferroni method.

Results: The main results were a significant improvement of intelligibility (IS) and mean intensity ($p < 0.001$) at EV3 and EV4 compared to both EV1 and EV2. The variation coefficient of Fo decreased significantly ($p = 0.008$) at EV3. MPT, jitter and shimmer did not differ significantly.

CONCLUSIONS: The findings provide evidence for the efficacy of the LSVT as well as the maintenance of these effects at least six months in patients with Parkinson's disease.

99

**The functional anatomy of bimanual coordination in Parkinson's disease.** W. Loichinger, W. Becker, J. Schwarz, A. Storch, E. Kraft, University of Ulm, University of Leipzig (Ulm, Leipzig, D)

Introduction: Clinical and behavioural data suggest impairment of bimanual coordination in Parkinson's disease (PD). Therefore the functional anatomy of bimanual coordination in PD and the effects of dopaminergic medication on this relevant motor skill are of great interest. We aimed to study the effect of L-DOPA on the motor areas involved bimanual coordination using fMRI.

Methods and patients: 13 right-handed patients (11 males, 2 females) with PD, mean age 59.3 ± 8.7 years (Hoehn/Yahr stages I-II) were studied on a 1.5 T scanner. Functional data were acquired using a T2* weighted EPI sequence. Patients performed bimanual power grip movements (grip force 10 N) with simultaneous and alternating movements of both hands. Movements were acoustically paced with a frequency of 1 Hz. Two fMRI sessions for each patient were performed, the first without medication for at least 12 hours (off-session) and the second 1 hour after taking 250 mg oral L-DOPA (on-session). Clinical testing using the UPDRS-motor score was obtained before and after taking L-DOPA. Each fMRI experiment was done in a block design with the motor tasks alternating with rest.

Results and conclusions: Contrasts of motor tasks with rest showed for the off- and on-session activity within a network of multiple motor areas including sensory motor cortex, lateral premotor cortex, supplementary motor area as well as putamen, thalamus and cerebellum. However, when contrasts of the motor tasks between off- and on-session were compared, increased activity was shown in the posterior putamen bilaterally for the alternating movement condition and to a lesser degree for the simultaneous movement condition. Our results suggest that the putamen in particular benefits from L-DOPA during bimanual coordinated movements. Our results also might suggest a possible role of the basal ganglia in controlling bimanual coordination.

100

**Amantadine for dyskinesias in patients affected by severe Parkinson's disease.** A. Thomas, D. Iacono, A. Luciano, B. Perfetti, K. Armellino, M. Onofrj, Neurophysiopathology (Pescara, I)

Some authors have indicated that Ama may ameliorate l-dopa-induced dyskinesias. From the first study emerges that the mild to moderate symptomatic "benefit effects" of Ama are generally transient, lasting about 30 days to 12 months. 40 patients with advanced PD complicated by motor fluctuations and l-dopa-induced dyskinesias were treated with Ama 300 mg/day or with placebo. All patients had been treated for 8 ± 2 years with l-dopa (800 ± 122 mg) and dopaminoagonists. The dyskinesias were assessed by the UPDRS subscale IV, item 32–34, and the Dyskinesias Rating Scale (DRS). The IGA (Investigator Global Assessment) of dyskinesias was utilised to evaluate changes during the follow-up on a seven point scale + 3 (marked reduction) to −3 (marked increase). All patients were assessed with the three different rating methods 15 days and every 30 days for 8 months during the study. Each evaluation was videotaped and dyskinesias were scored by a clinician unaware of previous ratings. Statistics were performed with Anova, Kaplan-Meier curve and Student's t test. Baseline UPDRS item 32–34 mean score was 10 ± 1.4 (SD). The mean value of the DRS scores was 19.5 ± 0.5. After 15 days of treatment a significant reduction of motor fluctuations and of total dyskinesia score reduced by 44 %, $p < 0.001$) was registered in the Ama group. Item 32–34 mean scores were 6 ± 1.2, $p < 0.001$, and DRS mean score was 7.5 ± 0.8, $p < 0.001$. IGA score was 2.1 ± 0.1. At the 30 day control UPDRS and dyskinesia scores were overlapping with scores obtained at 15 days evaluation. After 2–8 months of Ama treatment dyskinesia scores increased and no statistical differences could be evidenced as compared with baseline scores. After 8 months Ama was withdrawn in all patients, 2 patients experienced severe hyperthermia and therefore Ama was reintroduced, after 4 days hyperthermia subsided and amantadine was slowly tapered in 15 days without further adverse reactions. Our results demonstrate that 300 mg Ama reduce dyskinesias in PD by approximately 44 % but this benefit was transient and disappeared after 2–8 months. In our study the relatively short effect, with rebound effect of Ama, suggests that the administration of Ama cannot be viewed as a long lasting solution to the occurrence of dyskinesias and fluctuations in severe PD patients. Its short-term efficacy is however significantly powerful and suggests that further investigations are deserved on drugs acting on the glutamatergic modulation.

101

**Oral festination in Parkinson's disease.** C. Moreau, C. Ozsancak, J. L. Blatt, P. Derambure, A. Destée, L. Defebvre, CHRU de Lille (Lille, F)

Background: Festination is a tendency to speed up in parallel with a loss of normal amplitude of quick repetitive movement especially in gait, handwriting and speech, encountered in Parkinson's disease (PD). Freezing is a breakdown of repetitive voluntary movement emerging through festination or suddenly. The neural mechanism and pathology of festination is still unclear. Some authors believe that parkinsonian tremor may pace voluntary repetitive movements to go faster than intended while others suggest that festination and freezing are two close phenomena, related to akinesia.

Objective: To evaluate the relation between festination of speech and freezing of gait and tremor.

Subjects and methods: Ten healthy volunteers and 4 patients with PD were included. By means of an optoelectric movement analysis system, the displacement of the jaw during repetitions of the syllable /pa/ were recorded. Subjects were asked to synchronize labial diadochokinesis to sequences of periodic acoustic stimuli (1–7 Hz). Patients were considered to have oral festination if their executed frequency (EF) was higher than the EF of the volunteers + 2 standard deviations for at least one imposed frequency (IF).

Results: Healthy volunteers succeeded to synchronize their performance with the external cue up to 6 Hz. Variability of the performance during the trial was slight. In the parkinsonian group, two patients had oral festination: one when rhythm was over 2 Hz and the second over 3.5 Hz. The festination was accompanied with a greater variability of labial diadochokinesis. Freezing unabled them to reproduce IF > 5 Hz. The two other patients had normal mean and SD for EF up to 6 Hz for one and 7 Hz for the other. There was no difference of age or duration of the disease between the two types of patients. Patients with oral festination had severe freezing of gait, dysarthria and no tremor.

Conclusion: These preliminary results suggest that oral festination is related to the freezing phenomenon. It is probably secondary to akinesia and not due to an internal tremor pace maker.

# Session 17

## Neurogenetics – 1

102

**Homozygosity for CAG mutation in HD is associated with a more severe clinical course.** F. Squitieri, C. Gellera, M. Cannella, C. Mariotti, G. Cislaghi, D. Rubinsztein, E. Almquist, D. Turner, A. Bachoud-Levi, S. Simpson, M. Delatycki, V. Maglione, M. Hayden, S. Di Donato, IRCCS Neuromed, Istituto Neurologico C. Besta, Magenta Hospital, Cambridge Institute for Medical Research, Dept Medical Genetics, Dept Haematol Genet Pathol, Service de Neurologie, Childrens' Hospital, Murdoch Institute (Pozzilli, Milan, Magenta, I; Cambridge, UK; Vancouver, CAN; Adelaide, AUS; Creteil, F; Aberdeen, UK; Melbourne, AUS)

Huntington disease (HD) is an autosomal dominantly transmitted disorder characterized by motor, mood and cognitive signs caused by an expansion mutation beyond 36 CAG repeats in the IT 15 gene that is believed to confer a toxic gain-of-function on the mutant protein. Because patients homozygous for HD receive the gain-of-function mutation in a double dose, one would expect a more toxic effect in homozygotes than in heterozygotes, similarly to other poly(CAG) diseases. HD is widely believed, however, to be one of the rare genetic diseases that manifests "complete dominance", hence indistinguishable in homozygotes and heterozygotes. To compare clinical data in homozygotes and heterozygotes, we selected 8 homozygotes and a cohort of 75 heterozygous patients for all of whom data were available on disease course, age at onset, expanded CAG repeat number, and clinical decline since the onset of disease. Motor symptoms and behavioural changes were assessed clinically with the Unified Huntington's Disease Rating Scale (UHDRS). Disability score was rated with standard scales for disability, independence, and functional capacity. The disease stage was calculated according to the Total Functional Capacity (TFC) score. Disease progression was measured in units per year by the disability scores and TFC.

The age at onset of symptoms in the homozygote cases was within the range expected for heterozygotes with the same CAG repeat lengths, whereas homozygotes had a more severe clinical course. More rapid deterioration was evident by measures of neurological function and functional independence. In fact all homozygous patients reached a severe disability in a shorter time and displayed a significantly larger rate of TCF decline, in each disease stage, than heterozygotes. Also, a wider spectrum of neurological symptoms other than chorea was seen at onset in homozygote. The observation of a more rapid decline in motor, cognitive and behavioural symptoms in homozygotes was consistent with the extent of neurodegeneration as available at imaging in three patients and at the post-mortem neuropathological report in one case.

Our analysis suggests that though homozygosity for the HD mutation does not lower the age at onset of symptoms it affects the phenotype and the rate of disease progression. These data, once confirmed in a larger series of patients, point to the possibility that the mechanisms underlying age at onset and disease progression in HD may differ.

One possible explanation for the discrepancies found between homozygotes and heterozygotes with comparable CAG expansions can be that the polyglutamine mutation in huntington not only causes a toxic gain of function but also abolishes a putative protective function of the wild-type protein on neuronal survival. In this hypothesis, the homozygous condition would mimic a "recessive" loss of wild-type huntington function, in addition to the dominant toxic effect of the mutation.

103

**Huntington's disease like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes.** G. Stevanin, H. Fujigasaki, A. Lebre, C. Dodé, R. Bellance, A. Durr, A. Brice, INSERM U289, Hopital Cochin, CHU de Fort de France (Paris, Martinique, F)

We report a group of 252 Huntington's disease-like (HD-L) patients, including 60 with typical HD, who had tested negative for pathological expansions in the IT15 gene, the major mutation in Huntington's disease. They were screened for repeat expansions in two other genes involved in HDL phenotypes; those encoding the junctophilin-3 (JPH3/HDL2) and prion (PRNP/HDL1) proteins. In addition, because of the clinical overlap between patients with HDL disease and autosomal dominant cerebellar ataxia or dentato-rubro-pallido-luysian atrophy (DRPLA), we investigated trinucleotide repeat expansions in genes encoding the TATA-binding protein (TBP/SCA17) and atrophin-1 (DRPLA).

Two patients carried 43 and 50 uninterrupted CTG repeats in the JPH3 gene. Two other patients had 44 and 46 CAA/CAG repeats in the TBP gene. Patients with expansions in the TBP or JPH3 genes had HDL phenotypes indistinguishable from HD. Taking into account patients with "typical HD", their frequencies were evaluated to be 3 % each in our series of typical HDL patients. Interestingly, incomplete penetrance of the 46 CAA/CAG repeat in the TBP gene was observed in a 59 year old transmitting but healthy parent and his severely affected children with repeats of the same size. Furthermore, we report a new configuration of the expanded-TBP allele, carrying 11 repeats on the first stretch of CAGs. Expansions in the DRPLA gene and insertions in the PRNP gene were not found in our group of patients. Further genetic heterogeneity of HDL phenotype therefore exists.

104

**The large clinical spectrum of eIF2B mutations: from infant to adult white matter disorders.** A. Fogli, D. Rodriguez, E. Eymard-Pierre, E. Bertini, M. Pineda, R. Surtees, G. Uziel, E. Malaspina, M. Troncoso, R. Schiffmann, O. Boespflug-Tanguy, INSERM U384, Hôpital A. Trousseau, Bambino Gesu Children's Hospital, Hospital San Joan De Deu, Institute of Child Health, Carlo Basta Institute, Instituto di Clinica Padiatica, Hospital Clinico San Borja Arriaran, National Institute of Health (Clermont-Ferrand, Paris, F; Rome, I; Barcelona, E; London, UK; Milan, Bologna, I; Santiago, CL; Bethesda, USA)

The eucaryotic Initiation Factor 2B (eIF2B), constituted of 5 subunits (alpha to epsilon) is involved in the first step of protein synthesis and is highly regulated under cellular stress conditions like head trauma or fever. Mutations in the five genes, encoding these five eIF2B subunits, have been reported as the cause of autosomal recessive form of leukodystrophy, described as Childhood Ataxia with diffuse Central nervous system Hypomyelinisation (CACH) or Vanish White Matter (VWM) syndrome (Leegwater et al. 2001, Van der Knaap et al. 2002).

We searched for EIF2B mutations in a series of 76 patients (from 50 families) who had heterogeneous clinical symptoms but selected on the basis of neuroimaging abnormalities suggestive of CACH/VWM: diffuse abnormal signal of the cerebral white matter resembling the intensity of CSF on T1 and T2 weighted imaging. Mutations were found in 74 patients, more frequently in the epsilon (62 %) than in the beta (19 %) delta (15 %) and gamma (4 %) subunits, none in the alpha subunit. For 28 patients, clinical presentation was typical of CACH syndrome with a childhood onset (2–10 years), a progressive ataxia-diplegia with additional episodes of rapid deterioration following febrile infection or minor head trauma leading to death after 2 to 10 years of disease evolution. In all cases, extensive cavitations of the white matter were found on MRI using proton density or FLAIR sequences. Six patients presented a severe, fatal leucoencephalopathy with an onset before one year of age and death in 1 to 6 months. In contrast, 43 patients had an onset after 10 years of age with frequent school difficulties and behavioral problems as first reported symptoms. One patient had normal neurological and cognitive functions at 16 years of age when the occurrence of recurrent headaches led to the incidental discovery of white matter abnormalities on cerebral MRI. In 3 patients cognitive and motor disabilities appeared late (25–30 years). One had a transient episode of visual loss and one suffered from frequent and severe headache since the age of 31 years and died of epileptic status at 32 years. No genotype was strictly correlated with the severity of the disease, however patients carrying this R113H mutation tend to have a milder form of the disease with a juvenile onset form, suggesting that the R113H mutation has less deleterious effects.

In conclusion, among the large heterogeneous group of undetermined leukodystrophies, MRI analysis can help to select patients for EIF2B mutation. Further analyses are needed to understand the large spectrum of clinical presentation found in eIF2B related disorders.

105
**SCA genes analysis and CAG/CTG repeat expansion detection (RED) in 225 Italian families with hereditary spinocerebellar ataxia.** C. Gellera, A. Brusco, A. Saluto, C. Cagnoli, A. Castucci, C. Michielotto, C. Mariotti, V. Fetoni, N. Migone, S. Di Donato, F. Taroni, Istituto Nazionale Neurologico C. Besta, University of Turin, Predabissi Hospital (Milan, Turin, I)

The autosomal dominant spinocerebellar ataxias (ADCA) are a clinical and genetically heterogeneous group of progressive neurodegenerative diseases related to nineteen loci (SCA1–8, SCA10–17, 19, 21 and DRPLA). The relevant gene has been identified in ten cases (SCA1–3, 6–8, 10, 12, 17, DRPLA), all mutated through the expansion of a repeat sequence. We studied the relative prevalence of known SCA genes in a group of 225 Italian families with hereditary spinocerebellar ataxia. The large majority [190] presented a dominant transmission of the trait. SCA1 and SCA2 represent the majority of cases (21 % and 24 %). Though considered absent in Italy, we found two families with SCA3 and one with DRPLA. The frequency of these forms is very low but comparable to that found for SCA6–7–8 and 17; overall non SCA1–2 represent about 6 % of our cases, while SCA10 and 12 seem not to be present in Italy. Two homozygous expanded alleles were found in one SCA8 family. Notably, we found two SCA17 patients carring a 45-repeat disease allele, the shortest so far reported, and two patients with a 43-triplet allele, whose involvement in the pathology is still to be elucidated. Half of our familial cases (113/225) do not have expansions at known SCA genes. RED analysis on 110 patients from this group showed 20 % (22/110) of cases carrying more than 50 CAG/CTG repeats. All these expansions but one, could be tracked back to the ERDA1 (19 cases) or CTG18.1 (2 cases) polymorphic loci, which are irrelevant for the pathology. The remaining positive case, showing 60-repeat expansion at RED analysis, was considered of potential pathological relevance. The proband was a 46-yr-old woman who had manifested at 35 yr with a progressive cerebellar syndrome associated with cognitive impairment and choreoathetosis. Her sister had a similar phenotype. More careful examination of the family revealed that her two sons had been diagnosed as having cryptogenic focal epilepsy. This feature prompted us to perform the analysis for DRPLA expansion, which indeed disclosed the presence of a pathological allele of 63 repeats. This result was perfectly consistent with RED analysis and confirmed the efficacy of the technique to identify unknown pathological expansions larger than 50 repeats. Although our data seem to exclude unknown CAG/CTG expansions as cause of unclassified ADCA, short or interrupted repeats, such as those of SCA6 and SCA17, may be found pathogenic in future studies.

106
**Evidence for a founder Spg4 mutation and genetic heterogeneity in unrelated Scottish adhsp families.** A. Orlacchio, T. Kawarai, A. Totaro, W. Meschino, P. St George-Hyslop, A. Errico, E. Rugarli, G. Bernardi, IRCCS Santa Lucia, University of Toronto, North York General Hospital, TIGEM (Rome, I; Toronto, CAN; Naples, I)

Several causative genes for autosomal dominant hereditary spastic paraplegia (ADHSP) have been identified. Analyses of genotype/locus-phenotype correlations have revealed genetic and phenotypic heterogeneity in ADHSP. We report linkage and sequence analysis of the currently-known ADHSP causative genes in fifteen unrelated ADHSP families originating from Southern Scotland. Nine ADHSP families in our collection were linked to the SPG4 locus at 2p21-p24, where Spastin has been identified as the causative gene. Sequence analysis of the Spastin gene revealed a novel mutation in all of these nine families. Haplotype analysis suggested the existence of a common founder. Magnetic resonance image examination demonstrated unique features including thin corpus callosum and atrophy of cerebellum in patients. Expression of the mutant Spastin in transfected cells showed constitutive binding to microtubules and induced a redistribution of the microtubule cytoskeleton, as previously observed for other missense mutations located in the AAA domain. Linkage and sequence analyses excluded linkage to the currently-known ADHSP loci in the remaining six families, showing further genetic heterogeneity.

107
**Mutations in the paraplegin gene (SPG7) in patients with sporadic or autosomal recessive spastic paraplegia are associated with a clinically distinct phenotype.** F. Taroni, C. Mariotti, G. Casari, S. Baratta, C. Milanese, C. Gellera, S. Di Donato, M. Mora, L. Chiapparini, Istituto Nazionale Neurologico C. Besta, TIGEM (Milan, Naples, I)

Hereditary spastic paraparesis (HSP) is a group of heterogeneous neurodegenerative diseases, classified on the basis of clinical features, mode of inheritance, and genetic mapping. Clinically, they are classified into "pure" and "complex" forms (Harding, 1981). To date, 10 loci for autosomal dominant HSP, 3 loci for X-linked HSP and 6 loci (SPG5, SPG7, SPG11, SPG14, SPG15, and SPG20) for autosomal recessive HSP (AR-HSP) have been mapped in HSP pedigrees. However, only 6 autosomal and 2 X-linked disease-genes have been thus far identified. SPG7, encoding a mitochondrial protein named paraplegin, and SPG20, encoding spartin, are the only cloned genes associated with autosomal recessive HSP. Mutations in the paraplegin gene have been thus far reported in four families. In this study, we screened for paraplegin mutations a series of 28 unrelated patients, presenting with sporadic or autosomal recessive HSP. We have identified 1 frameshift, 1 nonsense and 2 missense pathogenic mutations in 5 cases (17.8 %). Age at onset ranged from 25 to 55 years. The study of the clinical features indicates that paraplegin mutations are associated with a slowly progressive neurodegenerative disorder, characterized by either pure spastic paraparesis, or, more frequently, by a complex form of the disease, in which cerebellar ataxia is the prominent sign. MRI studies confirmed the main involvement of the cerebellar structures. Skeletal muscle investigations and MR spectroscopy did not reveal morphological or biochemical abnormalities caused by defects of mitochondrial respiratory chain enzymes. In conclusion, our data indicate [1] that the frequency of mutations in the paraplegin gene may be higher than previously observed and [2] that paraplegin mutations appear to be predominantly associated with a "complex" phenotype.

# Session 18

## Neurogenetics – 2

108
**Clinical and genetic heterogeneity in autosomal progressive external ophthalmoplegia due to polymerase gamma-alpha subunit mutations.** M. Mancuso, M. Filosto, Y. Nishigaki, J. Pancruso, E. Bonilla, S. Shanske, M. Hirano, S. DiMauro, Columbia University (New York, USA)

Background: autosomal progressive external ophthalmoplegia (PEO) with mitochondrial DNA (mtDNA) multiple deletions has been linked to mutations in three genes involved in mtDNA maintenance or replication: adenine nucleotide translocator 1 (ANT-1), Twinkle and polymerase gamma (POLG). Mutations in POLG cause dominant or recessive PEO and are often associated with multisystemic disorders.

Objectives: to further investigate the frequency and genotype/phenotype correlations of mutations in the POLG gene, we screened 12 patients with PEO and mtDNA multiple deletions, but no mutations in ANT-1 and Twinkle genes.

Methods: we screened POLG by SSCP analysis and direct DNA sequencing of samples with abnormal SSCP patterns. The mutations were confirmed by RFLP analysis.

Results: mutations on POLG were found in 4 patients (33 %). The first had PEO and mental retardation and harbored a new heterozygous Gly1076Val mutation. The second patient had PEO and neuropathy, and is a compound heterozygous for Ala889Thr and Arg579Trp mutations. Two more patients, one with PEO and exercise intolerance and one with PEO, neuropathy, deafness and hypogonadism, harbored the same new mutation, an heterozygous Pro587Leu change. All the mutations were absent in 120 control alleles.

Conclusions: our results show the diseases associated with POLG mutations are both clinically and genetically heterogeneous. This study confirms that POLG mutations account for a significant proportion of patients (33 %) with PEO and mtDNA multiple deletions and suggests that defects in other genes must be involved in the etiology of this syndrome.

109
**A novel mutation in the spastin gene in an Italian family with hereditary spastic paraplegia.** A. Orlacchio, T. Kawarai, S. Merlo, A. Totaro, P. St George-Hyslop, G. Bernardi, IRCCS Santa Lucia, University of Toronto (Rome, I, Toronto, CAN)

Background: Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative disorders, characterised principally by slowly progressive weakness and spasticity of the lower extremities. To date, ten loci have been mapped in the autosomal dominant (AD) form of HSP and five causative genes have been identified including SPASTIN for the SPG4 locus.

Methods: Linkage and sequence analyses were used to discover the position and the nature of the genetic defect causing the disease in a large Italian pedigree with spastic paraplegia and additional neurological features including arachnoid cysts of the cerebellopontine angle, pes cavus and mental retardation inherited as an AD trait. The 17 coding regions and flanking intronic sequences of SPASTIN were analyzed by direct sequencing and compared between affected and normal individuals.

Results: The disease phenotype was associated to the SPG4 locus and all the other known AD loci were excluded for linkage. A novel missense mutation was found in the exon 17 of the SPASTIN gene.

Conclusions: A novel mutation in the SPASTIN gene was identified in a large Italian family with HSP and unique additional neurological features, suggesting a further clinical heterogeneity in SPASTIN mutations.

110

**Phenotype characteristics of metachromatic leukodystrophy with homozygosity for arylsulfatase A mutation P426L.** H. Rauschka, J. Berger, N. Baumann, J. Turpin, K. L. Lamers, R. A. Wevers, H. Bernheimer, M. Schmidbauer, Hospital Lainz, University Vienna, Salpetriere Hospital, University Hospital Nijmegen, General Hospital Vienna (Vienna, A; Paris, F; Nijmegen, NL)

Metachromatic leukodystrophy (MLD) is caused by genetic deficiency of arylsulfatase A (ASA). Mutations encoding inactive ASA cause early onset MLD, mutations encoding ASA with residual activity (R-alleles) cause late onset. Carriers of the R-allele I179S have been suggested to start with psychiatric disturbances, whereas the R-type mutation P426L seems to cause neurological symptoms at onset. To characterize the phenotype, we retrospectively studied the clinical presentation of 17 cases (including 3 pairs of siblings) homocygous for P426L. In addition, 4 cases of the literature homocygous for P426L, were included.

Neuroradiological examination showed diffuse supratentorial leukoencephalopathy in each case. Peripheral nerve conduction velocity was markedly reduced in all 19 symptomatic cases, the mean age at onset was 20 years (range 10 to 28). In 2 cases, which were still asymptomatic at the age of 14 and 29, electroneurography was not done. In 14/19 patients gait disturbance due to spastic-atactic paraparesis was predominant at presentation, whereas psychiatric symptoms were mild or absent. In the course of the disease mental deterioration got generally prominent and optic atrophy and various disturbances of brainstem function were occasional findings. In 2 further cases diminishing visual acuity due to optic nerve atrophy was predominant, but only 3/19 patients presented with pure psychiatric illness, although in every case a spastic-atactic syndrome developed in the years after onset.

In summary, we could show that MLD due to P426L homocygosity predominantly manifests with neurological symptoms in puberty or adult age, and, despite marked phenotype variability, the clinical core syndrome is a progressive spastic-atactic paraparesis.

111

**Clinical-molecular heterogeneity in mitochondrial sensorineural hearing loss.** F. Forli, S. Berrettini, A. Rocchi, M. Mancuso, G. Siciliano, University of Pisa (Pisa, I)

Several studies have indicated that a number of different mitochondrial DNA (mtDNA) mutations may be responsible for human pathologies. Sensorineural hearing loss (SNHL) may be associated with known syndromes (syndromal SNHL) or represent the only manifestation of mitochondrial damage (non-syndromal hearing loss). Moreover, mtDNA alterations may be responsible for aminoglycoside-induced deafness. In a group of 60 patients affected with idiopathic progressive SNHL, submitted to our protocol of study for progressive SNHL, we performed molecular analysis of mtDNA and found mtDNA alterations in five patients, one mtDNA single deletion and four mtDNA point mutations.

The patient with the mtDNA single deletion in skeletal muscle was affected with progressive SNHL of unknown origin, which started with a sudden adult-age onset bilaterally, although non-simultaneously and that was partially responsive to corticosteroids. Of the patients affected with mtDNA point mutations, three presented the A1555G mutation in peripheral leukocytes, and one the A3243G in skeletal muscle. One of the patients harbouring the A1555G point mutation was a 65 year old woman, affected with bilateral profound SNHL, started at age 24, after a treatment with streptomycin and slowly progressed over the following 40 years. It is notable that two out of her three daughters were also affected with SNHL of moderate entity and one of them presented the hearing loss after treatment with aminoglycosides antibiotics. The second patient affected with the A1555G mutation was a 33 year old woman, affected with a moderate SNHL started at age 15 and progressively worsened in the following years, with no history either for familiar deafness or ototoxic drugs assumption. The third patient with the A1555G point mutation was a young woman, affected with a moderate SNHL from her childhood, whose little daughter was affected with a severe bilateral congenital SNHL. The A3243G point mutation was detected in a 75 year old man, affected with a 20 years history of progressive SNHL, who was submitted to cochlear implantation. Family history was negative for deafness and clinical examination was normal except for a slight bilateral weakness of quadriceps. Molecular analysis on muscle specimen showed the mutation in a very low proportion in patient's muscle (3%) and urine sediment (< 1%), while the mutation was not detectable in blood and oral mucosa cells.

Our findings demonstrate that SNHL due to mtDNA mutations is not a rare event: it is generally progressive but may have a quite variable clinical presentation as regards the degree of hearing loss (one patient had a normal hearing function), the age at onset, family history, the eventual association with neurological dysfunction or diabetes. This can underline the importance to perform mitochondrial DNA analysis in cases of SNHL of unknown origin.

112

**Molecular pathogenesis of Friedreich's ataxia: is human frataxin a storage protein for mitochondrial iron?** V. Seveso, S. Levi, S. Consigli, P. Arosio, F. Taroni, Istituto Nazionale Neurologico C. Besta, Istituto Scientifico S. Raffaele, University of Brescia (Milan, Brescia, I)

Friedreich's ataxia (FRDA) is an autosomal recessive relentlessly progressive neurodegenerative disorder. It is the most common hereditary ataxia and is caused by a deficiency of frataxin, a 210-aa nuclear-encoded mitochondrial protein. Studies in both yeast and mammals have suggested that frataxin may play a critical role in mitochondrial iron homeostasis and free radical toxicity. It has been reported that purified recombinant mature yeast frataxin homologue Yfh1p may assemble in vitro in a macromolecular spherical complex of approx. 60 subunits and approx. 1.1 MDa, following the aerobic addition of iron (Adamec et al. 2000). These higher-order multimers would sequester iron maintaining it in a soluble, available, and non-toxic form. Similar role and similar structure have been proposed for the human counterpart. These observations prompted to hypothesize that frataxin may play a major role in the handling and storage of iron within mitochondria.

We have investigated the presence in vivo of frataxin macromolecular complexes and the iron binding capability of human frataxin by using 2-dimension blue-native electrophoresis (2D-BNE). Several cell lines (human HeLa cells, murine motor neurons, and human normal and patient-derived lymphoblasts) and rat heart were analysed. Both frataxin and control proteins were revealed by immunoblotting. Following 1stD-BNE, only a strong signal at low molecular weight (< 50 kDa) was detected, with no evidence of high-MW species. This observation was confirmed in the 2nd-D, where only the 16-kDa monomer could be detected. Culturing cells under iron overload conditions did not appear to promote the formation of high-MW complexes. Interestingly, the only experimental condition associated with detection of high-MW frataxin was when the human protein was overexpressed in simian COS1 cells. Under both normal iron and iron overload conditions, the most abundant form of frataxin was still a low-MW species, but a weak signal corresponding to a high-MW product could be observed, suggesting that some frataxin aggregation may occur under non-physiological condition at high protein concentration levels. To investigate the in vivo iron binding capability of human frataxin, HeLa cells were metabolically labeled with 55 Fe overnight and analysed by 2D-BNE. Autoradiography showed a single 55 Fe-containing band > 500 kDa, the intensity of which was not affected by immunoprecipitation experiments with saturating amounts of anti-human frataxin Ab. By contrast, immunoprecipitation with anti-cytosolic ferritin Ab resulted in a marked decrease of the radioactive band. These results were confirmed by quantitative analysis. In conclusion, our data do not lend support to the hypothesis that frataxin may act as a ferritin-like iron storage protein in the mitochondria, a role that could more likely be played by a recently identified mitochondrial ferritin (Levi et al. 2001). (Supported by a Telethon-Italia grant to FT)

# Session 19

## Multiple Sclerosis – 3

113
**The Multiple Sclerosis Database Network. An Italian source of valuable data for clinical and research studies.** M. Trojano, E. Granieri, G. Rosati, G. Savettieri, P. Livrea, G. Comi, University of Bari, University of Ferrara, University of Sassari, University of Palermo, Ospedale San Raffaele Università Vita e Salute (Bari, Ferrara, Sassari, Palermo, Milan, I)

Objective: To establish, in Italy, a Multiple Sclerosis (MS) database network (MSDN) to collect essential medical and demographic information of MS patients for clinical and research purposes.

Background: Standardised monitoring of large heterogeneous groups of patients with MS can provide important natural history information, and it is crucial to assess the long-term safety and effectiveness of currently used disease-modifying drugs (DMDs). In Italy, according to the prevalence rate, about 45,000–50,000 individuals are affected by MS; 35,000 of them visit one of the 200 MS centres.

Design/Methods: The MSDN is based on a new, user-friendly, electronic MS Patient Monitoring System (iMed) recently developed by Serono. Twenty-six specialized MS centres were selected in Italy on the basis of competence, experience and geographical distribution. Each was provided with a computer, ISDN line and smart card. Substantial staffing and technical support is available for retrospective and prospective data analysis. A designated scientific committee was responsible for the approval/validation of the database, training of data entry personnel, confirmation and analysis of data consistency, and publication policy. General clinical and demographic details are being collected for all patients (e. g. age, disease type/duration, relapse rate, functional system(s) [FSs] involved at onset, Expanded Disability Status Scale [EDSS] score, treatment type/duration). Patients are followed up every 6 months.

Results: Data collection began in July 2001. By February 2003, 10,078 MS patient records were available. Mean and median ages were 40 and 38.9 years, respectively; 65 % of patients were female, 75 % had relapsing-remitting (RR), and 15 % and 10 % had secondary (SP) and primary progressive (PP) course. Mean disease duration was 10 years, mean and median EDSS score were 3.1 and 2.5, respectively; 66 % of patients showed a monosymptomatic onset involving optic nerve (20 %) and brainstem (15 %), mainly. Oligoclonal IgG bands restricted to cerebrospinal fluid and MRI T2 lesions were found in 83 % and 90 % of patients. In 2.5 % of patients other autoimmune diseases were associated to MS. Sixty-eight % of patients received DMDs, in 41 % of them IFN-beta was administered. The mean progression index (EDSS/duration) of IFN-beta treated patients resulted significantly ($p < 0.01$) lower than that in untreated group. Being male ($p < 0.05$) and having longer disease duration, older age, PP course and a lower number of FSs involved at onset were all significantly ($p < 0.0001$) associated with a poor prognosis.

Conclusions: MSDN is the first Italian MS registry, and one of the largest MS data-base in Europe. It provides a valuable source of natural history information and long-term DMD safety and effectiveness data in MS.

Study supported by Cesare Serono Foundation.

114
**The epidemiology of multiple sclerosis in Ireland: does a latitudinal variation in prevalence exist?** C. McGuigan, A. McCarthy, C. Quigley, L. Bannan, S. Hawkins, M. Hutchinson, St. Vincent's University Hospital (Dublin, IRL)

Objectives: To compare the prevalence of MS in two Irish counties: Donegal in the north west of the island and Wexford in the south east.

Background: Northern Ireland has a high and rising prevalence rate of multiple sclerosis (MS). The most recent survey in 1996 found a rate of 168.7/100,000. Recorded prevalence rates for the south of Ireland, including County Wexford, have been markedly lower and seemed to suggest the existence of a prevalence gradient within the island.

Methods: Patients with clinically definite or probable MS (Poser criteria) who were resident within the county borders on the 1st January 2001 were considered prevalent cases for the study. Sources of case ascertainment included a postal survey of General Practitioners, County Physicians, Consultant Neurologists, respite facilities and local MS charities. Hospital coding lists and interferon prescription lists were also reviewed. Review of clinical case records and/or patient examination confirmed the diagnosis of MS.

Results: In County Donegal, 240 prevalent cases were identified giving a prevalence rate of 184.6 per 100,000 (95 % confidence interval: 162–209.5). In Wexford there were 126 prevalent cases resulting in a prevalence rate of 120.7 per 100,000 (95 % confidence interval: 100.5–143.7). The difference in prevalence rates is statistically significant ($Z = 3.94$, $p = < 0.001$).

Conclusions: There is a latitudinal variation in the prevalence rate of MS between the north of Ireland and the south. The north-south variation in prevalence may represent a variation in the genetic predisposition to MS between the background populations of the two counties.

115
**Computer-aided retraining of memory and attention in people with multiple sclerosis: results of a randomized controlled trial.** A. Solari, A. Motta, C. Pozzilli, G. Mancardi, M. Forni, E. Pucci, L. Mendozzi, for the CRIMS trial

Background: Cognitive dysfunctions are considered among main contributing factors to activity and participation restrictions in people with multiple sclerosis (MS). Computer-aided re-training programs have been used to improve memory and attention in people with MS, but their efficacy has not been conclusively demonstrated.

Objective: We assessed the efficacy of computer-aided re-training of memory and attention in MS outpatients in a randomised, double-blind, controlled study.

Methods: Seventy nine MS outpatients with mild to moderate cognitive impairment were treated on an individual basis for 45 minutes, twice a week for eight consecutive weeks. The experimental treatment consisted of the following Rehacom procedures: topological memory, and attention and concentration. The control treatment consisted of the visuo-constructional ability, and visuo-motor coordination procedures. The primary outcome criterion was the effect of treatment on cognitive impairment as measured by the Brief Repeatable Battery of Neuropsychological Tests (BRBNT). Secondary efficacy endpoints were changes in mood, and in health-related quality of life. The examining clinician, blinded to treatment assignment, administered the neuropsychological test battery at baseline and at weeks 8 and 16.

Results: With regard to the primary outcome measure, a greater than 20 % improvement in at least two BRBNT test scores occurred in 54 % of the control patients vs. 50 % of the study patients ($p = 0.72$). Figures at 16 weeks were 59 % (control patients) vs. 45 % (study patients) ($p = 0.21$). The study treatment was better than the control intervention only on the word list generation test at eight ($p = 0.002$) and 16 weeks ($p = 0.001$). Changes in secondary outcomes were also unaffected by treatment assignment.

Conclusions: The outcome measures showed no significant benefit of computer-aided re-training of memory and attention. Two previously-published studies had more encouraging findings, however both studies were open to bias: one had no control treatment (Plohmann 1998), and the other was a randomized controlled trial characterized by a multiplicity of outcomes (11 tests) and absence of blinding (Mendozzi 1998). Possible drawbacks of our trial are limited power, and a non-specific treatment effect; however the magnitudes of between group differences were small and often not in the predicted direction, so it is unlikely that the study failed to reveal a real benefit of the active intervention.

Study supported by the National Multiple Sclerosis Society (grant PP0798 to A. Solari)

116
**Simple and complex movement-associated functional MRI changes in patients at presentation with clinically isolated syndromes suggestive of MS.** M. Rocca, D. Mezzapesa, A. Ghezzi, A. Falini, V. Martinelli, G. Scotti, G. Comi, M. Filippi, Neuroimaging Research Unit, Ospedale di Gallarate, Department of Neuroradiology, Department of Neurology (Milan, Gallarate, I)

Although functional adaptive cortical changes have been detected in patients with established multiple sclerosis (MS), it is less clear how early in the course of the disease does this functional reorganisation occur and which are the pathological mechanisms underlying it. We performed this study to investigate, using functional magnetic resonance imaging (fMRI) and a general search method, whether movement-associated functional changes of the brain are present in patients that, most likely, are at the earliest stage of MS.

fMRI exams during the performance of three simple and one more complex motor tasks with fully normal functioning extremities were obtained from 16 patients at presentation with clinically isolated syndromes (CIS) suggestive of MS and paraclinical evidence of lesion dissemination in space. Fifteen sex- and age-matched healthy volunteers served as controls. fMRI analysis was performed using statistical parametric mapping (SPM99).

Compared to healthy volunteers, CIS patients had increased activations of the contralateral primary sensorimotor cortex (SMC), secondary somatosensory cortex (SII) and inferior frontal gyrus (IFG), when performing a simple motor task with the dominant hand. The increased recruitment of the contralateral primary SMC was also found during the performance of the

same motor task with the non-dominant hand and with the dominant foot. In this latter case, an anterior shift of the center of activation of this region was detected. During the performance of a complex motor task with the dominant upper and lower limbs, CIS patients had an increased recruitment of a widespread network (including the frontal lobe, the insula, the thalamus), usually considered to function in motor, sensory and multimodal integration processing. The comparison of brain activations during the performance of simple vs. complex motor tasks showed that the movement-associated somatotopic organisation of the cerebral and cerebellar cortices was retained in patients with CIS.

This study shows that cortical reorganisation does occur in patients at presentation with CIS highly suggestive of MS. It also suggests that local synaptic reorganisation, recruitment of parallel existing pathways and reorganisation of distant sites are all likely to contribute to the genesis of cortical adaptive changes since the earliest phase of MS.

117

**The prevalence of previously unrecognized depression in a community-based population with multiple sclerosis.** C. McGuigan, A. McCarthy, M. Hutchinson, St. Vincent's University Hospital (Dublin, IRL)

Objectives: To assess the prevalence of previously unrecognized depression in a community-based population with multiple sclerosis (MS) and examine the influence of potential contributing factors.

Background: Depression has been reported as a common symptom in patients with multiple sclerosis with a lifetime prevalence of up to fifty percent. Depression is more common amongst people with multiple sclerosis compared with similar age/sex matched controls with other long-term disabling conditions. Despite the frequent existence of mood difficulties, depressive symptoms are often concealed by patients and therefore often remain unrecognized and untreated.

Design/Methods: During the course of an epidemiological study on two counties in Ireland 367 people with MS (Poser diagnostic criteria) were identified. Patients with no prior history of clinical depression were enrolled in this study. Each patient was invited to attend interview with the primary author. Medical history and demographic information was recorded. Each patient had a full neurological examination. A kurtzke Expanded disability status score (EDSS) was applied and a multiple sclerosis functional composite score (MSFC) assessed. Participants also completed a Beck's Depression Inventory (BDI-II) questionnaire.

Results: One hundred and seventy five patients participated fully in the study and were included in the final analysis. The average age of participants was 43.6 years [19–69], male: female ratio – 1: 3.1, years since onset of MS 13.2 years, average EDSS 4.32 (range 0–9). Forty patients (22.8%) scored greater than 20 on the BDI-II and therefore were classified as having moderate/severe depression as assessed by the BDI-II. The existence of depression was independent of age, sex, duration of illness, disability level, social circumstances, treatment regimes, disease sub-type, physical symptoms and employment status.

Conclusions: This study concludes that up to one quarter of community based patients with MS in Ireland are suffering from unrecognized, and therefore untreated, depression as measured by the BDI-II. Disease severity etc. are not good indicators of the likelihood of developing depression. As health care professionals we need to be more active in assessing the mental state of patients with MS and treating appropriately.

118

**Automated brain MR segmentation in a large MS population.** G. Tedeschi, V. Bonavita, P. Livrea, C. Messina, A. Quattrone, G. Savettieri, B. Alfano, Federico II University of Naples, University of Bari, University of Messina, University of Catanzaro, University of Palermo, National Research Center (Naples, Bari, Messina, Catanzaro, Palermo, I)

Background and aims. In multiple sclerosis (MS), measures of lesion load and brain atrophy have been performed by a number of methods. The automated MR segmentation method (Alfano et al. MRM 1997) used herein allows an objective measure of lesion load (including T1, T2 and PD abnormalities), as well as of brain atrophy. The aims of the study were to measure lesion load and brain atrophy, and to validate the use of the automated brain MR segmentation method in a large population of MS patients. This was done by moving the MR machine to different locations and performing the same MR procedure on each individual.

Materials and methods. Eight MS centers from Southern Italy participated in the study. Inclusion and clinical evaluation criteria were kept at a very basic level, to achieve a fair concordance between MS clinicians. Diagnosis was based on McDonald's criteria (Ann Neurol, 2001). The main patient features taken into account were: age, age at disease onset, disease duration, course of the disease, therapy, number of acute attacks in the previous two years. Clinical evaluation included EDSS, fatigue severity scale (FSS) and MMSE. 453 MS patients and 72 controls were included in the study. A 1T GE machine for standard clinical use, hosted in a lorry, was moved to the eight clinical sites. MR segmentation data were acquired by a double interleaved SE sequence, slice thickness of 4 mm; T1 weighted with short RT and ET, PD and T2 weighted with long RT and double ET. The whole acquisition time was 20 min. The acquired data were stored and automated segmentation was performed off line (20 sec per study) and the following measurements were obtained: abnormal white matter (AWM, cc), white matter fraction (WMf, %), global white matter fraction (GWMf, %), gray matter fraction (GMf, %) and cerebro spinal fluid fraction (CSFf, %).

Results. Controls did show age related brain atrophy, so an age correction factor for GMf and CSFf was applied to MS patients. AWM was significantly different (t-Test, $p < 0.001$) in MS patients vs. controls [mean and SD: 19.5 (24.4) vs. 0.19 (0.33). WMf was significantly reduced ($p < 0.001$) in MS patients 32.0 (4.1) vs. 35.2 (2.5). GWMf was significantly reduced ($p < 0.001$) in MS patients 33.5 (3.0) vs. 35.3 (2.4). GMF was significantly reduced ($p < 0.001$) in MS patients 51.1 (3.1) vs. 53.2 (1.7). CSFf was significantly reduced ($p < 0.001$) in MS patients 15.6 (5.2) vs. 11.5 (2.5). Significant correlations were found between all MR segmentation parameters, as well as between MR segmentation parameters and age at onset, EDSS, disease duration, FSS and MMSE.

Conclusions. Brain MR segmentation is a very sensitive method to detect differences in lesion load and brain atrophy between MS patients and controls. MS is characterized by the involvement of both white and gray matter. Correlations between MR segmentation parameters and clinical data will be discussed.

Foundation Cesare Serono provided financial support.

# Session 20

## Multiple Sclerosis – 4

119

**MMP-9 microsatellite polymorphism and multiple sclerosis.** N. Fiotti, R. Zivadinov, N. Altamura, D. Nasuelli, A. Bratina, M. A. Tommasi, A. Bosco, L. Locatelli, A. Grop, G. Cazzato, G. Guarnieri, C. Giansante, M. Zorzon, Department of Clinical Medicine, Neurological Clinic (Trieste, I)

Background: Matrix metalloproteinase 9 (MMP-9) plays an important role in multiple sclerosis (MS) pathophysiology. A polymorphism (PM) in the microsatellite (14–27 AC repeats) of the promoter region modulates its expression.

Objectives: To determine if MMP-9 microsatellite PM is associated with MS susceptibility and with clinical and magnetic resonance imaging (MRI) characteristics of MS.

Methods: In 95 MS patients and 95 age- and sex- matched controls, MMP-9 PM has been determined. In MS patients, clinical (age, age of onset, disease duration, EDSS) and MRI (brain parenchymal fraction, T1- and T2-lesion load) characteristics have been evaluated according to MMP-9 PM.

Results: Patients with MS had different patterns of microsatellite PM (i. e. higher numbers of CA repeats) than controls ($p < 0.0001$). Prevalence of > 22 CA repeats in at least one allele was higher in patients than in controls (OR 3.4, 95% CI 1.7–6.8, $p < 0.0001$). When the patients were stratified according to the number of CA repeats in the MMP-9 promoter region no differences were found in the main clinical and MRI disease activity outcomes. An earlier age at disease onset characterized patients with > 22 CA repeats (33 SD10 vs 28 SD10, $p = 0.027$).

Accordingly, in multiple regression analysis, the only variable associated with the presence of at least one allele with > 22 CA repeats in the MMP-9 promoter region was a younger age at disease onset ($R2 = 0.29$, $p = 0.023$).

Conclusions: A PM in the microsatellite (> 22 CA repeats) of the MMP-9 region is associated with an increased risk of MS and with disease onset at younger age.

120

**The prevalence and significance of pain in multiple sclerosis.** L. V. Kalia, P. W. O'Connor, University of Toronto (Toronto, CAN)

Objective: To compare pain in multiple sclerosis (MS) to that in the general population and in other chronic conditions. To assess potential relationships between pain in MS, disability, mood, and health-related quality of life (HRQOL).

Background: Pain is an under-appreciated symptom in MS and its relationship to gender, disability, mood, and HRQOL is unclear. Given the importance of this symptom for patients with other chronic conditions (e.g. arthritis), it is important to study pain in MS.

Methods: Ninety-nine patients with definite MS were approached and all consented to participate in the study. The group represented a consecutive sample of patients attending St. Michael's Hospital MS Clinic, University of Toronto, Canada. Participants completed a self-administered survey including Medical Outcomes 36-Item Short-Form Health Survey (SF-36), Hospital Anxiety and Depression Scale, and Short-Form McGill Pain Questionnaire. Neurologic disability was rated using Expanded Disability Status Scale (EDSS). MS patients and comparator general populations were age- and gender-matched. Comparisons between SF-36 scores were made using t tests. Associations between pain, disability, mood, and HRQOL were determined using Pearson rank correlations.

Results: Patients had a mean (SD) age of 41.5 (10.2) years and 59% were women. Mean (SD) disease duration was 10.8 (7.3) years with 51% of patients having relapsing-remitting MS. Pain was the initial MS symptom in 11.1% of cases. Point prevalence of pain in MS was 26.3%. For males, SF-36 Bodily Pain scores were not significantly different between MS patients and US ($p = 0.86$) and Canadian ($p = 0.99$) norms. However, mean Bodily Pain score for females with MS was 56.2 vs. 70.8 and 72.3 for normative US ($p = 0.003$) and Canadian ($p < 0.0001$) samples, respectively. Importantly, gender-adjusted Bodily Pain scores were not significantly different between MS patients and osteoarthritis patients ($p = 0.40$). Only 38.4% of MS patients were receiving treatment for pain and treated patients had significantly higher pain levels than untreated patients ($p = 0.001$). Using general linear models, we found pain in MS to have no significant relationship to age or disease subtype. Pain was more strongly correlated with measures of mental health, such as SF-36 Mental Health ($r = 0.44$, $p < 0.0001$) and Social Functioning ($r = 0.46$, $p < 0.0001$) scores, vs. measures of disability, including EDSS ($r = -0.11$, $p = 0.29$) and SF-36 Physical Functioning ($r = 0.19$, $p = 0.06$) scores. Pain was significantly related to mood for females ($r = -0.53$, $p < 0.0001$) but not for males ($r = -0.24$, $p = 0.14$) with MS.

Conclusions: Pain is common in MS patients with an average intensity that does not differ significantly from that of arthritis. More severe pain is more likely to be treated. Presence and severity of pain are not related to age, disease subtype or disability but are strongly associated with measures of mental well-being. (Supported by MS Society of Canada)

121

**Analysis of the Asp299Gly polymorphism in the toll-like receptor 4 (tlr4) gene in patients with multiple sclerosis.** A. Kroner, K. Toyka, P. Rieckmann, M. Mäurer, University of Wurzburg (Wurzburg, D)

Objective: To evaluate the genotype frequencies for the Asp299Gly polymorphism of the TLR4 gene in MS patients.

Background: Human Toll-like receptor 4 (TLR4) transduces proinflammatory cytokine release by human cells in response to lipopolysaccharide (LPS). It was shown that a human gene polymorphism in the fourth exon results in the amino acid substitution of the Asp299Gly in the extracellular domain of the TLR4 and causes reduced expression and function of TLR4. Multiple sclerosis patients often suffer disease deterioration during bacterial infection. In particular urinary tract infections with LPS-positive bacteria are a common problem, we therefore investigated the influence of the TLR4 mutation on disease course and severity.

Material and Methods: DNA was obtained from whole blood samples of 370 unselected patients with multiple sclerosis (mean age 39 ± 10 years, range 13–71). All patients were tracked in our MS outpatient clinic under highly standardised conditions. Disability was assessed using EDSS and the rate of accumulation of neurological deficit was expressed by the progression index (PI = EDSS/duration, years). The A/G transition in exon 4 (Asp299Gly) was analysed by a nick-translation PCR (ABI Prism 7700) capable to differentiate single nucleotide exchanges. Results were confirmed by PCR-restriction fragment length polymorphism (RFLP) using mismatch primers and automatic sequencing.

Results: Genotyping of the 370 MS patients revealed 333 (90%) individuals with the wild-type (A/A) genotype, whereas 36 (9,73%) individuals were heterozygous and only 1 (0, 27%) individual was homozygous for the mutant allele. This allelic distribution is in accordance with the results reported recently in healthy German individuals. Complete clinical data were available in 314 patients. Two hundred and ninety two (93%) had bout onset of disease, and 22 (7%) had a primary progressive disease course. No significant association of different Asp299Gly genotypes with clinical MS subtypes and disease severity measured with the PI was found. Individuals carrying the mutant allele had an earlier disease onset (26 vs. 29 years), however this difference was statistically not significant.

Conclusion: Within this German MS population we found no association between Asp299Gly genotypes and disease course of multiple sclerosis looking at basic disease parameters. Nevertheless in clinical practice an association of bacterial infection and disease deterioration is often found. Therefore it is reasonable to look for alterations of the innate immunity in MS patients.

122

**The effect of corticosteroid on conduction in the visual pathways. A serial study using visual psychophysics.** E. M. Pye, S. J. M. Weatherby, D. Kesson, D. H. Foster, C. P. Hawkins, North Staffordshire Royal Infirmary for the Keele MS Research Group

Background: Acute relapses of MS are characterised by episodes of neurological dysfunction secondary to CNS inflammation, oedema and demyelination. Intravenous corticosteroid is the mainstay of treatment, hastens resolution of acute inflammatory change and promotes more rapid recovery. Visual psychophysics provides an established measure of sensory deficit, which may be selective for different functional pathways. A course of corticosteroid might be expected to improve signal conduction in affected pathways, leading to measurable improvements in vision.

Objectives: We wished to examine the nature of the sensory deficit and effect of corticosteroid on conduction in the visual pathways during an acute MS relapse in a serial study using visual psychophysics.

Methods: 5 patients (10 eyes) experiencing an acute, non-visual MS relapse having a three-day course of IV corticosteroid had visual psychophysics performed at baseline, after the third dose and again one week later.

Contrast thresholds were measured at three spatial frequencies (0.25, 1.0 and 4.0 cyc/deg) with counter-phase (chromatic-modulated) and in-phase (luminance-modulated) red-green gratings.

Results: At low spatial frequency (0.25 cyc/deg) throughout the study, chromatic thresholds were significantly lower than the corresponding achromatic thresholds ($p < 0.0003$). At low and medium spatial frequencies a non-significant improvement in both chromatic and achromatic thresholds from baseline was seen following the third dose and was sustained at one week. At high spatial frequency (4.0 cyc/deg) improvements in chromatic and achromatic thresholds from baseline were not seen until one week post-corticosteroid.

Conclusions: MS patients have subtle, subclinical deficits in vision that are not significantly improved by a course of corticosteroid. These chronic visual deficits may result from permanent structural injury e.g. gliosis, axonal loss. A degree of acute inflammatory change may still, however, be present as shown by the trend towards improvement in contrast thresholds. This trend favoured neither chromatic nor achromatic pathways.

123

**A functional MRI study of cortical activations associated with object manipulation and recognition in patients with MS.** M. Filippi, M. Rocca, D. Mezzapesa, A. Falini, B. Colombo, G. Scotti, G. Comi, Neuroimaging Research Unit, Department of Neuroradiology, HSR, Department of Neurology, HSR (Milan, I)

In this study, we used fMRI to compare the execution of two tasks with different levels of complexity and involving different cerebral pathways, to gain additional insights into the mechanisms of cortical functional reorganization in MS.

In 16 right-handed patients with MS (F/M = 12/4, mean age = 35.7 years, mean disease duration = 7.4 years, median EDSS score = 1.0) and 16 sex- and age-matched healthy volunteers, using fMRI, we investigated the performance of two different motor tasks. The first consisted of repetitive flexion-extension of the last four fingers of the right hand (simple task) alternated to epochs of rest, while the second consisted of manipulation of various complex daily-life objects (i.e., a pen, a glass, a toothbrush, etc.) as compared to manipulation of a single simple object (a rubber ball) (complex task).

During the performance of the simple task, compared to healthy volunteers, MS patients had more significant activations of the ipsilateral supplementary motor area (SMA), contralateral secondary sensorimotor area (SII), ipsilateral cerebellum, in a region located in the inferior semilunar lobule, superior parietal gyrus (SPG), bilaterally and contralateral inferior frontal gyrus (IFG). During the performance of the complex task, compared to healthy volunteers, MS patients had more significant activations of the SII, bilaterally, the contralateral cingulate motor area (CMA), bilateral cerebellum, in a region located in the inferior semilunar lobule, superior frontal gyrus (SFG), bilaterally, ipsilateral MFG, ipsilateral IFG, and contralateral inferior parietal lobule. When within-group and between-task comparisons were performed, both groups had increased activations of the contralateral primary sensorimotor cortex (SMC), ipsilateral cerebellum, SMA and basal

ganglia, bilaterally, when performing the simple task. MS patients also had increased activations of the contralateral IFG and the SII, bilaterally. Both groups had increased activations of the IPS, cerebellum, the IFG, the SFG, the CMA and MT/V5 with the complex task. While this pattern of activations was mainly involving the contralateral hemisphere in healthy subjects, in MS patients it had a more bilateral representation. MS patients also had increased activations of the middle occipital gyrus, bilaterally, the ipsilateral postcentral gyrus and the contralateral inferior parietal lobule.

During the performance of a simple motor task, MS patients activate cortical regions that are usually recruited in healthy subjects when performing a more complex task. This might yet represent an additional mechanism with the potential to limit the severity of the clinical outcome associated with MS-related tissue damage.

124

**Effect of intravenous methylprednisolone on the number, size and confluence of plaques in relapsing-remitting multiple sclerosis.** R. Zivadinov, M. Zorzon, R. De Masi, D. Nasuelli, G. Cazzato, Neurological Clinic (Trieste, I)

Background: There are some suggestions for a role of cyclical pulses of IV methylprednisolone (IVMP) as disease modifying therapy in both relapsing remitting (RR) and secondary progressive multiple sclerosis (MS). Recently, we demonstrated in a randomized, controlled, single blind, phase II clinical trial of IVMP in RR MS patients that prolonged treatment with pulsed IVMP slowed development of T1 black holes and delayed brain atrophy and disability progression.

Objective: To determine the effect of IVMP on the size, number and confluence of T2 lesions in patients with RR MS in the long-term.

Methods: Of 88 RR MS patients, randomly assigned to regular pulses of IVMP (1 g/day for five days with an oral prednisone taper) or IVMP at the same dose schedule only for relapses (IVMP for relapses) and followed-up without other disease modifying drug therapy for 5 years, 81 patients completed the trial as planned. Pulsed IVMP was given every 4 months for 3 years, and then every 6 months for the subsequent 2 years. Patients had cranial MRI scans at study entry and after 5 years, and standardized clinical assessments every 4–6 months. Calculations of number, size and confluence of T2 lesions have been obtained.

Results: At study entry the number and size of T2 lesions and T2-lesion load (LL) were well matched in the two study arms. Five years after, patients who received pulsed IVMP had significantly less confluent T2 lesions (105 vs 270, $p < 0.0001$), less large T2 lesions (> 10 mm) (165 vs 541, $p < 0.00001$), lower T2-lesion volume (LV) (21.4 ml vs 27.8 ml, $p = NS$), lower confluent T2-LV (5.4 ml vs 17.4 ml, $p < 0.00001$) and a lower T2-LV/N° T2 lesion index (0.52 vs 1.1, $p = 0.007$) compared to patients who received IVMP only for relapses.

Conclusions: In patients with RR MS treatment with pulses of IVMP prevents the confluence of T2 lesions. This may have contributed to slow disability progression in the long-term.

125

**Neutralising antibodies against IFN-beta in multiple sclerosis: antagonisation of IFN-mediated suppression of MMP expression.** R. Lindberg, F. Gilli, A. Bertolotto, University Hospital Basel, University of Turin (Basel, CH, Orbassano, I)

Objective: To 1) determine the transcriptional levels of matrix metalloproteinase-2 (MMP-2), MMP-9 and MxA in peripheral blood mononuclear cells of MS patients before and during IFN-beta (IFNB) treatment, and 2) to evaluate the impact of neutralising antibodies (NAb) against IFNB on these measures.

Background: Some MS patients may develop NAb in the course of IFNB treatment, but their clinical significance remains uncertain. The biological action of IFNB can be measured by the expression levels IFN-responsive proteins such as MxA, which is reduced in presence of NAb. MMPs are crucial effector molecules in several steps of MS pathogenesis. In MS serum levels of MMP-9 are increased, whereas IFNB suppresses MMP expression. We hypothesised that NAb would antagonise the IFNB effect on MMPs.

Methods: Transcriptional expression of MMPs and MxA were quantitated by real-time PCR in peripheral blood mononuclear cells from 39 treatment-naive MS patients (tested before (T0) and after the second IFNB injection (T12h)), and in 68 on-treatment patients. IFNB-induced NAb were evaluated with the cytopathic effect assay (CPE) every 3 months. Patients were categorised in three groups: NAb-negative (NAb–), persistent NAb-positive (pNAb+, ≥2 consecutive samples positive), and isolated NAb+ (iNAb+, single NAb+ or sporadic positivity during follow-up).

Results: treatment naïve patients: MMP-9 was expressed over a wide range in all treatment-naive patients (T0h). The mean MMP-2 expression was ~50 times lower than that of MMP-9 and not detectable in 22% (17/78) of samples. After two IFNB injections (T12h) expression levels of MMP-9 ($p = 0.4418$) and MMP-2 ($p = 0.4744$) remained unchanged. In contrast, mean MxA levels increased 10-fold after IFNB injection, as compared to baseline levels ($p < 0.0001$).

IFNB-treated patients: 22% (15/68) of IFNB-treated patients had pNAb+, 71% were NAb– and 7% had iNAb+ (2 were negative at the time of expression analysis (negative-iNAb+), 3 patients presented their single NAb+ sample concurrent to blood sampling for RT-PCR analysis (positive-iNAb+). During IFNB treatment MMP-9 expression was significantly lower than in treatment-naive patients ($p < 0.0001$) and levels were below the detection limit in 13% (9/68). pNAb+ patients showed significantly higher mRNA levels of MMP-9, as compared to other groups of IFNB treated patients (all $p \leq 0.044$) which reached levels similar to that in treatment-naive patients ($p > 0.25$). IFNB treatment suppressed MMP-2 expression below detection limit in all NAb– and iNAb+ patients, but remained detectable in 33% (5/15) pNAb+ patients. IFNB treated patients showed a 5-fold higher mean MxA expression as compared to T0h samples ($p < 0.0001$). Induction of MxA mRNA was observed in 74% (50/68) patients but remained at T0h levels in 26% (18/68). In 72% (13/18) of these patients NAb were present. In presence of pNAb+, MxA expression was significantly lower compared to NAb– ($p < 0.0001$) and negative-iNAb+ ($p = 0.0441$) patients and reached levels similar to those in T0h samples ($p = 0.1422$).

Conclusions: 1) Suppression of MMP expression is a long-term, but not an acute effect of IFNB therapy, 2) NAb antagonise the MMP suppressive effect of IFNB, 3) MMP-9 is the first marker of biological action of NAb against IFNB that has a role in MS pathogenesis.

# Session 21

## Muscle disorders – 1

126

**Muscle recruitment of multipotent circulating stem cells in dystrophic animal model.** M. G. D'Angelo, Y. Torrente, M. Belicchi, F. Pisati, M. Gavina, E. Negroni, A. C. Turconi, M. T. Bassi, N. Bresolin, IRCCS E. Medea, IRCCS Ospedale Maggiore Università di Milano (Bosisio Parini, Milan, I)

Background: The structural membrane protein, dystrophin, maintains the mechanical stability of the muscle fiber membrane during muscle contraction and relaxation. Dystrophin-deficient skeletal muscles can be more easily damaged than normal muscles by mechanical stresses such as physical exercise. Several studies documented the presence of multipotent Sca1+ cells in the adult mouse muscle, expressing both skeletal muscle and hematopoietic cells specific markers.

These muscle resident stem cells may partly be deriving from multipotent circulating stem cells. We analysed the behavior of Sca1+ cells obtained from peripheral blood and from muscle of mdx and wild type mice, in relation to muscular stress (swimming test).

Methods: Muscle-derived cells were isolated by mechanical and enzymatical dissociations of the hindlimbs of 1 month old and 3 months old mdx dystrophic and age-matched wild type mice. Each group was divided in two subgroups: one swimming and one kept at rest.

Sca1 positive were selected from muscle cultures after serial platings and from blood with a MiniMACS separation system using Sca1 antibody conjugate to magnetic beads. Cells were analysed for differentiative capability by immunocytochemical stainings.

ELISA analysis for cytokines was performed on muscle omogenates.

Results: Exercise did not influence the number of Sca1+ cells from blood and from muscle of 1 month old mdx and wild type mice. Cells expressed myogenic, myeloid, endothelial and neuronal markers in different medium conditions.

In 3 months old mdx mice, swimming induced a decrease of Sca1+ cells from blood (25.9% of Sca1+ cells in non swimming mice versus 10% in swimming ones). On the contrary, in muscles from exercised mice the number of Sca1+ cells increases.

In the 3 months old wild type mice, the number of circulating Sca1+ cells was higher in exercised than in non exercised mice, whereas there was no difference in the number of muscle derived Sca1+ cells.

ELISA showed a stronger increase of IL1beta, INF gamma and TNFalfa in the mdx than in normal muscles, particularly after swimming. Cytokines may facilitate the adhesion of circulating Sca1 cells to the vascular endothelium through the increase of ligands of adhesion molecules on these cells.

Conclusion: Exercise induces a series of events facilitating the recruitment of circulating stem cells more evident in dystrophic muscles than in non dystrophic ones.

127
**Localization of Th1 associated chemokines in inflammatory myopathies.** J. L. De Bleecker, B. De Paepe, University Hospital (Ghent, B)

Background: Diseases characterized by autoaggressive T-cells are thought to be associated with Th1 phenotype driven immune reactions. As the alpha-chemokine receptor CXCR3 is preferentially expressed on Th1 cells, we studied its distribution in the inflammatory myopathies (IM). We also characterized the expression of its ligands CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (I-TAC).

Methods: Muscle biopsies of controls (n = 10), polymyositis (PM, n = 10), sporadic inclusion body myositis (sIBM, n = 10) and dermatomyositis (DM, n = 6). Immunoperoxidase staining localizing chemokines and receptor, double labeling immunofluorescence with cell type specific markers. Western blotting of protein extracts.

Results: Strong membranous expression of CXCR3 was observed on inflammatory cells surrounding and invading nonnecrotic muscle fibers in PM and sIBM. The majority of cells present in perimysial inflammatory foci of DM were also CXCR3 positive.

CXCL10 was expressed by a subset of activated macrophages and T-cells in endomysial aggregates of inflammatory cells in PM and sIBM. Lower expression levels were detected in perimysial infiltrates of DM tissues. In all muscle specimens, CXCL10 positive blood vessels were present.

We found no CXCL9 immunoreactivity in control muscle nor in IM. For CXCL12, occasional blood vessels stained in muscle tissues from all diagnostic groups. Positive control tissues were Hodgkin lymphoma (CXCL9) and thymus (I-TAC) in which both immunostaining and Western blotting confirmed the reactivity of the antibodies used.

Conclusion: Earlier we described that T-cells of the memory lineage, characterized as CD45RO+, predominate in IM. In parallel with studies in other tissues, we here confirm CXCR3 expression on these cells as well as on the majority of macrophages, along with abundant expression of its ligand CXCL10. This further points to Th1 driven inflammation as the underlying mechanism responsible for IM. Our data further clarifies the selective usage of chemokine/chemokine receptor pairs. Chemokine receptors are particularly suited as targets for pharmacological immune therapy.

128
**Adult bone marrow-derived stem cells in muscle connective tissue and satellite cell niches: a novel finding with potential therapeutic impact.** F. Chrétien, P. Dreyfus, B. Chazaud, R. Gherardi, Hôpital Henri Mondor, INSERM EMI 0011 (Paris, F)

Postnatal growth and repair of skeletal muscle mainly result from activation, proliferation and fusion of satellite cells that are committed myogenic precursors residing beneath the muscle fiber basal lamina. Besides satellite cells, multipotent skeletal muscle stem cells, the origin of which is unknown have been recently identified in muscle connective tissue (Tamaki et al. J. Cell Biol 2002, 157, 571–577). These cells express stem cell markers and may differentiate into muscle cells, endothelial cells, or adipocytes. The distribution of bone marrow derived cells was investigated 1, 3, 6 months after transplantation of bone marrow from B6-TgGFP transgenic mice to normal irradiated B6 mice, the cytoplasmic green fluorescent protein (GFP) being used as an unambiguous marker of donor-derived cells in host muscle. Abundant GFP + mononuclear cells appeared in muscle tissue after transplantation and their number increased with time. GFP + mononucleated cells were located both inside and outside of the muscle fiber basal lamina. GFP + cells found in sublaminal satellite cell niches expressed unambiguous satellite cell markers (M-cadherin, Pax7, NCAM), their number increased with time and their myogenicity was assessed by appearance of scattered GFP + muscle fibers at 3 and 6 months post-transplantation. In addition, GFP + mononucleated cells expressing the stem cell antigens Sca1 and CD34 were detected in the interstitial connective tissue, usually in the vicinity of large and small vessels. As compared to satellite cells, interstitial bone marrow-derived muscle stem cells were less numerous and increased more slowly with time.

We conclude that both stem cell marker-expressing cells found in connective tissue and myogenic precursor cells located in sublaminal niches may be derived from bone-marrow in adulthood. Our findings extend the recent observation that bone marrow-derived cells can replenish the satellite cell niches previously emptied by irradiation (LaBarge and Blau, Cell 2002, 111, 589–601), and pave the way for future therapeutic strategies in muscle dystrophy.

129
**Non-invasive assessment of cardiac involvement and skeletal muscle metabolic alterations in DM2/PROMM by quantitative 31P-MRS and MRI.** C. Schneider-Gold, M. Beer, H. Köstler, S. Buchner, J. Sandstede, D. Hahn, K. V. Toyka, University of Dusseldorf, University of Wurzburg (Dusseldorf, Wurzburg, D)

Background: In the multisystem disorder myotonic dystrophy type 2 (DM2/PROMM) not only skeletal muscle but also cardiac muscle may be involved. We used a novel MRS method as well as cine MRI for non-invasive assessment of subclinical cardiac involvement.

Methods: Eleven patients with genetically confirmed DM2/PROMM were examined using a 1.5 T system (Magnetom VISION) and chemical shift imaging (CSI) for data collection. Skeletal muscle metabolism was analysed in the left gastrocnemius muscle. Cardiac metabolism was determined in the left ventricular myocardium. Absolute concentrations of PCr, ATP and Pi as well as PCr/ATP ratios were calculated by Spatial Localisation with Optimal Pointspread Function (SLOOP). Gradient echo 2D-cine MRI was used for the evaluation of cardiac morphology and function.

Results: In cardiac muscle, all DM2 patients showed reductions of PCr and ATP on MRS and increased LV systolic volumes on MRI which correlated with the severity of skeletal muscle weakness ($p < 0.05$).

Conclusions: Our MRS and MRI findings suggest that mild and frequently subclinical cardiac involvement seems to be a common feature in DM2/PROMM. Future studies are needed to define the sensitivity and specificity of these findings and their diagnostic value for identifying patients at risk for cardiac complications in the course of myotonic dystrophies and other multisystemic myopathies.

130
**Novel missense mutations and large deletion of GNE gene in two Italian families with autosomal recessive IBM.** R. Del Bo, P. Baron, A. Prelle, M. Serafini, M. Moggio, A. Di Fonzo, N. Bresolin, G. P. Comi, University of Milan (Milan, I)

Hereditary inclusion body myopathy (h-IBM) constitutes a heterogeneous group of neuromuscular disorders characterized by adult-onset slowly progressive distal and proximal weakness and refers to several syndromes with autosomal or dominant inheritance. The autosomal recessive form (AR h-IBM), first characterised in Jews of Persian descent, is a myopathy that affects mainly leg muscles, but with an unusual distribution that spares the quadriceps, so-called quadriceps-sparing myopathy.

The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) gene is the causative gene for AR h-IBM. GNE is a bifunctional enzyme known to be rate-limiting in the sialic acid biosynthetic pathway, a crucial function in many biologic processes, although the specific ones associated with AR h-IBM remain to be clarified.

Here, we report the genetic analysis of the GNE gene in two Italian families affected by AR h-IBM.

Histological, histochemical and ultrastructural analysis of muscle biopsy followed standard procedures. Genomic DNA was extracted from blood leukocytes of patients and unaffected family members. All the 13 exons of the GNE gene were amplified by PCR, using specific sets of primers, and directly sequenced with an automated sequencer 3100. All point mutations were confirmed by restriction analysis.

In family A, two sisters of 33 and 28 years of age were shown to be compound heterozygous for two novel GNE mutations: a large deletion involving exons 1–9 and a R162C aminoacid change in the epimerase domain of the protein. In family B, the single patient of 35 years of age revealed the presence of two different missense mutations, namely M171T and R177C.

Both R162C and M171T mutations have not been observed so far in h-IBM, while R177C has been recently reported in a Japanese patient. All the three missense mutations described in the present study are located in exon 3 within the epimerase domain of the protein, while the heterozygous deletion affects a large portion of GNE gene (spanning a genomic distance of at least 35.7 Kb) and represents the first deletion event observed in the GNE gene. In addition, since a decrease in the epimerase activity has been described in patients carrying mutations in the epimerase domain, the presence of a null mutation in one allele may severely impair the bifunctional enzyme activity, therefore supporting the notion that a markedly decreased GNE function is critical to the pathogenesis of AR h-IBM.

131
**Anticipation in an Italian family with myotonic dystrophy type 2: congenital myotonic dystrophy or casual association with early-onset mental delay?** V. Sansone, M. Cotelli, S. Cappa, R. Krahe, G. Meola, Istituto Policlinico San Donato, University of Brescia, University Vita e Salute, Dept. Molecular Genetics (Milan, Brescia, I, Houston, USA)

Background: Congenital myotonic dystrophy is characterized by distinct clinical features present at birth such as remarkable hypotonia, bilateral facial weakness, respiratory deficiency, feeding and swallowing difficulties, delayed motor and mental development, talipes and contractures. The more classical features of mytonic dystrophy type 1 (DM1), such as myotonia, progressive weakness and cataracts develop later on in adulthood. Transmission is generally maternal and is associated with CTG triplet expansions > 1000 in the myotonic dystrophy phosphokinase (DMPK) gene. The existence of a congenital form of myotonic dystrophy has so far been considered a distinctive feature of DM1 especially because no such form has been described in myotonic dystrophy type 2 (DM2).

Aims: To describe the clinical, neuropsychological and neuroimaging results of a 24-year-old male with delayed motor and mental development with genetically confirmed DM2.

Methods: The patient, son of a 49-year-old male belonging to a 5-generation family with genetically confirmed DM2, was subjected to full detailed family history, complete neurological examination including manual muscle strength assessment according to the MRC scale, EMG recordings and ocular screening for cataracts. In addition the patient was subjected to a battery of neuropsychological tests (MMSE, IQ, Wechsler, Token Test, Rey figure, Rey recall, Corsi supraspan) and to brain CT and MRI scans.

Results: Family history was suggestive for motor and mental developmental delay at birth apparently without known perinatal cause. Gestation had not been complicated and delivery was normal. Generalized epilepsy was an additional feature. Onset of muscle symptoms in the father had been in the 3rd decade with limb girdle muscle weakness. The father never complained of cognitive problems but the battery of neuropsychological tests demonstrated impaired visual spatial function. His brain MRI was normal. Manual muscle strength testing in our patient was normal as were bulk and tone of the limb muscles. Grip myotonia was absent. EMG showed myotonic discharges in all 4 limbs. No cataracts were found. Neuropsychological tests were consistent with moderate mental delay. Brain CT scan demonstrated diffuse cortical atrophy, diffuse thickening of the theca. Brain MRI confirmed CT results, no significant white matter hyperdense lesions were observed. Basal ganglia were normal. Genetic analysis demonstrated the DM2 mutation in leukocytes.

Conclusions: We describe a patient with genetically confirmed DM2 in whom moderate mental delay was present at birth. Perinatal suffering and other known causes of mental delay or epilepsy were not demonstrated. As previously described in some German DM2-linked families this is the first example of anticipation in an Italian family. We also hypothesize that this may be the first patient with congenital DM2 but a causal association may not be ruled out.

# Session 22

## Muscle disorders – 2

132
**Beneficial effects of aerobic training on exercise-related oxidative stress in mitochondrial myopathies.** G. Siciliano, M. Mancuso, A. Rocchi, F. Galluzzi, C. Kusmic, R. Barsacchi, A. Tessa, F. Santorelli, University of Pisa, Clinical Physiology-CNR, Bambin Gesù Hospital (Pisa, Rome, I)

In mitochondrial myopathies mutations of mitochondrial DNA are responsible for insufficient ATP production and deranged metabolism at the skeletal muscle level. Abnormal oxidative metabolism consequent to mitochondrial dysfunction is then implicated in production of reactive oxygen species (ROS) and peroxidation of membrane lipids, DNA and structural proteins within the cells. When there is an imbalance between ROS levels and antioxidant defense this realizes oxidative stress that may lead cell death with both apoptotic and not apoptotic pathways.

Mutations in mitochondrial DNA (mtDNA) are the most frequent cause of mitochondrial myopathy in adults. In the majority of cases mutant and wild-type mtDNA coexists, a condition referred to as mtDNA heteroplasmy. Aerobic training has been showed to improve oxidative metabolism in mitochondrial myopathies, probably acting on the balance between wild type and mutated mitochondrial DNA (mtDNA).

Aim of this study has been to evaluate, in a group of patients affected by chronic progressive external ophthalmoplegia (CPEO) and large-scale mtDNA rearrangements, the occurrence of in vivo oxidative stress related to exercise and to assess if aerobic training, other than improve oxidative metabolism, is able to reduce exercise-related ROS production from skeletal muscle. To do that, an indirect marker of oxidative stress, blood lipoperoxide level, measured at rest and during an incremental exercise test, was considered. This exercise test was performed before and after the proposed aerobic training, 10 weeks of supervised motor activity at near anaerobic lactate threshold.

Mean blood level of lipoperoxides was $382 \pm 38$ AU corresponding to a moderate oxidative stress according to Carratelli et al. During exercise blood level of lipoperoxides maintained high ($379 \pm 27$ AU at 40% of the maximal predicted normal power output, $387 \pm 27$ AU at maximal contraction level), while it was $375 \pm 26$ AU after a recharge period).

After the aerobic training the blood level of lipoperoxides was decreased by 13.7% at rest ($p < 0.01$), 10.4% at 40% of the power output, 8.6% at maximal contraction level and 8.5% after the recovery period, the absolute values of lipoperoxides now corresponding to a mild oxidative stress.

These data indicate that aerobic training can be beneficial also in those CPEO patients more severely affected by mitochondrial dysfunction. The fact that exercise-induced lipoperoxide production is reduced in our patients indicates that a lower exposure by skeletal muscle to peroxidation process occurs in such experimental conditions.

133
**Diagnosis and therapy of defects of mitochondrial fatty acid oxidation.** J. Schaefer, S. Jackson, J. Muehler, B. S. Andresen, H. Reichmann, Uniklinikum C. G.Carus Dresden, Leopoldina Hospital, University of Aarhus (Dresden, Schweinfurt, D, Aarhus, DK)

Hereditary defects of mitochondrial fatty acid oxidation are a group of inborn errors of metabolism manifesting early in infancy or rarely in adulthood. In childhood clinical symptoms usually consist of global metabolic derangement with hepatic encephalopathy (Reye-Syndrome) and hypoketotic hypoglycaemia, whilst adults generally present with muscle disease (exercise-induced myopathy, rhabdomyolysis). New diagnostic methods, in particular tandem mass spectrometry (tandem-MS) of beta-oxidation intermediates (acylcarnitines) and molecular genetic analysis of the underlying enzyme defect, have greatly facilitated the detection of these disorders which will in future allow early and more effective treatment.

In an adult female patient with an unusual clinical presentation of recurrent rhabdomyolysis associated with menstruation, demyelinating peripheral neuropathy and clinical symptoms resembling CPT-deficiency, we demonstrate the problems of differential diagnosis of these disorders. Using radio-hplc and tandem-MS in plasma and cultured fibroblasts from the patient, we could exclude CPT-deficiency and confirm a defect of very long-chain acyl-CoA dehydrogenase (VLCAD). Further molecular characterisation of the patient revealed in one chromosome 17 the simultaneous occurrence of a point mutation in the VLCAD gene and a duplication in the region around 17p11.2. The other chromosome 17 from the patient also had a point mutation in the VLCAD gene, but no duplication or deletion. Additional experiments in myoblast cultures and muscle biopsies provided information about the differential activation of VLCAD during muscle differentiation.

Additionally, using tandem-MS, we were able to show that the patient was able to metabolise medium-chain triglycerides (MCT), which are increasingly recommended for defects of long-chain fatty acid oxidation, supporting the beneficial effect of MCT supplementation in VLCAD deficiency.

134
**Muscle resonance imaging shows distinct diagnostic patterns in two common distal myopathies: Welander and tibial muscular dystrophy.** I. Mahjneh, A. E. Lamminen, A.E Paetau, O. Korhola, B. Udd, H. Somer, Pietarsaari Central Hospital, University of Helsinki, Vaasa Central Hospital (Pietarsaari, Helsinki, Vaasa, FIN)

Welander distal myopathy (WDM) and tibial muscular dystrophy (TMD) are the two most prevalent distal myopathies, both detected mainly from Sweden and Finland. We here report a longitudinal MRI study on 11 patients affected by WDM and 22 patients with TMD in order to define the pattern and characteristics of muscle involvement, and its changes over time in these two diseases. According to the muscle MRI findings the pattern of muscle involvement in WDM patients was characterised by early involvement of the

TA, EDL, gastrocnemius and soleus, as well as biceps femoris, semimembranosus and hip adductor muscles. TMD patients show much more selective involvement of the TA and EDL muscles in all patients, and most of the older patients showed semimembranosus and biceps muscle involvement. In TMD the semitendinosus muscle was affected in two patients, peroneus in two patients, gastrocnemius in eight patients and soleus in five patients. In both WDM and TMD, when affected, the gastrocnemius muscle was progressively more involved from the caudal to the cranial portions of the muscle. In some cases, the caudal portion of the gastrocnemius muscle was the only part affected. 24% of our patients showed asymmetry of muscle involvement. Surprisingly, these two disorders showed besides distal muscle involvement, also frequent involvement of the proximal muscles. The progression of muscle involvement was slow. We conclude that muscle MRI examination proved to be very useful in the determination of the exact pattern of muscle involvement and its changes over time in WDM and TMD. Clinical testing using the MRC scale is not enough to establish the pattern of muscle involvement in focal muscle diseases.

135

**Muscle Calpain 3 deficiency in quail eater's disease.** A. Toscano, O. Musumeci, R. Cagliani, M. Aguennouz, G. Comi, C. Messina, G. Vita, University of Messina, University of Milan (Messina, Milan, I)

A rhabdomyolysis outbreak after quail meat ingestion has sometimes been described in individuals resident in the Mediterranean countries. Etiopathogenesis is still unknown but it has been suggested that animals or vegetable toxins could be responsible.

We describe two unrelated Italian patients (F/40 yrs and M/31 yrs) who were admitted to our Neuromuscular Center because of sudden onset of vomiting, myalgias, stiffness and pigmenturia one day after wild quails ingestion. Clinical examination was normal except for diffuse limb muscle pain. Laboratory investigations revealed markedly increased serum CK (33981 IU/L and 10500 IU/L respectively) and myoglobin (7610 mg/L and 3207 mg/L -n. v. 0–70). EMG was normal. HLA haplotypes did not show any similarity in patients. A muscle biopsy was taken in both cases. Histochemical studies only revealed mild lipid storage in case 1. Biochemical analysis of mitochondrial, glycogen and lipid pathways as well as myoadenilate deaminase activity did not show any specific enzyme deficiency. Immunohistochemical studies showed normal expression of dystrophin, sarcoglycans, dysferlin and caveolin-3. Western blot revealed an abnormal pattern of calpain-3 in both patients with a significant decrease of the 94 Kd and 60 Kd bands. Molecular genetic investigation of CAPN3 gene did not show any pathogenic mutation. Both patients promptly recovered within one week after intense i. v. hydration. A one year clinical follow up was unremarkable.

This is the first report of Italian patients with quail eater's disease. We postulate that a secondary calpain 3 deficiency, due to an unknown toxin, can be responsible massive muscle damage.

136

**Antisense therapeutics in myasthenia gravis.** D. McKee, S. Agus, H Soreq, O. Ben Joseph, S. Brawer, J. Sussman, Z. Argov, Greater Manchester Neuroscience Centre, Hadassah University Hospital, Hebrew University, Ester Neuroscience (Salford, UK, Jerusalem, Tel Aviv, IL)

Introduction: EN101 is a 20 base antisense oligodeoxynucleotide, modified for stability in vivo, and with a sequence complementary to a coding region of the human acetylcholinesterase (AChE) gene. It has the ability to bind selectively to AChE mRNA preventing its translation into protein, and has been shown to both suppress AChE production in vitro and improve performance in rats with autoimmune experimental myasthenia gravis. We report results from an open label trial of EN101 in patients with myasthenia gravis (MG).

Methods: Patients with stable generalised MG were recruited, all of whom required AChE inhibitors for symptom control. After hospitalisation, AChE inhibitors were discontinued for a washout period of 12–18 hours, followed by one-day titration period during which escalating oral doses of EN101 were given. Single daily oral doses of EN101 were then given at 500 micrograms/kg for the following three days. Following the final dose of EN101, AChE inhibitors were reinstituted when signs of deterioration were observed. Neuromuscular performance was regularly assessed using the Quantitative Myasthenia Gravis Score (QMG).

Results: In 15/16 patients there was a clear deterioration in myasthenic symptom control on withdrawal of AChE inhibitors, followed by a clear symptomatic improvement after administration of EN101. The single non-responder whose condition failed to improve on EN101 remained demonstrably responsive to pyridostigmine. Analysis of QMG scores in responders showed a statistically significant sequential improvement throughout the treatment period, with a decrease in average QMG from 13.2 at baseline down to 6.0 following the final dose of EN101 ($p = \leq 0.001$), and mean percentage improvements in QMG of up to 53.4% ($p = \leq 0.05$). Improvements in performance were reflected in individual elements of the composite score, demonstrating differences, which in addition to being statistically significant were also of clear, and in several cases dramatic, clinical significance. Improvements in QMG scores following the final dose of EN101 were sustained for up to 72 hours. No serious adverse events were recorded, and cholinergic side effects were conspicuous by their absence.

Discussion: The AChE system in MG is an elegant model in which to investigate the antisense therapeutic approach, offering a human disease paradigm in which the expression of a single gene product is rapidly translated into relatively easily measured clinical parameters. We have shown that orally administered EN101 appears to be powerfully effective in improving symptoms in patients with myasthenia gravis. In addition to the marked scientific significance of this observation, the apparent magnitude of effect and the suggestion of reduced cholinergic side effects suggest that EN101 offers considerable therapeutic advantages over conventional AChE inhibitors, and the results justify further study in a randomised controlled trial.

137

**Neuromuscular transmission studies in Na/Ca exchanger type III deficient mice reveal deficits highly suggestive of Lambert-Eaton myasthenic syndrome.** S. Sokolow, M. Manto, J. M. Vanderwinden, A. Herchuelz, S. Schurmans, Free University of Brussels (Gosselies, Brussels, B)

The roles played by the Na/Ca exchanger type III at the neuromuscular junction remain to be elucidated. We have generated a mouse strain deficient for the NCX3 isoforms of the Na/Ca exchanger. Repetitive nerve stimulation (RNS) in NCX3 deficient mice (NCX3–/–) and control mice were performed. The compound muscle action potentials (CMAPs) of the gastrocnemius muscle were recorded in response to direct supramaximal stimuli applied to the sciatic nerve. During each test, five stimuli were given. A positive decremental or incremental response was defined as a decrease or an increase of more than 10% of the amplitude of fifth response compared to the first response. The means and SEM of the decrement (or increment) at 10 Hz (LRS: low frequency repetitive stimulation) or 30 Hz stimulation (HRS: high frequency repetitive stimulation) for 8 NCX3–/– mice and 5 control mice were computed. Responses evoked in gastrocnemius with LRS elicited decremental CMAPs. The mean decrement stimulation was larger in NCX3–/– ($11.78 \pm 1.19\%$) than in control mice ($0.03 \pm 0.3\%$) (intergroup difference: $p < 0.001$). During HRS, incremental CMAPs were recorded for four of the eight NCX3–/– mice ($51.69 \pm 2.76\%$). Incremental CMAPs were never recorded in control mice. In addition, in order to improve the sensitivity of our investigations, we analysed the post-exercise facilitation. The testing program was the following: [1] a single repetitive stimulation at 10 Hz was recorded at rest; [2] electrical stimulation of sciatic nerve at 10 Hz for 20 min (exercise); [3] CMAPs were recorded after 10-Hz repetitive stimulations, immediately after exercise, and every 30 sec during 5 min; [4] after a resting period of 30 min, the same animals (5 control mice and 5 NCX3–/– mice) were tested with the same program at 30-Hz stimulation. At LRS, a slight decrease of CMAP was confirmed in the NCX3–/– mice. Immediately after the exercise period, the decrease of the CMAP following 10 Hz stimulation was significantly higher in the knock-out group ($p < 0.05$). After resting, a marked increment was observed at 30 Hz in the NCX3–/– mice. In conclusion, the RNS test revealed findings highly suggestive of Lambert-Eaton Myasthenic Syndrome (LEMS) in NCX3–/– mice: decremental response at LRS, incremental response at HRS, and postexercise facilitation at HRS. These observations strongly suggest a possible role of the NCX3 isoform of the Na/Ca exchanger in the pathogenesis of the Lambert-Eaton Myasthenic syndrome.

138

**No association between acetylcholine receptor epsilon subunit polymorphisms and myasthenia gravis.** D. M. Bonifati, A. Abdelgany, J. Cossins, N. Willcox, A. Vincent, D. Beeson, Weatherall Institute of Molecular Medicine (Oxford, UK)

Background: Myasthenia gravis (MG) is an autoimmune disorder characterised by antibodies against the acetylcholine receptor (AChR) and associated with certain immune response genes. Recently, a patient with a congenital myasthenic syndrome (CMS) due to mutations in the AChR e subunit, subsequently developed typical early onset autoimmune MG (EOMG).

Aim: To test if mutations or polymorphisms in the e subunit of the AChR are associated with development of EOMG.

Materials and methods: We searched for AChR mutations, Y15H and Y15X, and for AChR polymorphisms by single strand conformation polymorphism (SSCP) in 110 patients with EOMG (88 female and 22 male) and

in a group of controls (100 CMS patients and healthy control). We also analysed a common polymorphism in intron 11 (IVS11 + Del20) that changes the reading frame introducing 15 new aminoacids before finding a stop codon. Chi Square test was applied.

Results: Neither of the e subunit mutations (Y15H and Y15X), previously found in the CMS patient who developed autoimmune MG, were found in the additional 101 EOMG patients. SSCP analyses detected the following polymorphisms in MG patients: C459T (A153A); G −8T (G-3V), C906T (C302C); G1342C (V448L) with a frequency of 6.7%, 9%, 3% and 2% respectively, but no statistical differences were found compared with the controls. One new polymorphism G-8 C (G-3A) was identified.

The frequency of the homozygous IVS11 + 20Del 20 polymorphism was 2.3% in 167 MG patients compared with 0.5% in 199 controls ($p = 0.11$), while that of heterozygotes was 12.3% in MG patients and 8% in controls ($p = 0.07$). Since two of the homozygote and two of the heterozygote MG patients were non-caucasian, we searched for the IVS11 + 20Del 20 polymorphism in a group of 53 non-caucasian controls. 24.5% of these subjects were heterozygous compared with 8% of Caucasian controls ($p = 0.0008$) confirming an increase frequency of the polymorphism in Non-caucasians.

Conclusion: We conclude that polymorphisms or mutations in the AChR e subunit are not, overall, involved in the development of autoimmune MG. Our work, however, emphasises the importance of a large and ethnically matched population when association studies with polymorphic markers are conducted.

# Session 23

## Neuro-oncology – 1

139

**Voltage-gated potassium channel antibodies and limbic encephalitis.** P. Pozo-Rosich, L. Clover, A. Saiz, F. Graus, A. Vincent, Hospital Clinic Barcelona, John Radcliffe Hospital (Barcelona, E; Oxford, UK)

Limbic encephalitis (LE) is usually associated with lung cancer or other neoplasms. In rare instances a tumour is not found after a long follow-up. Voltage gated potassium channel (VGKC) antibodies have been described in two patients with reversible LE (one of them associated with thymoma). To investigate the frequency and clinical course of VGKC antibodies in LE, we studied 15 patients, screening for onconeural antibodies (Hu, Yo, Ri, Tr, CV2, Ma2) by immunohistochemistry and immunoblot, and for VGKC antibodies by radioimmunoassay.

Eleven LE patients did not have VGKC antibodies. Eight of these had onconeural antibodies (Hu, $n = 6$; Ma2, $n = 2$), and three were seronegative. Nine had lung and one prostate cancer. No tumor was found in one patient who died a few months after the onset of the LE. Only the two patients with Ma2 antibodies obtained a partial remission.

Of the four patients with VGKC antibodies, the two patients with low VGKC antibodies (170 pM, 300 pM; controls < 100 pM), had lung cancers, the LE did not improve or followed a relapsing-remitting course, and there was little change in VGKC antibody over time in one patient. By contrast, in the two patients with high levels (both > 800 pM), there were VGKC antibodies in the CSF (56 pM, 69 pM, controls < 10 pM), no tumour detected up to 3 and 5 years later, and their LE went into remission after treatment with cycles of intravenous immunoglobulins and high dose (1 gram) methylprednisolone with a reduction in antibodies to 0 and 474 pM respectively.

We conclude that VGKC antibodies occur in 57% (4/7) of patients with LE without onconeural antibodies. VGKC antibodies have now been identified in 10 further patients with LE (Vincent, Schott, Palace, Rosser in preparation). Whereas low levels of VGKC antibodies may be found in paraneoplastic LE, it appears that high VGKC antibodies may indicate a nonparaneoplastic form of LE with a remitting course.

140

**Autoantigen diversity in the opsoclonus-myoclonus syndrome.** L. Bataller, M. Rosenfeld, F. Graus, J. Vilchez, N.-K. Cheung, J. Dalmau, University of Arkansas for Medical Sciences, Hospital Clinic, Hospital La Fe, Memorial Sloan-Kettering Cancer Center (Little Rock, USA; Barcelona, Valencia, E; New York, USA)

Background: Although circumstantial evidence suggests that idiopathic and paraneoplastic opsoclonus-myoclonus (OM) are often immune mediated, no specific autoantigen has been identified.

Objectives: To identify target neuronal antigens recognized by the serum of patients with idiopathic and paraneoplastic OM.

Methods: The sera of 21 patients with OM (10 idiopathic, 5 associated with small-cell lung cancer, 6 associated with neuroblastoma) were selected for probing a brainstem cDNA library. Recombinant fusion proteins of the isolated clones were obtained, and tested by western blot with the sera of all 21 OM patients, and controls.

Results: We isolated 37 clones coding for 25 proteins that were classified into three groups: 1) proteins of the postsynaptic density (adenomatous polyposis coli or APC, and others); 2) proteins with expression or function restricted to neurons, including RNA or DNA-binding proteins and zinc-finger proteins (Hu, Zic, Promyelocitic leukemia zinc finger protein, and others); 3) a miscellaneous group comprising ubiquitously expressed, unspecific or unknown proteins. Each antigen was recognized by one single OM serum, except for the APC protein that was recognized by the sera of two patients with idiopathic OM and two control patients with paraneoplastic brainstem and cerebellar or limbic encephalitis.

Conclusions: 1) There is an heterogeneous immune response directed to an extensive variety of target neuronal antigens in patients with OM, without a single specific antibody marker. 2) The postsynaptic density is a frequent source of novel autoantigens, with several proteins of this complex targeted by antibodies of OM patients. 3) The detection of antibodies to onconeuronal antigens (ie, Hu proteins) in some patients with paraneoplastic OM is probably related to cancer-induced immunity rather than to the neurological syndrome.

141

**Enhanced accumulation/persistence of Pt-DNA lesions in dorsal root neurons underlie cisplatin-induced polyneuropathy.** A. Dzagnidze, J. Makhalova, Z. Katsarava, B. Liedert, J. Thomale, V. Limmroth, University Hospital Essen, Institute of Cell Biology (Essen, D)

Background: Sensory polyneuropathy is one of the major dose limiting side effects of chemotherapy with DNA reactive drugs such as Cisplatin. Recently, we have shown that Cisplatin-induced DNA lesions represent the primary molecular basis of neurotoxicity. Their accumulation in the dorsal root ganglion (DRG) neurons together with the activity of DNA repair strongly correlates with the extent of morphological damage following Cisplatin exposure. In the present study we investigated the correlation between the accumulation of Pt-DNA-adducts in neuronal cells of WT and DNA repair deficient mice and functional impairment in peripheral nerves.

Methods: C57Bl/6 mice (wild type) and XPA-knockout mice (DNA repair deficient) were treated with Cisplatin, 0.5 mg/kg ip twice a week or sterile saline ip (controls). Electrophysiological tests (M- and H-responses, motor [MNCV] and sensory nerve conduction velocity [SNCV] of the sciatic nerve) were performed in WT mice at cumulative doses of 10 and 16 mg/kg; in XPA−/− mice at cumulative doses of 4 and 8 mg/kg. Further escalation of the Cisplatin dose in knockout mice led to acute impairment of vital functions. Frozen tissue sections were prepared from DRG and spinal cord. Neuronal cell types were identified histochemically, sections were immunostained for Cisplatin-DNA lesions with adduct-specific monoclonal antibodies. The amount of Pt-DNA-adducts was measured by quantitative image analysis (fluorescent microscopy).

Results: In Cisplatin treated WT mice at cumulative dose of 10 mg/kg electrophysiological parameters did not differ from the control WT mice; at cumulative dose of 16 mg/kg both, the amplitude of H-reflex and SNCV were significantly reduced compared to the saline-treated littermates ($p < 0.01$ and $p < 0.05$ respectively).

In XPA-/- mice, starting at cumulative dose of 4 mg/kg Cisplatin, the amplitude of H-reflex was reduced ($p < 0.01$) and SNCV began to slow down. After the cumulative dose of 8 mg/kg a highly significant decrease in both sensory parameters compared to the controls ($p < 0.01$) could be observed. M-response and MNCV remained unaffected in both experimental groups.

The accumulation of Pt-DNA-adducts was dose dependent and approx. 2-fold higher in DRG neurons than in the spinal cord cells. The XPA−/− mice showed significantly lower adduct removal, which caused a dramatic increase in DNA lesion persistence. To achieve an adduct level comparable to that in XPA−/− mice at a cumulative dose of 8 mg/kg Cisplatin, WT mice had to receive a two-fold higher cumulative dose.

Conclusions: The enhanced accumulation/persistence of Pt-DNA adducts in DRG cells was strongly associated with an early functional impairment as shown in vivo using neurophysiological techniques. Our data suggest that the amount of persisting DNA lesions determines the development of Cisplatin-induced polyneuropathy.

142
**Outcome and psychological problems after brain tumours in developmental age.** E. Castelli, G. Poggi, M. R. Liscio, S. Strazzer, A. R. Adduci, M. Massimino, L. Gandola, F. Fossati Bellani, IRCCS 'E. Medea', National Tumour Institute (Bosisio Parini, Milan, I)

Background: tumors of posterior fossa are the most frequent brain tumors in developmental age. Because of the longer survival times, attention is now being paid to the sequelae of the disease and its treatment. In particular survivors are at risk for complex and multiple disorders.

Goals: to identify the main psychological problems in children and adolescents with a diagnosis of tumor of posterior fossa and their clinical features according to age so as to establish specific psychological and rehabilitative intervention lines.

Methods: 65 patients were studied who received a diagnosis of cerebellar tumor (45 medulloblastomas, 11 astrocytomas, 9 ependymomas) before age 18 years. We divided the patients into three groups: 11 patients aged 4–6 years (mean age: 5.2 years), 29 patients aged 7–13 years (mean age: 9.8 years) and 25 patients aged over 14 years (mean age: 25 years). All the patients received a multidisciplinary clinical assessment including daily living autonomy, cognitive and quality of life evaluation. Each patient's neurological history was scored using the Neurological Severity Score (NSS). Quality of life was evaluated using Bloom classification. A standardized battery of psychological tests was administered according to their age: the Child Behavior Checklist for Ages 4–18 years, the Vineland Adaptive Behavior Scales, the Anxiety and Depression Scales and the Cognitive Behavior Assessment.

Results: At neurological evaluation 42% of patients had pyramidal deficits while 80% had ataxia. Mean NSS was 4.2 SD 1.8 (range 1–10). IQs were lower in the older group and about half patients had learning disabilities. From a psychological perspective, the three groups of patients mainly presented with Internalizing problems whose proportions increased with age (36%, 65,5% and 70,8% respectively). Younger patients showed a greater impairment in all social adjustment areas, in particular communication skills, while older patients showed a greater impairment of social skills. Main factors associated to onset of psychological problems were the time elapsed since diagnosis and evaluation, the radiotherapy and the level of cognitive disorders.

Conclusions: Our data confirm a high frequency of psychological and behavioral disorders in patients treated for brain tumor. While younger patients exhibit behavioral problems to a greater extent, older patients show frank psychopathological disorders.

143
**A new approach to identify tumour antigens in patients with malignant brain tumours.** S. Dapper, F. Vogel, O. Bozinov, S. Cepok, S. Stei, K. Büssow, O. Grauer, J. Maurer, U. Sure, B. Hemmer, University of Marburg, MPI für Molekulare Genetik, University of Regensburg, RZPD (Marburg, Berlin, Regensburg, D)

Malignant gliomas are among the most common primary brain tumors. Since these tumors are highly resistant to conventional therapies, recent research has been focused on developing alternative treatment strategies, particularly immunotherapy. However, in contrast to systemic tumors, only few glioma antigens have been defined so far. Here, we use a novel protein array approach to search for glioma-associated antigens. The protein array, containing 35,000 expression clones from a cDNA library of the human fetal brain, was used to determine immune responses in the serum of 5 patients with malignant brain tumors. The immune reactivity was compared to patients with inflammatory and non-inflammatory disorders of the nervous system. We identified a high immunoreactivity to ten proteins in these tumor patients, which were not present in controls. The cDNA insert was defined for each expression clone and the immune response to the purified protein confirmed by western blot. Interestingly, one of the identified proteins was the known CTCL tumor antigen se14–3. Ongoing studies will address the frequency of immune responses in a larger group of patients to determine the role of these antigens for the host response against primary brain tumors.

144
**Delta-24 adenovirus induces powerful anti-cancer effect in medulloblastomas with disruption of the Shh pathway.** R. Stolarek, C. Gomez-Manzano, G. Suttle, C. Conrad, W. Yung, J. Fueyo, MD Anderson Cancer Center (Houston, USA)

Brain tumors are the second most frequent solid tumor in children. Medulloblastomas account for the majority of brain tumors in pediatric patients and are resistant to conventional therapies. Since the Rb regulatory network is abnormal in these tumors through abnormal activation of the sonic hedgehog pathway, this network of proteins offers a suitable target for the development of novel therapeutic approaches for medulloblastomas. We have previously characterized a replication competent adenovirus, termed Delta-24, whose replication phenotype is restricted to cancer cells with inactivation of the Rb function. Our previous studies showed that Delta-24 replicates efficiently in human glioma cells in vitro and in vivo inducing complete tumor regression in an intracranial animal model of gliomas. In this study we aimed to test the anti-cancer activity of Delta-24 in medulloblastomas. In vitro experiments were performed using the medulloblastoma cell line Daoy. Infection of Daoy with Delta-24 resulted in accumulation of cells in the S phase of the cell cycle as demonstrated by flow cytometric analyses of the DNA content. The infection of Daoy cell with Delta-24, at a doses of 0.1, 1.0, 2.5, 5.0 and 10 MOIs decreased the cell viability in a dose-dependence manner as assessed by crystal violet and trypan blue exclusion test (from 72.2% at 0.1 MOI to 0% at 10 MOI). On the contrary, Delta-24 was unable to replicate, and therefore to induce cell death, in both normal fibroblasts and astrocytes. Importantly, blocking the sonic hedgehog pathway with cyclopamine resulted in Rb activation and prevented adenoviral replication, demonstrating that Delta-24 targets this specific pathway in medulloblastomas. Animal experiments to test the in vivo efficacy of Delta-24 in a medulloblastoma animal model are in progress.

This study represents the first attempt to test oncolytic therapy in the most common type of brain tumor in children. Our results indicate that infection of medulloblastoma cells with conditionally replicative oncolytic adenoviruses exploits the genetic abnormalities of these tumors and results in potent and selective anti-cancer effect.

# Session 24

## Neuro-oncology – 2

145
**Characterization of the U-87 MG animal model for its use in oncolytic therapy for gliomas.** C. Gomez-Manzano, H. Jiang, F. Lang, W. Yung, G. Fuller, J. Fueyo, MD Anderson Cancer Center (Houston, USA)

Animal models to test the efficacy of conditionally replicative adenoviruses require the use of human tumor xenografts implanted intracranially in nude mice. Following intracranial injection of 5x10e5 U-87 MG cells into the right basal ganglia of nude mice, all animals die by day 25 post-implantation. PCNA staining showed high proliferation activity starting from a few hours after implantation to the last day of the experiment. Four days after cell implantation, vessels surrounding the tumor displayed changes in morphology, including an enlarged diameter and disorganized structure. Some vessels interacted physically with peripheral tumor cells. From day 15 to day 20 post-implantation, we observed massive and hypervascularized tumors. After day 20, the vessels were numerous but thinner than those observed at previous time points. We did not observe infiltration of normal tissue at any point during tumor growth. The tumor caused the death of the animals by mass effect. Treatment of the U-87 MG tumors with increasing doses of a tumor selective oncolytic adenovirus resulted in dose-dependent increases of animal survival. Examination of the expression of early and late adenoviral genes showed a progressive spread of the adenovirus inducing an enlarging area of central necrosis. Doses higher than 10e7 pfu resulted in total suppression of tumors with production of microcalcifications and microcystic formations. In this study, we have performed a time point analysis of the evolution of angiogenesis in the U-87 MG animal model and established correlations with the histology and growth patterns of the xenografts. These data indicate that the growth and kinetics of U-87 MG tumors make the model suitable for adenovirus-based therapies for gliomas.

146
**PCV chemotherapy for newly diagnosed low grade oligodendroglial tumours: final results of a phase II study.** R. Rudà, A. Costanza, M. Nobile, R. Mutani, R. Soffietti, S. Giovanni Battista Hospital (Turin, I)

Background: PCV chemotherapy is highly effective in anaplastic and aggressive oligodendrogliomas and oligoastrocytomas, whereas its role in the treatment of low grade tumors is still unclear.

Objective: The objective of this study was to determine the benefits and toxicity of PCV chemotherapy in patients (pts) with newly diagnosed nonenhancing low grade oligodendroglial tumors.

Patients and methods: Patients with newly diagnosed after biopsy or partial resection oligodendrogliomas and oligoastrocytomas (grade II WHO) were treated with up to six cycles of standard PCV. Before chemotherapy all patients had nonenhancing tumors on MRI and a Karnofsky score ≥ 70. Volumetric estimates of T2-signal abnormalities on axial MR images were determined and tumor response was evaluated by the conventional criteria of Macdonald.

Results: Twenty-four patients (17 oligodendrogliomas and 7 oligoastrocytomas), with a median age of 37 years (range 24–61) are evaluable for response: complete response (CR) in 0/24; partial response (PR) in 6/24 (25%); stable disease (SD) in 18/24 (75%); progressive disease (PD) in 0/24. Among patients with SD, 5/24 (21%) showed a reduction of tumor volume of 20–40% (minor response, mR). Overall partial + minor responses account for 46% of patients (11/24). We observed early responses (maximum response within the first 3 cycles) in 7/11 patients and delayed responses (maximum response after 6 cycles) in 4/11 patients. A reduction or disappearance of seizures was observed in 5/6 patients (2 PR, 2 mR, 1 SD). Median time of follow up is 34 + months (10+ – 61+ months), with 22/24 patients still without tumor progression.

Conclusions: Standard PCV is active in low grade nonenhancing oligodendroglial tumors, even if at a lesser degree than in the anaplastic and aggressive forms. Patients with epilepsy may benefit from chemotherapy. New tools for evaluating response to treatments of these slow growing tumors are needed: PET with methionine and 1HMRS spectroscopy are under evaluation.

147
**Second line PCV chemotherapy in recurrent oligodendroglioma after first line Temozolomide chemotherapy.** V. Triebels, M. Taphoorn, A. Brandes, J. Menten, M. Frenay, J. Kros, C. van der Rijt, R. Enting, I. van Heuvel, M. van den Bent, Canisius Wilhelmina Hospital, UMC, Universital Hospital, UZ Gasthuisberg, Antoine Lacassagne, Erasmus MC (Nijmegen, Utrecht, NL; Padua, I; Leuven, B; Nice, F; Rotterdam, NL)

Background: Both PCV (Procarbazine CCNU Vincristine) chemotherapy and Temozolomide (TMZ) are active in recurrent oligodendroglial tumors after prior radiation therapy (RT), with 55–70% of patients (pts) responding. The results of second line TMZ after prior PCV are less favourable, with approximately only 25% of pts responding which may be due to cross resistance between these alkylating agents. No data exist on second line PCV in patients treated in first line with TMZ. We investigated the objective response rate and time to progression to second line PCV in recurrent oligodendroglioma after RT and first line TMZ chemotherapy in a cohort of pts treated within a prospective clinical trial (EORTC 26971) on first line TMZ in this tumor type.

Patients and methods: Pts were eligible if they had been treated within EORTC 26971 study, had progressive disease after TMZ (regardless of the response to TMZ), had measurable disease (enhancing lesion of at least 1 cm diameter on neuro-imaging) and adequate bone marrow, renal and hepatic function. Response was assessed every two cycles with MRI according to MacDonald's criteria. Toxicity was assessed according to the NCI-CTC criteria.

Results: Of the 38 pts eligible in EORTC study 26971, 3 pts were still free from progression, 2 pts were lost to follow up at the time of progression, 2 pts received other second line treatment, 5 pts deteriorated too rapidly to receive further treatment, 2 pts refused further treatment and 24 pts received second line PCV. Thirteen of the latter 24 pts responded to first line TMZ, which is similar to the 54% overall response rate observed in EORTC study 26971. In total 83 cycles of PCV were administered (median 3). Four of 24 pts (17%, 95% CI [5–37%]) showed an objective response to second line PCV: complete response (CR): 2, partial response (PR): 2. In 11 pts stable disease was achieved. Median time to progression (TTP) for all patients was 6 months (mo) and 10 mo for the 15 CR, PR and SD pts. Median survival has not yet been reached. Six pts discontinued PCV because of grade III/IV haematological toxicity. Other side effects were in general mild. Information about the allelic loss of chromosome 1p and 19q of these 24 pts will be presented at the meeting.

Conclusion: Although only 17% of patients responded to second line PCV chemotherapy, a significant number of pts stabilized and at 6 months 50% of pts were still free from progression.

Therefore, PCV can be considered as second line treatment for recurrent oligodendroglial tumors after first line TMZ.

148
**The peripheral T-cell repertoire in patients with malignant gliomas – extensive clonal expansions of CD4+ and CD8+ T-cells.** F. Vogel, V. Grummel, S. Cepok, O. Bozinov, O. Grauer, U. Sure, B. Hemmer, University of Marburg, University of Regensburg (Marburg, Regensburg, D)

Malignant brain tumors – in particular malignant gliomas – represent one of the major therapeutic challenges in neurology. Since these tumors are highly resistant to conventional therapies, recent research has been focused on developing alternative treatment strategies. Tumor-specific T cell therapies have been applied to a variety of tumors, most successfully to malignant melanoma. However, little is known about the T cell response in patients with malignant gliomas. Histopathological studies, demonstrating microglia activation, macrophage and T cell infiltrates, suggest, that an acquired immune response is primed against the tumor, although it is not sufficient to control tumor growth. In this study, we investigated the T-cell receptor expression of CD8 + and CD4 + peripheral T cells using a flow cytometric and spectratyping approach. The peripheral expression profiles of 30 glioma patients were carefully compared to healthy age matched controls. We found significant repertoire alterations more frequently in patients than controls, involving both CD4 + and CD8 + T cells. These repertoire changes in glioma patients were caused by clonotypic expansion of T cells. Using spectratyping and clonotypic rtPCR we are currently investigating, which of those peripherally expanded clonotypic T cells accumulate in the tumor. Identification of tumor-associated T cells may provide a basis for the development of future immune based therapies for malignant gliomas.

149
**Brain metastases from non small-cell lung cancer: efficacy of fractionated stereotactic radiotherapy.** A. Costanza, G. Beltramo, E. Laguzzi, C. Mantovani, M. Nobile, R. Rudà, R. Soffietti, S. Giovanni Battista Hospital (Turin, I)

Objective: We have prospectively evaluated the efficacy and toxicity of Hypofractionated Stereotactic Radiotherapy (HSRT) in patients with brain metastases from non small-cell lung cancer (NSCLC).

Background: HSRT delivers a high conformal dose distribution in few fractions using a relocatable stereotactic frame. Modeling studies have demonstrated a potential biologic advantage for HSRT over radiosurgery in the treatment of brain metastases.

Design/methods: Eligibility criteria for HSRT were: 1) age ≥ 18 years; 2) Karnofsky Performance Score ≥ 70; 3) controlled primary tumor and systemic disease; 4) 1–3 brain metastases on MRI; 5) lesion diameter ≤ 3.5 cm. From April 1998 to May 2002 we enrolled 40 patients (32 males, 8 females, with median age 59.5 years, range 43–77). We treated 46 lesions (41 supratentorial, 5 infratentorial, with median lesion volume 7.2 $cm^3$, range 0.4–24.3). The primary tumor histology was squamous cell carcinoma in 24 patients (60%), adeno-carcinoma in 12 (30%), large-cell carcinoma in 2 (5%) and mixed cell carcinoma in 2 (5%). A median dose of 24 Gy (range 16–36 Gy) was delivered in 3 fractions to the Gross Tumor Volume plus a 2 mm margin with a X 6 MV linear accelerator modified for HSRT. Local tumor control was defined as no increase in the tumor's maximal diameter on axial plane images on MRI. Age, gender, baseline KPS, histology, volume, location and total dose were analysed for their influence on local tumor control by univariate and multivariate Cox regression analysis.

Results: The median follow-up period was 12 months (range 2–35). The actuarial 1-year local tumor control rate was 69%, the actuarial overall survival rate at 12 and 24 months was of 54% and 33% respectively. The median survival time was 16 months. No significant acute toxicity was seen. Multivariate analysis revealed the following factors to be statistically significant predictors of local tumor control: histology (local control rate 65% in adenocarcinoma vs 50% in squamous cell carcinoma at 30 months, $p < 0.002$), location (local control rate 65% in supratentorial lesions vs 20% in infratentorial at 30 months, $p < 0.01$) and volume ($p < 0.04$).

Conclusions: Overall, high local control and low morbidity rates suggest that HSRT is an effective and safe modality, especially in patients with small lesions, supratentorial location and adenocarcinoma histotype.

# Session 25

## Cerebrovascular disorders – 3

150
**PC-trial – patent foramen ovale and cryptogenic embolism.** H. Mattle, B. Meier, S. Windecker, Inselspital Bern on behalf of the PC Trial Group

Introduction: Several studies have shown an association of cryptogenic stroke and embolism with patent foramen ovale (PFO). The question how to prevent further events is unresolved. The PC-Trial compares percutaneous PFO closure and best medical treatment.

Methods: The PC-Trial is a prospective, multi-center, randomized clinical trial comparing the efficacy of percutaneous PFO closure using the Amplatzer PFO occluder with best medical treatment in patients with cryptogenic embolism, i.e. mostly cryptogenic stroke. Warfarin for 6 months followed by antiplatelet agents is recommended as medical treatment. Randomisation is stratified according to patients' age (18–45 versus 45–60 years), presence of atrial septal aneurysm (ASA) and one or more embolic events before randomisation. Primary endpoints are death, nonfatal stroke and peripheral embolism.

Principal investigators: Bernhard Meier, Cardiology, bernhard.meier @insel.ch and Heinrich Mattle, Neurology, heinrich.mattle@insel.ch, Bern, Switzerland.

Study Management: Kurt Quitzau, InterCorNet, kq@intercornet.com, Zürich, Switzerland.

Protocol: http://www.drabo.de/dl/pctrial_ch.pdf

Sponsor: AGA Medical, Golden Valley, MN, USAResults. Recruitment started with 20 patients in 2000. As of Jan. 18, 2003, 119 patients had been randomized. Their mean observation time is 1.3 years. At present 31 centers in Australia, Austria, Canada, Denmark, Germany, Poland, Slovakia, Switzerland, and the United Kingdom have ethical approval. Seventeen centers are actively randomizing patients.

Discussion: Randomization is planned until 2004. With 410 patients and a projected reduction of the annual event rate from 3 % to less than 1 % the power will be 80 % at a level of 0.049 to provide in 2008 the answer whether percutaneous PFO closure represents an alternative to antithrombotic treatment to prevent paradoxical embolism.

151
**Donepezil-treated patients demonstrate cognitive benefits: a comparison of Alzheimer's disease versus vascular dementia.** D. Wilkinson, R. Pratt, C. Perdomo, Moorgreen Hospital, Eisai Inc. (Southampton, UK; Teaneck, USA)

Objective: To compare the cognitive effects of donepezil and placebo treatment, as assessed by the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog), in vascular dementia (VaD) versus Alzheimer's disease (AD) patients.

Methods: Data from a combined analysis of two 24-week, double-blind studies in patients with probable or possible VaD, together with results from a similar study in patients with probable AD, are presented. Patients were randomized to receive placebo or donepezil (5 or 10 mg/day). Data are presented separately for those patients that showed any improvement from baseline and those who showed any decline from baseline on the ADAS-cog for the intent-to-treat population at Week 24 (LOCF analysis).

Results: 1219 VaD patients (placebo, n = 392; donepezil 5 mg/day, n = 406; 10 mg/day, n = 421) and 473 AD patients (placebo, n = 162; donepezil 5 mg/day, n = 154; 10 mg/day, n = 157) were enrolled. More donepezil- than placebo-treated patients showed improvement, both in VaD (placebo, 37 %; donepezil 5 mg/day, 53 %; 10 mg/day, 54 %), and in AD (placebo, 27 %; donepezil 5 mg/day, 38 %; 10 mg/day, 54 %). Fewer donepezil- than placebo-treated patients declined, both in VaD (placebo, 19 %; donepezil 5 mg/day, 12 %; 10 mg/day, 12 %), and in AD (placebo, 42 %; donepezil 5 mg/day, 20 %; 10 mg/day, 19 %).

Conclusions: Although there were a variety of responses observed within all treatment groups, both in AD and in VaD, donepezil-treated patients were more likely than placebo-treated patients to show cognitive improvements, as measured on the ADAS-cog. The number of patients showing a response to donepezil (10 mg/day) was similar in VaD and AD patients, with > 50 % improving by 4 or more points and > 80 % showing no decline. Placebo-treated VaD patients were less likely to show decline than placebo-treated AD patients. Therefore, differing treatment effect sizes in VaD versus AD may be explained by differences in placebo response, since in VaD patients significant treatment effects have to be driven by improvement.

152
**Cerebral atherosclerosis in sleep-disordered breathing.** D. Kaynak, B. Göksan, H. Kaynak, N. Degirmenci, S. Daglioglu, University of Istanbul (Istanbul, TR)

Introduction: Epidemiological studies have shown a strong association between sleep-disordered breathing (SDB) and cerebrovascular disease. This association could be due at least in part to an increase in the progression of the atherosclerosis process at the level of cerebral arteries. We investigated atherosclerotic degeneration at the level of carotid arteries in subjects with SDB to evaluate whether there is a link between SDB severity and atherosclerosis.

Subjects and methods: We prospectively studied 114 consecutive male patients between 40–65 years old, referred to our sleep laboratory for the evaluation of snoring or daytime sleepiness. Subjects were examined with a standard protocol that included an interview, clinical examination, polysomnographic and ultrasonographic evaluation. Major vascular risk factors were determined. Subjects were divided into 3 groups; habitual snoring, mild-moderate obstructive sleep apnea syndrome (OSAS), and severe OSAS respectively. Measurement of intima-media thickness (IMT) and the presence of plaque was determined by ultrasonographic evaluation.

Statistics: Analysis of variance with post-hoc comparisons (Tukey HSD) was used to determine whether demographic, polysomnographic, clinical and ultrasonographic data variables varied across the RDI categories. Categorical data were analyzed with the chi-square test. Multiple linear regression analysis and logistic regression analysis were performed to identify demographic, clinical and polysomnographic predictors of IMT and plaque.

Results: OSAS groups had significantly higher IMT values compared to the habitual snoring group ($F = 12{,}08, < 0.001$). There was no significant difference however between the mild-moderate and severe OSAS groups with respect to IMT values. Three groups were significantly different with regard to prevalence of plaque ($p < 0.001$).The age and BMI were found to be significantly associated with IMT (Beta = 0.02, $p < 0.005$ and Beta = 0.03, $p < 0.05$ respectively) while age and RDI were found to be most likely predictors of plaque ($B = 0.08$, $p < 0.05$ and $B = 0.04{,}52$, $p < 0.05$ respectively). There were no significant differences between three groups with respect to age, prevalence of hypertension, diabetes, smoking, total cholesterol and total triglyceride levels. Three groups were significantly different in BMI and duration of sleep related breathing disorder ($F = 5{,}43$ $p < 0{,}05$, $F = 14{,}83$ $p < 0{,}001$ respectively). Post hoc testings revealed that severe OSAS group had significantly higher BMI than habitual snoring and mild-moderate OSAS groups and two OSAS groups had significantly longer duration of sleep related breathing disorder than habitual snoring group.

Conclusion: There is a dose-response relationship between the severity of sleep-disordered breathing and the IMT and the prevalence of stenoocclusive lesions. OSAS predispose an atherosclerosis process in OSAS patients and precipitate stenoocclusive lesions particularly when associated with higher RDI.

153
**Delirium in acute subarachnoid haemorrhage.** C. Menger, L. Caeiro, J. M. Ferro, R. Albuquerque, M. L. Figueira, Hospital Santa Maria (Lisbon, P)

Background: Delirium may be a presenting feature in acute subarachnoid haemorrhage (SAH).

Purpose: The aim of this study was to investigate the presence and the risk factors for delirium in the first four days after SAH onset and to analyse the relation between delirium and location and amount of haematic densities.

Patients and Methods: We assessed delirium in a sample of 50 consecutive patients (mean age 56 years old) with an acute (≤ T4 days) subarachnoid haemorrhage (25 with an aneurysm, 4 with a perimesencephalic haemorrhage and 21 with no aneurysm), previous to surgery, using DSM-IV-R criteria and the Delirium Rating Scale (DRS). DRS score was related to: 1) the median of the haematic densities at 10 basal cisterns/fissures and the median of the 4 ventricles, using the Hijdra et al. rating scale 2) the haematic densities in the prepontine cistern and the convexity of the brain and 3) hydrocephalus, using the bicaudate index, obtained from review of admission CT scans.

Results: Nine acute SAH patients presented delirium. An alertness disturbance ($x^2 = 14.7$, $p = 0.001$, OR = 32.0, 95 %CI = 3.0 to 345.9), the presence of aphasia ($x^2 = 14.7$, $p = 0.001$, OR = 20.5, 95 %CI = 1.8 to 230.5) and a higher score on the Hunt and Hess scale ($x^2 = 10{,}05$, $p = 0.02$, OR = 6.8, 95 %CI = 1.2 to 36.9) were associated with a higher frequency of delirium. Higher amounts of intraventricular haematic densities ($U = 74.5$, $p = 0{,}006$) and hydrocephalus ($U = 86.5$, $p = 0.02$) were associated with higher DRS scores. Two delirious patients had basofrontal haematomas.

Conclusion: Delirium was detected in 18 % of acute SAH patients. Intra-

ventricular bleeding, hydrocephalus and basofrontal haematomas contributed to the pathogenesis of delirium, through damage to anatomical structures that integrate networks subserving sustained attention, declarative memory and the expression of emotional behaviour.

154
**Stroke and illicit drug abuse: five observations and a review of 401 cases.** N. Weiss, S. Crozier, M. Obadia, Y. Samson, Hôpital de la Salpêtrière (Paris, F)

Stroke may occur after use of cocaine or other illicit drugs but the type and the frequency of drug-related stroke remains poorly known. For example, the percentage of drug-related stokes vary in the literature from 2 to 39% in young adults. We report 5 cases of ischemic stroke after drug abuse and we reviewed 78 papers about 401 cases and 3 case-control studies. This review revealed a majority of men (68% vs. 32% of women). Mean age was 33.6 years and was similar in ischemic and hemorrhagic stroke. We found 54% of hemorrhage and 39% of ischemic events. Most strokes occurred in the first 24 hours (92%) after drug intake. Cocaine hydrochloride and crack were the most frequent used drug (76%). New drugs like ecstasy and other amphetaminic derivatives are less implicated in stroke perhaps because of the difficulty to prove the presence of the substance (standard biological analysis positive only for 5 days after intake).

Statistical analysis revealed significantly more hemorrhagic than ischemic stroke for amphetamines and cocaine, respectively 5 and 2 times more ($p < 0.05$), whereas the same proportion of hemorrhage and ischemic events was found for crack.

This review seems to confirm the association between illicit drug abuse and stroke, and suggests that different types of drugs may cause different types of stroke (i. e. ischemic vs. hemorrhage). It also shows that the real incidence of drug-related stroke remains poorly known. Knowledge may be improved by programs of systematic research of drug abuse by interview and toxicology analysis, in young adults admitted in stroke centers.

155
**Does secondary prevention differ in practice after cerebral ischaemia and coronary heart disease? Results of a pilot hospital-driven study.** M. Girot, D. Deplanque, F. Pasquier, A. Destee, D. Leys, Hôpital Roger Salengro (Lille, F)

Background: Ischemic strokes and coronary heart diseases (CHD) share similar pathophysiological mechanisms. Preventing subsequent vascular events is an important issue in both disorders. Secondary prevention consists of controlling vascular risk factors and introducing antithrombotic therapy. The question of whether the quality of secondary prevention differs in practice between both disorders remains unsettled. The aim of this study was to compare the secondary prevention within 6 years between patients with history of CHD and of cerebral ischemia.

Method: We included 107 consecutive patients with history of cerebral ischemia and 85 consecutive patients with history of CHD. We compared in both groups presence and management of risk factors, blood pressure and biological parameters during hospitalization, the type of follow-up, and whether antithrombotic therapy was still prescribed. Whether patients were properly treated or not, was determined by a comparison between their current treatments and guidelines.

Results: Secondary prevention was not appropriate in 76 of 107 patients (71%) with previous cerebral ischemia and 73 of 85 patients (86%) with CHD. With the exception of arterial hypertension which was more often diagnosed in CHD patients, identification of other risk factors, presence of antithrombotic therapy, proportion of patients with a blood pressure > 140/90, 150/90, or 160/90 mmHg; with a glycemia > 126 mg/dl; with a total cholesterol > 190, or 250 mg/dl did not differ between both groups. Patients with previous CHD were two-fold more likely to have stopped smoking after their vascular event ($p < 0.05$).

Conclusion: Many patients were not properly treated in both groups. Differences between practice and guidelines were more important in the CHD group, because guidelines are more strict, but this did not lead to significant differences in the proportion of patients who still received antithrombotic drugs and in the proportion of patients with untreated risk factors.

# Session 26

## Cerebrovascular disorders – 4

156
**Prevention from embolisation during carotid angioplasty with the Parodi anti-emboli system.** K. Rabe, H. Gödel, R. Perron, C. Rubel, W. Pfeil, K.-F. Beykirch, R. Theis, H. Sievert, Cardiovascular Center Bethanien, Bethanien Hospital (Frankfurt, D)

Background: The majority of neurological complications during carotid angioplasty are caused by debris which embolizes to the brain. To prevent these events we used the Parodi Anti-Emboli System during the intervention.

Patients: Carotid angioplasty and stenting under flow reversal was attempted in 54 patients (age 69 ± 9). Diameter stenosis ranged from 51 to 95% (77 ± 10), the length of the lesion ranged from 0,2 to 25 mm. Three lesions contained visible fresh thrombus. 5 patients had a contralateral stenosis of > 50% and one patient had a contralateral occlusion. One lesion was in the carotid siphon.

Methods: Reversal of blood flow in the internal carotid artery was achieved by occlusion of both the common and the external carotid artery during the procedure using the Parodi Anti-Emboli System. The blood flew through the guiding catheter and after passing a filter outside the body into the contralateral femoral vein. Conventional wires and balloons were used. A stent was implanted in all lesions.

Results: The device could easily be introduced into the common carotid artery. Balloon occlusion of the common carotid artery as well as the external carotid artery was achieved in all patients. The occlusion time ranged between 3 and 37 min (14 ± 7) and was tolerated reasonabl in all patients except three, in whom the balloon had to be deflated repeatedly during the procedure. Seven other patients experienced transient neurological symptoms during balloon occlusion without need for interruption of the procedure. In all patients angiographic success was achieved without immediate complications. In one patient a PRIND occurred several hours later, another patient suffered from amaurosis fugax two weeks after the procedure. Macroscopic visible debris was found in the filter in 50/54 patients.

Conclusions: Flow reversal in the carotid artery for embolism protection during carotid angioplasty is feasible in the majority of patients. Arteriosclerotic debris is kept back efficiently. If the balloon occlusion is not tolerated, the procedure can be completed by deflating the balloon intermittently.

157
**Spontaneous intraluminal thrombus in the common carotid artery. A retrospective study of 8 patients.** S. Bouly, J. M. Blard, E. Touze, D. Leys, J. L. Mas, J. P. Neau, A. Le Bayon, M. Dauzat, V. Gautier, O. Delhaume, G. Castelnovo, P. Labauge, Department of Neurology, Department of Radiology (Nimes, Montpellier, Paris, Lille, Poitiers, F)

Objective: To determine the natural history of patients with intraluminal spontaneous thrombus in the common carotid artery.

Background: Spontaneous intraluminal thrombus diagnosis is based on ultrasound aspects, consisting of normal-medial thickness, adherent thrombus, regular inner surface with isoechoic or hypoechoic composition, aspect of cigar-shaped filling defect. They are a rare cause of strokes. Prognosis and treament are not well known.

Design/methods: This multicentric retrospective study has included patients with a diagnosis of spontaneous intraluminal thrombus of the common carotid artery and acute stroke. Following complementary exams were mandatory: brain CT scan or MRI, MRA or conventional angiography, color duplex flow imaging, extensive research of a coagulopathy, transoesophageal echocardiography. Follow up consisted of clinical evaluation and color duplex flow imaging. Exclusion criteria: atherosclerosis of the carotid artery, embolic cardiac source, dissection of the carotid arteries.

Results: Eight patients were included in this study (6 women/2 men), mean age: 49.5 years old (range: 47–52). Cardiovascular risk factors: 0 (4 patients), 1 (3 patients), 2 (1 patient). Seven patients presented a stroke and one a TIA. The mechanisms of the stroke/TIA were embolic (n = 6) or hemodynamic (n = 2). Thrombus involved common, internal and external carotid artery in all of the patients. Occlusion was incomplete in 6 patients and complete in 2. Initial treatment: all of the patients were treated by intravenous/subcutaneous anticoagulants. Short term evolution under anticoagulant: absence of new strokes or TIA (n = 7); new TIA (n = 1). Only one patient was treated by surgery, because of new TIA on the 5th day. Initial ultrasound follow up (mean: 8 days, range: 6–10 days): complete dissolution of the thrombus (n = 5), incomplete (n = 1), absence of change (n = 1). Long

term follow up (mean: 12 months): all of the patients were treated by oral anticoagulants then by antiaggregants. No recurrence of TIA/strokes was observed. Etiology of the thrombus was unknown (n = 3); leukemia relapse (n = 1), microcytic iron deficiency anemia (n = 4). Patients with anemia were all women. The mean hemoglobin level was 9.25 g/dl (range: 8.7–10 g/dl). Anemia was associated with thrombocytosis in 3 cases (mean platelets count: 449 000/mm$^3$; range 408 000–479 000/mm$^3$). Etiology of the anemia was menorrhagia (n = 2) or unknown (n = 2).

Conclusion: Spontaneous intraluminal thrombus of the carotid artery is a rare cause of stroke. Initial treatment has to be based on anticoagulant. Thrombectomy is not usually mandatory. Half of the cases revealed iron deficiency anemia.

158

**Aspirin non-responders in stroke and myocardial infarction prevention.** V. Karepov, V. Karataev, G. Tolpina, L. Trofimova, Medical Center "M. E.T.E.O.R" (Bat-Yam, IL)

Background: Aspirin (ASA) non-respondence is one of the causes of the low clinical benefit of this antiplatelet agent in ischemic stroke (IS) and myocardial infarction (MI) prevention. This phenomenon must be determined and corrected in the practice before the vascular catastrophe has happened.

Objective: The aim of the study was to evaluate the platelet functions in the patients with high risk for IS and MI and ASA using for primary prevention, as well as in the patients using ASA for secondary IS and MI prevention after the first-ever ischemic events.

Materials and Methods: Citrate whole blood samples were drawn from 25 ASA users with high risk for IS and MI (group A), from 25 ASA users after first-ever MI (group B), and from 25 ASA users after single IS (group C). Blood samples were studied using platelet function status (PFS) analyzer. The method is based on an evaluation of the closure time for apertures occlusion after epinephrine (EPICT) and adenosine-5-diphosphate (ADPCT) tests. The normal time for these tests in our laboratory was 80–170 sec for EPICT, and 71–114 sec for ADPCT test. The results of the PFS analysis were defined as normal or abnormal (prolonged or shortened EPICT or ADPCT). The patients were determined as aspirin non-responders if their EPICT was less than 150 sec, and/or ADPCT was 66 sec or less. Statistical analysis was done using the Student's t-test. The results were compared between three clinical groups: A, B and C.

Results: Of the 25 patients in group A, 7 patients (28 %) have no response to ASA. In groups B and C (secondary MI and IS prevention), the number of ASA non-responders were 9/25 (36 %) and 8/25 (32 %), respectively, n. s.

Conclusions: ASA preventive therapy was not sufficient in 28–36 % of the patients from the different groups of the population. ASA non-respondence can explain the clinical phenomenon of ASA failure: occurrence of MI and IS while patients are on ASA. This method of PFS evaluation could be helpful for antiaggregant therapy monitoring and for more effective use of ASA doses.

159

**Monitoring systemic thrombolysis with transcranial colour-coded duplexsonography.** G. Gahn, A. Kunz, V. Pütz, U. Becker, T. Goldhagen, H. Reichmann, University of Technology (Dresden, D)

Objective and Background: To assess revascularisation rate of middle cerebral artery (MCA) occlusion in patients with acute cerebral ischemia treated with systemic thrombolysis.

Methods: We prospectively monitored MCA-occlusion in 22 consecutive patients with repeated transcranial color coded duplexsonography (TCCD). All patients had TCCD evaluation before, 30, 60, 90 minutes, and 12–24 hours after initiation of thrombolysis. Twelve patients had echo enhanced TCCD (galactose palmitic acid based echo enhancing agent) because of limited acoustic temporal bone windows. Patients were eligible for systemic thrombolysis within the six hours time window after onset of cerebral ischemia fulfilling the clinical and CT-criteria of ECASS II and proven MCA-occlusion by CT-angiography or TCCD. Sixteen patients underwent thrombolysis with rt-PA 0.9 mg/kg, four patients with rt-PA 0.45 mg/kg combined with Abciximab 0.125 mg/kg bolus followed by a 12 hrs infusion of Abciximab 0.125µg/kgxmin. Two patients received Abciximab alone 12 hours after beginning of ischemic symptoms.

Results: Mean time interval between onset of symptoms and initiation of thrombolysis was 232,4 ± 60 min (median 200, range 90–720 min). Mean NIHSS on admission was 18,4 ± 6,7 (NIHSS unknown in one patient because of early intubation secondary to aspiration, before intubation suspected NIHSS 7). In three patients TCCD depicted MCA-recanalisation within 90 minutes, in eight others within 24 hours. Nine out of these 11 patients had remarkable improvement in NIHSS and a favourable outcome [Rankin scale (RS) 0–2]. Eleven patients had persistent MCA-occlusion. Ten out of these 11 showed no improvement in NIHSS or deteriorated (RS 4–6). One had delayed recanalisation after 72 hours and regained functional independency (RS 1). Symptomatic hemorrhagic infarction occurred in one patient with persistent MCA-occlusion. Another one died from pulmonary embolism.

Conclusion: In this ongoing prospective study, TCCD showed early recanalisation of MCA-occlusion after systemic thrombolysis in 13,6 % and delayed recanalisation in additional 36,4 % of patients. Out of these patients, 81,8 % had a favourable outcome, all other patients had a poor outcome. Recanalisation of MCA-occlusion within the first 24 hours after onset of cerebral ischemia appears to be a predictor of functional outcome.

160

**Carotid angioplasty as treatment of cerebrovascular occlusive disease.** K. Rabe, H. Gödel, R. Perron, C. Rubel, W. Pfeil, K.-F. Beykirch, R. Theis, H. Sievert, Cardiovascular Center Bethanien, Bethanien Hospital (Frankfurt, D)

Introduction: Carotid angioplasty and stenting is a routine procedure in many centers worldwide. However, information about the long-term results is still limited.

Methods: Since February 1994, we have treated 308 high-grade lesions of the internal carotid artery in 276 consecutive patients. In 229 lesions the procedure was performed under embolic protection. 325 stents were implanted: Acculink [15]; BARD [32]; Carotid Wall [76]; Precise [46]; NexStent [16]; Smart [27]; Wallstent [106]; others [10]. Follow-up investigations included a neurological examination and duplex ultrasound after 6 and 12 months, thereafter annually.

Results: The patients' age ranged from 40–90 years with an average of 70 ± 9 years. 135 lesions were symptomatic. 139 patients had a contralateral stenosis or occlusion. 65 % of the patients suffered from coronary heart disease, 67 % from hypertension, 28 % from diabetes mellitus, 50 % from hyperlipidemia and 50 % were previous or current smokers.

Mean lesion diameter was 77 ± 10 %, mean length of lesion 9 ± 5,5 mm.

The procedure was technically successful in 306/308 lesions. 11 patients had one of the following acute or subacute (< 30 days) complications: Cerebrovascular death in 0, non-fatal major stroke in 3, minor stroke in 2, central retinal artery occlusion in 1, asymptomatic occlusion in 1, non-cerebrovascular death in 4 patients. During follow-up (311 patient years) 4 major, 2 minor strokes and 17 asymptomatic re-stenoses occurred. 18 patients died from non-cerebral causes.

Conclusion: Carotid angioplasty and stenting leads to results which are at least comparable to the results of endarterectomy. Re-stenoses and late complications are rare.

161

**Do prognostic markers and stroke care organization influence intracerebral haemorrhage outcome?** S. Escalante, E. Díez Tejedor, M. Blanco, B. Fuentes, R. Navarro, J. M. Laínez, J. Vivancos, J. Castillo, R. Leira, J. Tejada, A. Arboix, I. Casado, C. Muñoz, J. Masjuan, T. Del Ser, J. Díaz, F. Díaz, Hospital La Paz, Ictus Project on behalf of the Spanish Cerebrovascular Study Group (SCSG)

Introduction: A large number of factors are associated with intracerebral haemorrhage (ICH), we analyse their influence on ICH outcome. Benefits of stroke units (SU) on acute stroke management have been widely demonstrated as compared to general wards. There are few studies evaluating these benefits on ICH management. We evaluated organised inpatient care importance on ICH.

Methods: Prospective, multicentric, observational study. Inclusion criteria: ICH detected in CT Scan < 24h. Exclusion criteria: GCS 6, previous illness with a life expectancy < 6m, SAH, recent craneoencephalic injury.

BP, temperature, glycemia, fibrinogen, BSR, volume, location, canadian score scale (CSS), GCS at admission and modified Rankin Scale (mRS) and CSS at 3m. Distribution: general ward, Stroke team and SU. We analysed mortality and 3m functional outcome and prognostic markers. Chi square, t student and multivariate logistic regression were carry out.

Results: 108 patients; 63,6 %/36,4 % (M/F); age 69.6–11.7y. Etiology: 73.3 % HBP, Volume: 50.95–58.4cc. Distribution in SU 24.1 %, ST 34.4 % and general ward 43.5 %. Mortality: 13.9 %. Hyperglycaemia ($p = 0.009$) and lower GCS and CSS, were related with mRS > 2 ($p < 0.000$). Volume ($p = 0.005$) and lower GCS and CSS were related with mortality. No significant differences with the other variables. Between groups, there were no significant differences in mRS neither in mortality, but we observed a trend to lower mortality at SU, in patients with worse initial situation (hyperglycaemia and lower GCS were detected at SU ($p = 0.055$)).

Conclusions: Although hyperglycaemia and CSS are related with mor-

tality, only the age and CSS were independent factors and volume with mortality. No significant differences regarding the organization model were found but a trend to higher survival in SU was observed, even on patients with worse initial situation.

162
**Endovascular stent-assisted angioplasty for symptomatic middle cerebral artery stenosis.** T. H. Lee, D. H. Kim, B. H. Lee, H. J. Kim, D. S. Jung, C. H. Choi, Pusan National University Hospital, Chosun University Hospital, Metrohospital Anyang (Pusan, Kwangju, Anyang, KOR)

Purpose: To assess the feasibility, safety, and effectiveness of stenting for symptomatic middle cerebral artery stenosis.

Subjects and Methods: Stent-assisted angioplasty was performed on 12 patients with symptomatic middle cerebral artery stenosis (> 50 %). We retrospectively analyzed the technical success rate of stenting, the procedure-related complications, clinical and angiographic outcome for 2 to 17 (mean 5.7) months of follow-up.

Results: Stent-assisted angioplasty was techinically successful in 10 of 12 (83.3 %) patients. It failed in two (16.7 %) patients, one of whom died of the arterial rupture during the balloon inflation and the other whomse stent catheter did not reach to the stenotic portion due to the tortuosity of carotid artery. In 10 patients with successful stenting, the postprocedural angiography showed restoration of normal luminal diameter with smooth inner margin. The procedure-related complications included acute thrombosis within stent (n = 5), stent migration (n = 1), and tear of artery (n = 1). Acute thrombosis in the stent was lysed with intraarterial administration of abciximab in all five patients. Stent migration was solved by the implantation of second stent. Tear of artery could be controlled by the ballooning and the implantation of second stent over the tear site. Two of ten patients had periprocedural stroke, which was relieved by intravenous heparin administration or intraarterial thrombolysis. All 10 patients were neurologically stable at the clinical follow-up. Angiographic follow-up (n = 3) revealed mild restenosis (20 %) after 10 months in one asymptomatic patient.

Conclusion: Endovascular stent-assisted angioplasty for symptomatic middle cerebral artery stenosis was technically feasible and effective in alleviating symptoms and improving cerebral blood flow. However, long-term follow-up study would be necessary to evaluate its prolonged effectiveness on the prevention of stroke and durability.

# Session 27

## Extrapyramidal disorders

163
**Age of onset is a significant factor in determining the phenotype of primary torsion dystonia.** S. O'Riordan, T. Lynch, M. Hutchinson, St. Vincent's University Hospital (Dublin, IRL)

Objective: Primary torsion dystonia (PTD) is clinically and genetically heterogeneous. The study aim is to test the hypothesis that while different gene mutations are responsible for PTD, clinical phenotype is determined by age of onset.

Methods: 1. Fifteen multiplex families with PTD were ascertained and videotaped examinations of all consenting individuals were rated for affected status and distribution by three neurologists. 2. A systematic review was performed of series of patients with sporadic PTD published on MEDLINE in the last 25 years (using PTD synonyms as search terms). Surveys detailing ages and sites of onset were included.

Results: 41 affected individuals in the 15 families, when grouped by site of onset showed a significant caudal to cranial increase in median age of onset ($p = 0.0002$; lower limb 10 years, upper limb 20.5, cervical 43 and cranial 75). In the analysis of 33 published series of sporadic PTD a similar significant distal to proximal trend was observed ($p < 0.0001$; upper limb 38 years, cervical 41.1, laryngeal 46.2 and cranial 55.8).

Conclusion: Both the family study and published series of sporadic PTD confirm a significant effect of age-at-onset on phenotypic presentation of dystonia with a distal to proximal anatomical gradient in the mode of presentation with increasing age.

164
**Effect of oral creatine supplementation on clinical and 1H-MRS parameters in HD patients.** A. Bender, D. Auer, R. Reilmann, T. Merl, P. Saemann, A. Yassouridis, J. Bender, A. Weindl, M. Dose, T. Klopstock, University of Munich, Max-Planck-Institute of Psychiatry, University of Munster, Technische Universitaet, Bezirkskrankenhaus Taufkirchen (Munich, Munster, Taufkirchen, D

Introduction: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by a triplet repeat expansion. It is clinically characterized by progressive development of cognitive impairment, movement disorder, personality changes, and a fatal course, leading to death after 15–20 years. At present no effective treatment is available.

There is increasing evidence that impairment of energy metabolism and glutamate (Glu) mediated excitotoxicity play an important role in the pathogenesis of HD. This is reflected by findings of decreased activities of respiratory chain complexes and proton spectroscopic findings of elevated brain lactate levels, and raised Glx (combined glutamate and glutamine) to creatine (Cr) ratios in HD brains.

Administration of Cr has proven to be an effective means of neuroprotection in toxic and genetic rodent models of HD and other neurodegenerative diseases. As a natural compound of the energy homoeostasis system, Cr can improve the function of energy-dependent processes such as the reuptake of Glu, thereby potentially reducing excitotoxicity. We report on the results of a multicenter open clinical trial, investigating the effect of oral Cr supplementation on clinical symptoms and brain metabolite levels in 21 HD patients.

Materials and methods: 21 patients with symptomatic HD (10 male, 11 female, average age 45 ± 7.5 years, mean duration of symptoms 3.7 ± 2.2 years; 44.2 ± 2.7 CAG repeats) were included in the trial. Proton Magnetic resonance spectroscopy (1H-MRS) and clinical scales for disease severity (UHDRS motor sections & MMSE) were performed before (t0) and 8 (t1) weeks after oral Cr was started (20 g/d for 5 days, then 6 g/d, pausing on Sundays). Spectra were acquired at 1.5T using a PRESS sequence (TR = 4000ms, TE = 35ms) with a voxel placed in the mesial parieto-occipital cortex. Metabolite quantification was done using an automated fitting program. A MANOVA repeated measure design was applied to test for treatment effects.

Results: Glx levels were significantly reduced after 8 weeks of Cr treatment compared to initial values (4.41 ± 0.17 vs. 3.85 ± 0.18; $p < 0.05$). No other metabolite was significantly altered by Cr treatment. There was no effect on clinical rating scales. 25 % of the patients reported an improvement in general well being (open question) during the course of the trial.

Discussion: The main finding of this pilot study was a significant decrease in Glx levels in the occipital cortex after 8 weeks of Cr treatment. In contrast to other publications, we could not detect a well defined lactate peak in our spectra. Also, to our surprise, oral Cr supplementation did not result in an increase of brain Cr levels, which might be due to an increased shift towards Cr-phosphate in the Cr-kinase (CK) reaction.

The reduction of potentially toxic Glx levels by Cr is a promising result in a disorder where glutamatergic overstimulation is presumed to be of pathogenetic importance.

165
**Muscle spindle dysfunction following Botulinum toxin-A injection in spasmodic torticollis.** P. Urban, University Hospital (Mainz, D)

Background: The only moderate degree of muscle weakness after BTX injection in focal dystonia does not fully explain the reversal of involuntary movement. It has thus been speculated that mechanisms other than denervation of extrafusal muscle fibre endings might account for this phenomenon. From animal studies motor denervation of the muscle spindles has been shown, but it is not clarified if botulinum toxin (BTX) leads to muscle spindle dysfunction in man.

Methods: We applied a vibration-magnetic stimulation paradigm in 20 normal subjects and 10 patients with spasmodic torticollis. Three patients were investigated before the first BTX application (de novo patients) and 7 additional patients during an ongoing BTX treatment. In all patients the sternocleidomastoid muscle (SCM) was treated unilaterally. The motor cortex was stimulated with an intensity 30 % above motor threshold at rest and MEPs were recorded from the relaxed SCM. Vibration of the SCM was performed with a pneumatic vibrator at a frequency of 85 Hz.

Results: In patients the increase in the amplitude and area of the MEPs in the clinically not affected and untreated SCM did not differ significantly from controls. At baseline measurement, the vibration-induced increase in the affected patient SCM was significantly lower than in the control group, as well as than in the not affected SCM. Six weeks after BTX application, the observed facilitation decreased significantly (amplitude and area: $p = 0.005$) compared with baseline values. Twelve weeks after BTX application, facilita-

tion was increased for the amplitude ($p = 0.025$), and the area values ($p$ = n. s.) compared to the 6-week examination.

Conclusions: Vibration applied to a muscle or its tendon mainly excites the primary muscle spindle endings, most effective with frequencies from 80 to 100 Hz. We therefore used the facilitation factor between pre-and post-vibration MEPs as a surrogate for muscle spindle function and Ia-afferent input. This approach is supported by the observation that in contrast to the SCM, only minor facilitation (median 130%, own unpublished observation) of the MEPO is observed in facial muscles, which have only few or no muscle spindles. In the SCM of torticollis patients we could demonstrate that the facilitation significantly decreases 6 weeks after BTX application and again increases after 12 weeks. This observation suggests for the first time in man that BTX not only denervates extrafusal fibres, but also denervates intrafusal fibres.

166

**Symmetry and distribution of lesions in neurologic presentation of Wilson's disease.** D. Kozic, M. Svetel, R. Semnic, V. Kostic, Institute of Oncology, Institute of Neurology (Sremska Kamenica, Belgrade, YU)

Purpose: To reveal the sites and symmetry of abnormalities by brain magnetic resonance imaging (MRI) in patients with neurologic manifestation of Wilson's disease (WD).

Patients and methods: Thirty-six patients with neurologic manifestation of WD were examined using fast-spin-echo T2/PD-weighted sequence in axial plain and T1-weighted sequence in sagittal plain with 1.5T magnetic resonance imager.

Results: Symmetry of lesions was evident in all patients (100%) with involved putamen, nucleus caudatus, thalamus, midbrain, tegmentum of pons and cerebellum and in 94% of patients in whom base of the pons was affected. Putaminal lesions, mostly presented with a pattern of bilateral symmetric and concentric T2-weighted hyperintensities were most frequently revealed (77% of patients). At least one of four, relatively specific signs for WD, like peripheral putaminal sign, bright claustrum sign, "giant panda sign" or ventral nuclear mass of thalamus sign were seen in approximately 30% of patients. Brain stem involvement with no abnormally increased T2W signal in the basal ganglia or thalami was identified in 14% of patients.

Conclusion: Our study shows that frank symmetry of T2W signal elevation in striatum in appropriate clinical setting should be very significant criteria to suggest further investigation for WD while initial establishing of diagnosis is significantly more difficult from neuroradiologic perspective in patients with exclusive brain stem involvement and spared basal ganglia. Since WD is progressive and fatal if untreated, frequently missed by clinicians according to atypical clinical presentation and taking into consideration the fact that unlike wide spectrum of other genetic disorders can be well treated the role of neuroradiologist for early diagnosis is important.

167

**Chewing pattern in patients with Meige's syndrome.** M. M. Mascia, M. Gómez-Choco, J. Valls-Solé, Hospital San Giovanni di Dio, Hospital Clinic (Cagliari, I; Barcelona, E)

Introduction: Cranial dystonia has several possible expressions, including blepharospasm and oromandibular dystonia. Both aspects of cranial dystonia may coexist or present as a separate entity, but they share similar neurophysiological alterations, i. e., enhanced blink reflex recovery to paired stimuli or facial muscle dystonic spasms. In this study, we have investigated the characteristics of the chewing pattern of patients with Meige's syndrome, which may be a distinctive feature of these patients in comparison to other forms of dystonia.

Subjects and Methods: The study was carried out in 8 normal volunteers and 7 patients with Meige's syndrome. Subjects sitting comfortably in an armchair were instructed to munch and swallow a piece of soft cake while electromyographic activity was recorded with surface electrodes attached over the masseter (MAS) and orbicularis oris (OOr) muscles of the dominant side. They were advised to do 3 to 5 masticatory movements followed by one swallowing movement.

Results: In normal subjects we recorded a regular alternating phasic pattern of muscle activity in MAS and OOr during mastication and a co-activation pattern during swallowing. Mean duration of the EMG bursts was 368 ms (SD = 93 ms) for the OOr and 318 ms (SD = 77 ms) for the MAS. Mean interval of MAS and OOr EMG bursts was 153 ms (SD = 59 ms). In patients the pattern of EMG activity was irregular, with large variability in the duration of the bursts, ranging from 120 ms to 3000 ms. The pattern was not regularly alternating, but there was frequent co-contraction between MAS and OOr. A persistent EMG activity with superimposed irregular bursts of the muscles under study was found in four patients. Not clear simultaneous co-activation was found when swallowing.

Conclusion: The alternating pattern between MAS and OOr seen in normal subjects during mastication is probably the consequence of activation of a learned motor pattern in which both muscles act as functional antagonists. The absence of a similar pattern of EMG activity in patients appears to be the expression of the excessive and overlapping activity in agonist and antagonist muscles, which is a consistent finding in dystonic patients. This is a simple way of characterising electrophsiologically one aspect of oromandibular dystonia of patients with Meige's syndrome.

168

**Dysautonomic side effects after Botulinum toxin B injections in patients with cervical dystonia, resistant to Botulinum toxin A.** M. H. Marion, D. Wren, St George's Hospital (London, UK)

We have treated 8 patients (6 females, 2 males) with cervical dystonia, resistant to botulinum toxin A (BTX-A), with injection of BTX-B. The mean age of patients was 52 years [39–62], duration of disease: 12 years [7–29], age of onset: 40 years (21–52. The mean delay to develop resistance to BTX-A was 36 months (10 months–3 years). All patients were diagnosed resistant after a Frontalis test (40 units Dysport on one side) and had a BTX free interval of 2 years on average. All patients were injected with a starting dose of BTX-B of 10000 units Neurobloc divided in 2 or 3 cervical muscles.

Clinical benefit was variable with no significant improvement in 4 cases after the first injection. Two patients were improved between 50 and 60% and 2 patients more than 75%. None of the patients developed atrophy of the injected muscles, in particular of the sternomastoid muscle. Duration of benefit in the 4 patients with significant improvement was 8 weeks.

Short term side effects were present in all 8 patients, and were dry mouth (n = 8), weird unpleasant taste (n = 2), dry lips (n = 3), and dysphagia for solid (n = 6). Three patients had systemic dysautonomia with symptomatic orthostatic hypotension (n = 3), dry skin of the hands (n = 2), blurred vision (n = 1), heavy eyelids (n = 1) constipation (n = 3), nausea (n = 2) and urinary hesitancy (n = 2). Three patients have been reinjected 2 or 3 times at 3 month intervals. Two of the 3 patients with mild side effects the first time received 10000 units a second time with a similar response (benefit and side effect). One patient received a third injection of 10000 units, but lost her clinical response (about 50% benefit previously) and did not have any side effect.

In conclusion, it is too early to judge the clinical efficay but there is an unusual range of side effects, with BTX-B compared to BTX-A. Autonomic side effects with BTX-B had been already reported by Dressler et al. (2003). Patients with underlying dysautonomia or cardiovascular disorders should be carefully screened. Early resistance to BTX-B, in patients already resistant to BTX-A needs to be studied.

169

**Patterns of EMG-EMG coherence in limb dystonia.** P. Grosse, M. Edwards, M. Tijssen, A. Schrag, A. Lees, K. Bhatia, P. Brown, Institute of Neurology, Academic Medical Center (London, UK; Amsterdam, NL)

Dystonia of the limbs may be due to a wide range of aetiologies and may cause major functional limitation. We investigated whether the previously described pathological 4–7 Hz drive to muscles in cervical dystonia (Tijssen et al. 2000) is present in patients with aetiologically different types of dystonia of the upper and lower limbs. To this end, we studied 12 symptomatic and four asymptomatic carriers of the DYT1 gene, six patients with symptomatic dystonia due to focal basal ganglia lesions and 11 patients with fixed dystonia. We evaluated EMG-EMG coherence in the tibialis anterior (TA) of these and 15 healthy control subjects. 10 out of 12 (83%) of symptomatic DYT1 patients had an excessive 4–7 Hz common drive to TA evident as an inflated coherence in this band. This drive also involved gastrocnemius leading to co-contracting EMG bursts. In contrast, asymptomatic DYT1 carriers, patients with symptomatic dystonia, patients with fixed dystonia and healthy subjects showed no evidence of such a drive nor any other distinguishing electrophysiological feature. Moreover, the pathological 4–7 Hz drive in symptomatic DYT1 patients was much less common in the upper limb, where it was only present in two out of six (33%) of patients with clinical involvement of the arms. We conclude that the nature of the abnormal drive to dystonic muscles may vary according to the muscles under consideration and, particularly, with aetiology.

# Session 28

## Clinical neurophysiology – 1

170
**Abnormal cortical activation to voluntary movement in Alzheimer's disease: EEG evidence.** L. Leocani, G. Magnani, M. Natali Sora, M. Falautano, A. Martins da Silva, E. Schiatti, R. Mossini, G. Comi, Scientific Institute Hospital San Raffaele (Milan, I)

Objective: To investigate the presence of subclinical motor involvement in mild/moderate Alzheimer's Disease (AD).

Background: Involvement of motor function is considered a late finding in AD. Movement-related potentials (MRP) and event-related desynchronization (ERD) of the EEG sensorimotor rhythms are considered indicators of cortical activation during movement preparation and execution, while beta event-related synchronization (ERS) after movement is considered a sign of cortical inhibition. We investigated motor function in AD using motor tapping and MRP/ERD analysis.

Design/Methods: Nineteen patients with probable AD (mean age 69 ± 8 yrs) with mild moderate AD (MMSE 20.4 ± 2.8) and 17 normal control subjects of comparable age were studied. Finger tapping was measured 3 times for each side. MRP and mu and beta ERD/ERS to self-paced right thumb movement were studied on 59 channel EEG.

Results: Compared to control subjects, AD patients showed significantly slower motor tapping for both sides ($p < 0.006$) and reduced MRP amplitude to motor preparation ($p < 0.05$). ERD/ERS over the primary sensorimotor area were not significantly different between the two groups, while beta ERD was significantly more widespread in AD patients being larger over frontal electrodes ($p < 0.05$).

Conclusions: The finding of abnormal motor tapping confirms that subclinical motor involvement may be present in relatively early stages of AD. These abnormalities are related to changes in the activation of cortical motor circuitries during motor preparation (reduced MRP) and execution (increased activation ERD over frontal areas). These changes, particularly increased frontal ERD, may be related to a compensation of motor deficit and/or to abnormal organisation of the cortical motor circuitries involved in motor control.

171
**Cerebral glucose metabolism of vestibular and visual cortex during acute vestibular neuritis (PET study).** M. Dieterich, S. Bense, M. Lochmann, P. Schlindwein, K. Prange, T. Brandt, P. Bartenstein, Johannes Gutenberg-University, Ludwig-Maximilians University (Mainz, Munich, D)

Vestibular neuritis (VN) is an acute unilateral vestibular failure that causes a tone imbalance with severe rotational vertigo, spontaneous horizontal-rotatory nystagmus, postural imbalance, and nausea. Signs and symptoms resolve over 2–6 weeks. Earlier positron-emission tomography (PET) water activation studies showed that caloric vestibular stimulation in normal subjects elicited regional cerebral blood flow (rCBF) increases in several vestibular areas and while simultaneously decreasing rCBF in visual cortex areas. These findings supported the hypothesis of a reciprocal inhibitory vestibular-visual interaction. The aim of this fluorodeoxyglucose (FDG)-PET study was to determine how glucose metabolism in the vestibular and visual areas is affected by acute unilateral vestibular loss.

FDG-PET (ECAT EXACT, Siemens, Germany) was performed in six patients (5 m, 1 f) A) in the acute phase of VN 5.8 days (mean) after symptom onset and B) after clinical recovery due to central compensation 3 months later, laying supine with the eyes closed. Categorical comparison of the two conditions was done using SPM99b ($p < 0.0001$ not corrected).

Categorical comparison (A vs. B) showed significant differences due to increased glucose metabolism for the acute phase bilaterally in the posterior insular region (parieto-insular vestibular cortex, PIVC) and adjacent superior temporal gyri (BA 22), anterior insula, paramedian and dorsolateral thalamus, hippocampus, middle or superior frontal gyrus (BA 9/6, representing ocular motor areas), and the anterior cingulate gyrus (BA 32/24). Differences due to decreased glucose metabolism (B vs. A) were found bilaterally in the visual cortex (BA 17/18/19/7) and the somatosensory cortex in the postcentral region.

Thus, during the initial symptomatic phase rotational vertigo and nystagmus were associated with a significant increase of the cerebral glucose metabolism in vestibular (PIVC in the posterior insula, thalamus) and ocular motor areas, whereas a decreased metabolism was seen in the visual and somatosensory cortex. The vestibular disorder caused a transient up-regulation of the vestibular cortex and down-regulation of the visual and somatosensory cortices which is compatible with the reciprocal inhibitory sensory-sensory interactions as found earlier.

172
**Reciprocal inhibition between ankle flexor and extensor muscles in patients with a mutation in the glycine receptor.** C. Crone, J. B. Nielsen, N. Petersen, J. van Dijk, M. Tijssen, Rigshospitalet, University of Copenhagen, Leiden University Medical Center, Academic Medical Center (Copenhagen, DK; Leiden, Amsterdam, NL)

Background: During normal voluntary movements in man antagonistic motoneurones (MNs) are inhibited in parallel with activation of agonist muscles. This reciprocal inhibition is presumed to be essential for the performance of smooth coordinated movements and it has thus been proposed that changes in this inhibitory mechanism may play a role in the development of spasticity. And several studies have indicated that reciprocal inhibition between agonist/antagonist muscles is decreased in patients with spasticity. Thus the activity in the disynaptic Ia inhibitory pathway is decreased in spastic patients suffering from multiple sclerosis (Crone et al. 1994) and the disynaptic inhibition is even replaced by a facilitation in the paretic leg in hemiplegic stroke patients (Crone et al. 2003). However, no clear correlation between the degree of spasticity and the lack of reciprocal inhibition has been found in these patients and it is therefore still not clear whether a causal relationship exists between development of spasticity and decrease of reciprocal inhibition.

Disynaptic reciprocal inhibition is mediated by the neurotransmitter glycine. And patients with hereditary startle disease have a mutation in the gene encoding for the alpha1-subunit of the glycine receptor. One aim of the study was therefore to measure the degree of disynaptic reciprocal inhibition in these patients in order to gain knowledge about the contribution of reciprocal inhibition to muscle tone.

Patients: 6 patients with the major form of hereditary hyperekplexia who had a known point mutation in the gene encoding for the alpha1-subunit of the glycine receptor were tested clinically and electrophysiologically. Four of the six patients suffered from pronounced muscle stiffness in the legs and all 6 patients had extra beats when testing for ankle clonus. All patients experienced regular startle reactions with falls.

Methods and results: The degree of disynaptic reciprocal inhibition of soleus MNs (elicited by peroneal nerve stimulation) was measured by stimulating the peroneal nerve and measuring the ensuing change in size of the soleus H-reflex and the change in soleus EMG activity (stimulus-triggered averaging of rectified EMG). The degree of inhibition was compared with results from similar testings in 25 healthy subjects.

A clear disynaptic reciprocal inhibition of the soleus MNs was seen with both techniques in all healthy subjects whereas it was clearly decreased, not present or even replaced by a facilitation in the six patients.

Conclusion: This strengthens the hypothesis that spinal glycinergic inhibition plays an important role in the control of muscle tone in normal subjects and the present findings do together with other observations (see abstract by JB Nielsen et al.) support the idea that lack of disynaptic reciprocal inhibition may be one of several contributing factors to the development of spasticity.

173
**Neurophysiological investigation of executive function in Alzheimer's disease.** G. Magnani, L. Leocani, E. Schiatti, M. Natali Sora, A. Martins da Silva, M. Franceschi, A. Barbieri, G. Comi, Scientific Institute Hosp. San Raffaele (Milan, I)

Objective: To evaluate motor function in mild cognitive impairment (MCI) and in mild-moderate Alzheimer's disease.

Background: Motor dysfunction is considered to occur at a relatively late stage of AD, even though impairment of fine motor skills, such as finger tapping, has been reported at earlier stages. The involvement of motor function in MCI has yet to be clarified.

Design/Methods: Twelve patients with MCI (7 females, mean age 68.6 ± 10), 24 patients with AD (16 females, age 70 ± 9) mild-moderate (MMSE > 14) and 12 normal control subjects participated in the study. Patients had no motor symptoms or signs at neurological examination. All patients and subjects were right handed according to the Edinburgh scale. Finger tapping on a mouse button was performed 3 times for each side, and the frequency of motor tapping and the right/left ratio were measured using a computerized system.

Results: Compared to control subjects, both patient groups showed significantly slower finger tapping ($p < 0.02$) for both sides (normal subjects: 4.7 ± 0.8 right, 3.8 ± 0.6 left; AD: 3.1 ± 1.0 right, 3.0 ± 1.0 left, MCI: 3.0 ± 0.67 right, 2.8 ± 0.5 left). Moreover, both groups showed a reduced right/left

dominance ($p < 0.008$) compared to the normal group (normal: $1.2 \pm 0.07$, AD $1.06 \pm 0.2$, MCI $1.09 \pm 0.12$).

Conclusions: The finding of a reduced frequency of motor tapping in our patients with mild-moderate AD confirms the presence of subclinical motor impairment in relatively early stages of AD. The finding of similar abnormalities in MCI patients suggests that involvement of the motor system may be present even before a diagnosis of AD is made and suggests the need for a larger follow-up study in order to assess whether the presence of motor involvement may have a predictive value for the future development of AD.

174

**Reciprocal inhibition between wrist extensor and flexor muscles in patients with a mutation in the glycine receptor (hereditary hyperekplexia or familial startle disease).** J. B. Nielsen, C. Crone, V. Marchand-Pauvert, H.S Pyndt, M. A. J. Tijssen, J. G. Van Dijk, University of Copenhagen, Hapital de la Salpêtrière, University of Amsterdam, University of Leiden (Copenhagen, DK; Paris, F; Amsterdam, Leiden, NL)

Disynaptic reciprocal inhibition has been reported to be absent in the arm (Floeter et al. 1996) and the leg (Crone et al. 2001; and abstract by Crone et al. this meeting) in patients with hereditary startle disease (hyperekplexia). It had been verified in the study by Crone et al. (2001) that all patients suffered from a mutation of the gene coding for the glycine receptor, whereas it is unclear whether this was also the case for the patients investigated by Floeter et al. (1996). Furthermore, Crone et al. (2001) found that most of their patients suffered from pronounced muscle stiffness in the legs, which they proposed could be linked to the absence of reciprocal inhibition. However, the patients showed no signs of muscle stiffness or increased reflex excitability in the arms, which would have been expected, if absence of reciprocal inhibition were universally linked to increased muscle stiffness. We therefore re-investigated reciprocal inhibition in the arm of patients with hyperekplexia using the same patients as in the study by Crone et al. (2001).

6 patients with the major form of hereditary hyperekplexia who had a known point mutation in the gene encoding for the $\alpha$1-subunit of the glycine receptor were tested clinically and electrophysiologically. None of the patients showed any sign of muscle stiffness or reflex hyperexcitability in the arms.

The degree of disynaptic reciprocal inhibition of flexor carpii radialis (FCR) motoneurones (MNs) was measured by stimulating the radial nerve and measuring the ensuing change in the size of the FCR H-reflex and the change in FCR EMG activity (stimulus-triggered averaging of rectified EMG). The degree of inhibition was compared with results from similar testings in 12 healthy subjects.

A clear disynaptic reciprocal inhibition of the FCR MNs was seen with both techniques in the healthy subjects. An inhibition was also observed in the FCR EMG in 4 of the patients and in the FCR H-reflex in the two subjects in whom this reflex could be elicited.

However, the inhibition was somewhat smaller and delayed by around 3–4 ms as compared to the healthy subjects.

The data demonstrate that reciprocal inhibition in the arm is preserved, but weak and delayed in patients with identified mutation of the glycinergic receptor. The presence of the inhibition may explain why the patients do not show signs of increased muscle stiffness in the arms.

175

**Separation of habituation from fatigue in a repetitive simple motor task: a study with movement-related cortical potentials.** G. Dirnberger, C. Duregger, G. Lindinger, W. Lang, University of Vienna (Vienna, A)

Objectives: The Movement-Related Cortical Potential (MRCP) is a widespread EEG potential that precedes voluntary movements and is caused by the neural processes involved preparing and executing the commands to move. The aim of this study was to examine changes in the amplitude of this potential during the prolonged performance of a very simple motor task, and to determine how such changes can be explained by neuropsychological variables psychomotor performance and fatigue.

Methods: MRCPs were recorded in 33 right-handed healthy subjects from electrodes F3, Fz, F4, C3, Cz, C4, P3, Pz and P4. Subjects made 100 self-paced unilateral button presses with their left or right index finger, and then continued on the other side of the body for another 100 movements. For the entire experiment, the mean time interval between two consecutive index finger movements was constant at $8 \pm 2$s.

Results: [1] The amplitude of the MRCP decreased over the right hemisphere in the course of the tasks irrespective of the side of movement. [2] Lower than average scores in a test of psychomotor performance (the Digit Symbol Test) were associated with lower MRCP amplitudes over right lateral electrodes, whereas scores after the button press tasks were equivalent for the two subgroups of subjects. [3] Subjects who reported a larger increase of fatigue when they had finished the motor tasks had more negative amplitudes of the late MRCP across the entire scalp.

Conclusion: The right hemisphere may predominate motor activation or attentional demands directed towards movement execution or somatosensory inputs even in very simple motor tasks. Fatigue and habituation which occur in the course of a simple motor task exert dissociable effects on brain activation as measured via MRCPs.

# Session 29

## Clinical Neurophysiology – 2

176

**Intracortical inhibition and facilitation in natural human sleep as assessed by transcranial magnetic stimulation.** F. Salih, R. Khatami, O. Hummel, A. Kühn, P. Grosse, Charité (Berlin, D)

Previous studies using transcranial magnetic stimulation (TMS) showed that corticospinal excitability is reduced in all stages of human sleep compared to wakefulness with significant differences between sleep stages (Grosse et al. 2002). To date, however, it has not been unequivocally elucidated to what extent cortical and/or spinal inhibitory mechanisms contribute to the decreased level of corticospinal excitability in human sleep. Here we investigate whether intracortical inhibition (ICI) and facilitation (ICF) are present in sleep at all and if so, whether differences among the different sleep stages exist.

We studied 9 healthy subjects using the conventional TMS paired-pulse paradigm (Kujirai et al. 1993) to determine ICI at interstimulus intervals of 2 and 3 ms, and ICF at 8, 10 and 12 ms, respectively, in NREM2 (5 subjects), NREM4 (4 subjects), and REM (4 subjects) and compared their results to wakefulness. Motor evoked potentials were recorded from FDI with magnetic stimuli being delivered over the contralateral hand area of the motor cortex using a focal coil. Statistical analysis was performed using a repeated measures general linear model with sleep stages and state of vigilance (sleep-awake) as main effects. When appropriate a post hoc paired 2-tailed t-test was applied.

In all three sleep stages examined ICI corresponds to ISIs of 2 and 3 ms with amplitudes of conditioned stimuli well below the test stimulus. Conversely, ICF can be seen in ISIs of 8, 10 and 12 ms and amplitudes of conditioned stimuli above that of test stimuli. In all sleep stages ICI was enhanced compared to wakefulness (NREM4 > REM > NREM2). Whereas in NREM4 ICI enhancement was statistically significant ($p < 0.01$) compared to wakefulness and NREM2, this was not the case in NREM2 and REM sleep. In contrast, ICF was almost unchanged in all sleep stages compared to wakefulness.

We can show that ICI and ICF are present in all sleep stages in a comparable fashion to wakefulness. However, the degree of enhanced ICI depends on the sleep stage as it is almost maximal in NREM4. We conclude that the changes in ICI reflect varying degrees of synaptic inhibition along the sleep cycles as it has been shown that ICI depends on GABAergic transmission (Ziemann et al. 1996). Our findings support the hypothesis derived from animal data (Timofeev et al. 2001) that GABAergic synaptic inhibition plays an important role for cortical inhibition in NREM4, and also possibly in REM and may be involved in the maintenance of human sleep in these sleep stages. This data may provide a basis for further insight into the pathophysiology of sleep-bound neurological disorders like the epileptogenesis of sleep-bound epileptic seizures.

177

**The effect of voluntary activity on the maximum motor response: Enlargement of the Mmax during exercise is followed by a long gradual decrease in size.** B. Andersen, B. Westlund, C. Krarup, Rigshospitalet, Copenhagen University Hospital (Copenhagen, DK)

In connection with our interest in the effect of voluntary activity on function of the central and peripheral nervous system, the maximum motor response (Mmax) evoked in the abductor digiti minimi muscle during and after series of voluntary contractions was studied in 10 healthy subjects.

During an initial 30 min period Mmax responses were recorded every 3 min at rest. Subsequently, the subjects performed intermittent digit V abduction at 50 % maximal voluntary force (MVC) for periods of 30 s alternating with 15 s of rest. At the endurance point where the target force could no

longer be maintained the subjects continued the contraction until exhaustion, defined as 30% of MVC. At this point the subjects relaxed and after 25 min of rest the subjects repeated the contractions while at a lower duty cycle (15 s contraction, 15 s rest). Mmax responses were recorded at 5 s intervals during every 15 s rest period between the contractions and at 10 s to 1 min intervals during the 25 min recovery period.

The Mmax decreased slightly in area by 10 ± 1% (SEM) and by 4–5% in amplitude and duration during the initial rest period. During exercise Mmax increased markedly in size, affecting both area, amplitude and duration of the response. The change in area during the first exercise period amounted to 28 ± 4%, while increases in amplitude and duration were 24 ± 4% and 19 ± 4%, respectively. There was a very rapid decline in size of Mmax from the first to fourth stimulus elicited during the 15 s rest periods between the contractions. After exercise the size of Mmax returned to its initial values measured at the beginning of the experiment within 1–2 min, but then continued to decrease while at a slower rate. At the end of the 25 min rest period the changes in area, amplitude and duration were 44–28%, respectively. During repeated exercise the enlargement of Mmax was even more pronounced than that observed during the first exercise period.

The study showed that voluntary activity is associated with prominent fluctuations in size of Mmax even after long time of rest following the contractions. Important activity-dependent changes within the muscle fibres may contribute to these phenomena.

178

**Cortical hyperexcitability after head trauma in humans.** L. Boffa, M. G. Palmieri, P. L. Galizia, M. A. Pierantozzi, L. Brusa, F. Ursini, M. D. Caramia, D. Centonze, II University Tor Vergata (Rome, I)

Background: We applied paired transcranial magnetic stimulation (pTMS) to patients presenting post-concussional symptoms after mild head trauma. The purpose of the study was to determine the potential abnormality of cortical excitability in this pathological condition.

Methods: Fifteen traumatic brain injury (TBI) patients (8 women and 7 men, aged between 18 and 47 years) suffering from headache underwent paired TMS within 6 months from the trauma. Paired TMS stimulation, according to the conditioning-test paradigm employing interstimulus intervals (ISIs) from 1 to 6 msec, was used in order to investigate the time course of intracortical inhibition (ICI).

Results: All patients exhibited a significantly lower MEP inhibition than controls at 2, 3 and 4 ms ISI. The statistical analysis of paired-pulse TMS protocol showed a significant effect ($F = 107.25$; $p < 0.001$) of the factor "group". TBI patients showed a mean "conditioned" MEP amplitude higher than that observed in controls throughout all the six ISI analysed. The "ISI" factor was also significant ($F = 76.01$; $e = 0.72$; $p < 0.0001$) with the mean "conditioned" MEP amplitude increasing from 25.5% to 130.3% as the ISI increased from 1 to 6 ms. Finally, the interaction of "group " with "ISI" was also significant ($F = 18.83$; $e = 0.72$; $p < 0.001$), showing that the condition of TBI was able to affect the MEP amplitude at different ISIs.

Conclusions: Our results demonstrate that the episode of head trauma can give rise to abnormalities in the inhibitory and/or excitatory intracortical motor circuits that are in keeping with both experimental and anatomopathological evidence of increased glutamergic activity inducing overstimulation of brain excitatory pathways in the wake of mechanical trauma to the brain.

179

**Dynamic tibial nerve SSEP in patients with lumbar spinal stenosis and surgical decompression.** J. Koehler, S. Delank, S. Vollhardt, J. D. Rompe, P. Eysel, Johannes Gutenberg University, University Hospital (Mainz, Cologne, D)

Introduction: The aim of the study was to investigate the correlation of dynamic somatosensory evoked potentials (SSEP) after tibial nerve stimulation and radiological findings with signs and symptoms before and after spinal decompression.

Methods: Dynamic tibial nerve SSEPs were recorded at spinal level L2 and Cz-Fz before and after exercise in 12 patients before and post surgery. The degree of spinal stenosis at lumbar level was measured by computertomography before surgery. Signs and symptoms were evaluated by clinical examination, Oswestry-Low-Back-Pain-Disability Questionnaire, Visual Analogue Scale (VAS) for pain and time of walking without rest before and after surgery. Surgical therapy was indicated if combined physical and drug treatment over a period of 4 weeks failed to improve the spinal claudicatio symptoms. Two groups of patients were defined: 1) patients with stable or improved dynamic SSEP and 2) patients with increased abnormalities of the dynamic tibial nerve SSEP.

Results: In 6 patients unchanged or shortened of tibial nerve SSEP latencies were recorded after exercise (group 1). Group 1 showed a clear improvement of the mean Oswestry Scale (before surgery: 18.5; after surgery 4.0) as well as mean VAS (before surgery: 6.0; after surgery: 0.167) and mean time of walking (before surgery: 13.3 minutes; after surgery: 83,3 minutes). In contrast the 6 patients with increased abnormal dynamic tibial nerve SSEP recordings after exercise showed less improvement after surgery of the Oswestry Scale (30.3 vs. 14.8), VAS (6.3 vs. 4) and mean time of walking (10.0 minutes vs. 21.7 minutes). Radiological findings showed no differences between these two groups of patients.

Conclusions: Radiological findings can not support the decision of surgery whereas dynamic tibial nerve SSEP could help to estimate the prognostic outcome of a surgical decompression. Patients with no increased abnormalities of the dynamic tibial nerve SSEP before surgery showed a pronounced improvement of symptoms after surgery compared to patients with abnormal dynamic tibial nerve SSEP.

180

**Quantitative evaluation of different botulinum toxins on sweating.** T. Schlereth, I. Mouka, G. Eisenbarth, M. Winterholler, F. Birklein, Johannes-Gutenberg-University, Friedrich-Alexander-University (Mainz, Erlangen, D)

Botulinum toxin (BoNT) is widely used in the treatment of muscle disorders like cervical dystonia. In the treatment of muscle disorders BoNT/A is 20 to 50 times more effective than BoNT/B (Sloop et al. 1997). Besides motor nerves also autonomic fibres can be blocked (Rand and Whaler 1965). Accordingly, the reduction of sweating in patients suffering from focal hyperhidrosis is a new indication for BoNT. Both BoNT/A and BoNT/B have been used successfully (Schnider et al. 1999), but the comparison of BoNT/A to BoNT/B in the therapy of hyperhidrosis is missing.

In order to compare the effects of two different BoNT/A-preparations (Dysport®, Ipsen Pharma, UK and Botox(R), Merz, USA) with a BoNT/B-preparation (Neurobloc®, Elan Pharmaceuticals, Dublin, Ireland) on sweating, different doses of each BoNT were injected subcutaneously into the lateral aspect of lower legs in healthy subjects. The study was divided into three subgroups: 15 subjects received Dysport® in doses of 2.5–120 MU (mouse units), 21 Botox® in doses of 0.3–80 MU and 15 Neurobloc® in doses of 1–125 MU. For evaluation the area of anhidrosis three weeks after injection of BoNT was visualized by iodine starch staining and planimetrically analyzed (Minor 1927). Effects of the different BoNT-preparations were identified by regression analysis.

Each preparation of BoNT reduced sweating dose dependently at the injection sides. The slopes of the regression lines were 0.4 for Dysport®, 0.1 for Botox® and 0.2 for Neurobloc®. Accordingly, to yield an anhidrotic skin area of 20 $cm^2$ 31 MU Dysport®, 59 MU Botox® or 51 MU Neurobloc® had to be injected.

In conclusion, we could show that the effect on sudomotor function of BoNT/A and BoNT/B preparations is similar, in contrast to the effects on motor nerves.

181

**Abnormal motor responses to peripheral stimulation in patients with cerebellar ataxia.** S. Tamburin, G. Zanette, S. Marani, P. Manganotti, A. Andreoli, A. Fiaschi, University of Verona (Verona, I)

Objective: To examine the sensorimotor interactions in cerebellar patients.

Methods: We investigated the effects of electrical stimulation of the second (D2) and fifth (D5) fingers on the amplitude of motor evoked potentials (MEPs) to transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (TES) in the relaxed right abductor digiti minimi muscles of 7 patients with pure cerebellar syndromes and of 14 age-matched controls. The digital stimulation was set at 3 times the sensory threshold and preceded brain stimulation at interstimulus intervals (ISIs) of 10–100 msec.

Results: D5 stimulation produced significant MEP inhibition in normal subjects at ISIs of 20–50 msec, while D2 stimulation resulted in a non-significant inhibitory trend at the same intervals. In contrast, digital stimulation had no effect on MEP amplitude in cerebellar patients. A significant difference was found between patients and controls at ISIs of 20–50 msec with D5 stimulation. TES conditioning induced MEP inhibition only at ISIs < 40 msec.

Abnormalities of MEP responses to peripheral stimulation were correlated to clinical symptoms' severity.

Conclusions: Digital stimulation appears to modulate motor system excitability less effectively in cerebellar patients. MEP inhibition by cutaneous afferences is reduced in response to digital stimulation and the somatotopic distribution of cutaneomotor inhibition is absent in patients. These abnor-

malities may contribute to the genesis of cerebellar motor symptoms and their time course suggests involvement of subcortical and cortical sites.

# Session 30

## Multiple Sclerosis – 5

182

**Conventional and magnetization transfer MRI predictors of clinical multiple sclerosis evolution: a long-term follow-up study.** M. Rovaris, F. Agosta, M. Sormani, M. Inglese, V. Martinelli, G. Comi, M. Filippi, Neuroimaging Research Unit, Department of Neurology, HSR (Milan, I)

The correlation between conventional MRI lesion load accumulation and multiple sclerosis (MS) clinical evolution is only modest. The assessment of brain parenchymal volume and of its changes over time may provide adjunctive MRI-derived markers reflecting the more disabling aspects of MS pathology. Magnetization transfer (MT) MRI is sensitive to "occult" MS-related brain damage and might also contribute to overcome the clinical/MRI paradox. In this study, we assessed the value of conventional and MT MRI-derived metrics in predicting the long-term clinical evolution of patients with different MS phenotypes.

We studied 73 patients, with relapsing-remitting (RR) MS (n = 34), secondary progressive (SP) MS (n = 19) and clinically isolated syndromes suggestive of MS (CIS) (n = 20), and 16 healthy subjects. Brain dual-echo, T1-weighted (only in patients) and MT MRI scans were obtained at baseline and after 12 months. T2-hyperintense and T1-hypointense lesion volumes, normalized brain volume (NBV) and average lesion MT ratio (MTR) were measured. MTR histograms from the whole brain tissue were also obtained. Clinical MS evolution and neurological disability were re-assessed in all patients after a median follow-up of 4.5 years. A multivariate analysis was performed to establish which clinical and MRI-derived variables were significant predictors of neurological deterioration at the end of the study period.

At the end of the study period, 34 patients showed significant neurological deterioration. The final multivariable model included average brain MTR percentage change after one year ($p = 0.02$, Odds Ratio -OR = 0.86) and baseline T2-hyperintense lesion volume ($p = 0.04$, OR = 1.04) as independent predictors of long-term disability accumulation. In this cohort of patients, abnormal values of average brain MTR changes showed a relatively high specificity (76.9%) and positive predictive value (59.1%) for EDSS deterioration in individual cases.

In patients with MS, a comprehensive estimation of the short-term changes of both conventional and MT MRI-detectable lesion burden might provide useful prognostic information for the long-term clinical disease evolution.

183

**Clinical and MRI outcome 3 years after autologous hematopoietic stem cell transplantation (AHSCT) in MS.** Y. Blanco, A. Saiz, E Carreras, J. Berenguer, M. Rovira, T. Pujol, C. Martinez, T. Arbizu, F. Graus, Hospital Clínic de Barcelona (Barcelona, E)

Introduction/objectives: AHSCT is presently evaluated as potential treatment for severe cases of MS. We report the clinical outcome and MRI evolution of 15 patients treated with AHSCT after a median follow-up of almost 3 years.

Methods: Fifteen MS patients (9 SP, and 6 RR), with a median age: 30 years [22–45], median EDSS: 6.0 (4.5–6.5), median annual relapses: 3 [1–7], and median worsening of the EDSS in the previous year of 1.0 (0.5–4.5) were treated and followed with an AHSCT protocol previously published (Neurology 2001;56:1084–1089). The 3-year progression-free survival was defined as no increase in the EDSS score after AHSCT as compared with baseline. The 3-year disease activity-free survival was defined as the absence of progression of any type, which included increase on EDSS at last assessment as above, increase on EDSS after initial improvement even if the worsening did not reach the baseline EDSS, increase by one point of the ambulatory index, and absence of objective relapses.

Results: Stem cells mobilization failed in one patient. There was no major systemic toxicity in the 14 remaining patients. Neurological deterioration related to the procedure was observed in 3 patients (2 transient, and 1 persistent). Two patients developed uveitis and thyroiditis (months +2 and +15). After a median follow-up of 35 [12–53] months, the EDSS remained stable in 8, improved in 4, and worsened in 2 patients (in one the deterioration was related to the procedure). The 3-year progression-free survival was 86% (95% CI: 60–90), and the 3-year disease activity-free survival was 46% (95% CI: 24–76). Only 2 patients presented relapses treated with steroids. No enhanced T1 lesions were detected since month +1 after AHSCT in any of the follow-up MRI studies. The mean percentage reduction of T2 lesion volume at 3 years was 20.2% compared with baseline. A decrease of the corpus callosum area was observed throughout the study with a mean reduction of 12.7% at 3 years compared with baseline. However, the reduction was only of 0.37% between the first and second MRI and 0.20 between the second and third year MRI. Similar figures were observed when brain atrophy was measured by brain volume technique.

Conclusions: The protocol of AHSCT was well tolerated by our patients. The 3-year result of this trial AHSCT deserves further evaluation in the setting of multicenter randomised controlled trials.

184

**Interferon beta-azathioprine association activity on immunological marker in vitro and in multiple sclerosis.** T. Biagioli, B. Mazzanti, A. Aldinucci, C. Ballerini, M. Vergelli, L. Massacesi, University of Florence, CSF (Florence, I)

Interferon-b (IFN-b) and Azathioprine (AZA) are drugs active on T-cell functions and used in the therapy of Multiple Sclerosis (MS). Combined (IFN-b/AZA) therapy with these two drugs is currently under investigation. However, 6MP (the active AZA metabolite) is thought to inhibit the nucleic acid synthesis, and immunomodulatory effects of IFNb are believed to be modulated by "de novo" protein production. Therefore a possible interference between the two drugs can be hypothesized. To investigate this issue we studied in vitro IFNb and 6MP combined activities on mitogen and antigen-specific T-cell proliferation and cytokine production. Stimulation of T cells under these experimental conditions demonstrated a dose-dependent suppression of antigen specific (MBP peptide 80–99, Flu-HA) as well as of mitogen (PHA) driven T cell proliferation. The production of TNFa was also significantly reduced. The production of IFNg was unaltered by 6MP, but was decreased when IFNb alone or in combination with 6MP was added. IL-10 secretion was increased by IFNb in a dose dependent manner and these effects correlated with the reduction of the T cell proliferation. On the other hand 6MP did not affect the production of IL-10 by MBP specific TCL. Combination of both drugs increased IL-10 production compared to IFNb alone. Immunomodulatory effects of IFNb, AZA and the combination of both drugs were investigated also ex vivo in patients with relapsing-remitting MS. T cell response to various mitogens (PHA, ConA, OKT3, PWM), cytokines (IL-2, IL-7) and recall antigens (PPD, TT, Candida) were investigated in 18 MS patients that underwent IFNb, AZA or combined AZA/IFNb therapy, both in term of proliferative response and of cytokine production, before and 6 months after the beginning of therapies. Treatment with AZA significantly decreased the frequency of positive T cell proliferation in response to recall antigens ($p = 0.027$). This pattern of reactivity was maintained in the MS group that underwent combined therapy ($p = 0.04$). These results indicate that AZA treatment determines in vivo an activation induced cell death (AICD) and therefore that its activity on the immune system is selective and potentially tolerogenic. In addition AZA activity on gene expression does not influence the IFNb activity based on protein production and therefore IFNb immunomodulatory effects are not reduced and sometimes enhanced by AZA.

185

**Oral prednisone taper has no effect on neurologic recovery following intravenous methylprednisolone for the treatment of a multiple sclerosis relapse.** C. Caon, W. Ching, A. Tselis, E. Sonenvirth, R. Lisak, O. Khan, Wayne State University (Detroit, USA)

Objective: To examine the effect of oral prednisone taper (OPT) on neurologic outcome after treatment with intravenous methylprednisolone (IVMP) for a relapse in RRMS patients compared to patients who received no OPT after treatment with IVMP.

Background: MS relapses are often treated with IVMP with or without OPT. However, it is not clear if treatment with OPT improves neurologic outcome during recovery from a relapse. Methods: This was a retrospective analysis of 285 consecutive relapses in RRMS patients who received treatment with IVMP at a dose of one gram a day for five days with or without OPT (which was typically started at 80 mg/day and tapered off over 14 to 21 days). All patients were on disease-modifying therapy for at least 6 months, and had neurologic examination immediately prior to, 6, and 12 months after treatment with IVMP.

Results: 285 relapses were confirmed with neurologic examination including worsening on EDSS by 1 or more points in 264 RRMS patients and treated with IVMP with or without OPT. Mean age and disease duration (DD) was 35.6 years and 5.6 years. 179 patients were women. Mean baseline EDSS (n = 264) recorded 3 to 6 months prior to relapse was 2.29 and at the time of confirmation of relapse was 4.33. Mean EDSS (n = 264) was 3.21 and 3.17 at 6 and 12 months, respectively, after treatment with IVMP. To examine the effect of OPT, patients were divided into two groups. 152 (57.6 %) of 264 patients received OPT following IVMP for the treatment of 171 (60 %) of 285 relapses. Mean age and DD were 37.4 and 5.9 years. EDSS at baseline, relapse confirmation, and at 6 and 12 months after treatment with IVMP was 2.37, 4.42, 3.31, and 3.24, respectively. In the group receiving no OPT (n = 112 patients with 114 relapses), mean age and DD were 35.4 and 5.3 years, respectively. Mean EDSS at baseline, relapse confirmation, and at 6 and 12 months after treatment with IVMP was 2.28, 4.29, 3.28, and 3.14, respectively. There was no difference in the mean EDSS between the two groups at any time point before or after treatment with IVMP. However, 12 months after treatment, there was a significant increase in the mean EDSS in both groups compared to baseline ($p < 0.05$).

Conclusions: Oral prednisone taper after treatment with IVMP for an MS relapse appears to have no effect on neurologic disability after one year of follow up. Our study suggests that relapses may significantly contribute to neurologic disability early in the course of the disease.

187

**Glatiramer acetate reduces activation markers and TNF-a production of monocytes in vitro and in vivo.** M. S. Weber, C. Farina, M. Starck, E. Meinl, R. Hohlfeld, Ludwig-Maximilians University, Max-Planck Institut for Neurobiology, Marianne-Strauss-Klinik (Munich, Kempfenhausen, Martinsried, D)

Objective: To investigate the effect of Glatiramer acetate (GA) on monocytes in vitro and in vivo.

Background: GA is an approved therapy for relapsing-remitting multiple sclerosis (RRMS). Its immunomodulatory effect is thought to be mainly mediated by induction of GA-specific Th 2-cells which mediates bystander suppression. However, little is known about GA effects on other cell-types such as antigen presenting cells.

Methods: In vitro studies: Peripheral blood mononuclear cells (PBMCs) from healthy donors were preincubated for 1.5 h with different concentrations of GA [12.5, 25, 50 µg/ml] and stimulated with inflammatory mediators (GM-CSF/IFN-g) or toll-like-receptor (TLR) ligands for 18 h. Ex vivo studies: PBMCs from 8 healthy donors, 6 untreated MS patients and 8 GA-treated MS patients were stimulated with different concentrations of LPS [0.75, 0.15, 0.3, 0.6, 1 ng/ml] for 18 h without preincubation. Induction of activation markers on monocytes was measured as the percentage of positive stained monocytes by flow cytometry and the frequency of TNF-a producing PBMCs by Elispot assay.

Results: In vitro GA-preincubation blocked dose-dependently the induction of SLAM, CD 25, CD 69 and CD 83 and TNFa-production of monocytes mediated by GM-CSF, IFN-g, LPS, LTA, PGN or Flagellin. Monocytes from the 8 GA-treated patients showed a decreased LPS-mediated SLAM-induction and TNFa-production compared to monocytes from untreated MS-patients and healthy controls. At a concentration of 1ng/ml LPS the percentage of SLAM-positive monocytes was significantly lower in the GA-treated MS patient group (25.4 ± 6.9; mean ± SD, $p < 0.001$) than in the untreated MS patient group (38.7 ± 2.9) and the healthy donors group (45.7 ± 6.9).

Conclusions: [1] At non-toxic concentrations GA can inhibit the activation of monocytes in vitro. [2] Monocytes from GA-treated patients are less susceptible to activation by LPS.

# Session 31

## Multiple Sclerosis – 6

188

**The clinical impact of interferon beta neutralizing and binding antibodies in relapsing-remitting multiple sclerosis.** P. Gallo, P. Perini, M. Calabrese, G. Biasi, Multiple Sclerosis Centre, Dept of Experimental Pathology (Padua, Ancona, I)

Objectives: To analyze the kinetics and clinical impact of binding antibodies (BAbs) and neutralizing antibodies (NAbs) to 3 interferon beta (IFNb) products in patients with relapsing-remitting multiple sclerosis (RRMS).

Background: Treatment with IFNbs has been associated with the development of BAbs and NAbs. However, the clinical significance of BAbs and NAbs remains controversial.

Methods: Patients with RRMS received 1 of 3 treatment regimens for up to 4 years: IFNb1b 8 MIU subcutaneously (SC) every other day, IFNb-1a 30 mcg intramuscularly (IM) once weekly, or IFNb1a 22 mcg SC 3 times weekly. Serum samples were collected before initiation of therapy and every 3 months during treatment. BAbs to IFNb were measured using enzyme-linked immunosorbent assay, and positive BAb samples were subsequently analyzed for neutralizing activity using an antiviral cytopathic effect assay. High BAb titers were defined as > 1:500; patients identified as positive for NAbs had titers > 20, with high NAb titers defined as > 1:100. In addition, the impact of BAbs and NAbs on relapse rate and Expanded Disability Status Scale (EDSS) score was evaluated.

Results: A total of 90 patients were enrolled, with 30 patients allocated to each of the 3 treatment groups. Over the course of the study, 83 % of patients developed BAbs to IFNb1b, 13 % to Avonex, and 47 % to Rebif. Forty percent of patients developed NAbs to IFNb1b, 6.7 % to Avonex, and 26.7 % to Rebif. Of 22 NAb-positive patients, 10 patients (45.5 %) demonstrated high titers of both NAbs and BAbs (20 % IFNb1b, 3.3 % Avonex, 10 % Rebif). Although BAbs developed earlier than NAbs, their overall kinetics were similar in patients treated with IFNb1b or IFNb1a. There was a significant positive correlation between high NAb titers and relapse rate (i. e., > 2 relapses after NAb appearance) ($p = 0.03$). However, an even higher significance level ($p < 0.001$) was observed for the correlation between high titers of both NAbs and BAbs and relapse rate. In 10 patients with high titers of both NAbs and BAbs, an increase in mean EDSS score from 2.2 ± 0.8 at baseline to 3.6 ± 1.2 at Year 2 ($p < 0.01$) was observed. NAb-negative patients showed no significant change in EDSS score at Year 2.

Conclusions: These findings demonstrate that high titers of both BAbs and NAbs reduce the clinical efficacy of IFNb in patients with RRMS, which is important for the long-term efficacy of these drugs.

189

**Do neutralising antibodies to interferon-beta disappear?** M. Capobianco, A. Sala, S. Malucchi, A. Di Sapio, F. Gilli, R. Bottero, S. Morando, F. Marnetto, A. Bertolotto, Centro di Riferimento Regionale Sclerosi Multipla (Orbassano, I)

A percentage of Multiple Sclerosis (MS) patients treated with interferon-beta (IFN-b) develop anti IFN-b Binding and Neutralizing antibodies (NAbs). We conducted a follow-up survey of NAbs titer in 315 IFN-b treated MS patients in our Centre. NAbs were tested every three months during a period of 10–92 months, using the cytopathic (CPE) assay. 26 patients (8 %) (11 Betaferon, 14 Rebif-22 and 1 Avonex) were evaluated as persistent NAb positive (Nab +) showing at least 2 consecutive positive samples and abolished bioavailability, as tested by MxA mRNA quantification. The mean time that was necessary for NAbs development was 12 months. During IFN-b treatment NAbs disappeared in 10 out of 26 patients (38 %) (4 Betaferon 36 %, 6 Rebif-22 43 %). Seroconversion was confirmed in 5 patients by increased MxA mRNA, indicating the presence of IFNb bioavailability. Moreover, seroconversion was observed in patients showing mean NAbs titre < 200 LU; higher NAbs titres were associated with a long positive period. 62 % of NAb+ patients (16/26) were still positive at the last detection (mean 22 months; range 4–67). None of the patients showing a mean NAb titre higher than 200 LU became negative.

Conclusion: A. NAbs positivity is a long lasting phenomenon. B. 2 out of 3 NAb + patients did not seroconvert. C. Seroconversion is inversely correlated to NAb titre. D. Seroconversion is associated with reappearance of IFNb bioavailability.

190

**Three-year follow-up and clinical course after change of immunomodulating therapy in relapsing-remitting multiple sclerosis.** C. Caon, M. Din, M. Zvartau-Hind, A. Tselis, R. Lisak, O. Khan, Wayne State University (Detroit, USA)

Objective: To determine the long term clinical outcome in RRMS patients who switch immunomodulating therapy (IMT).

Background: Several IMT are available for the treatment of RRMS. Usual reasons for switching IMT are lack of efficacy or unacceptable toxicity. We have previously reported an 18-month prospective follow up on the clinical course in 85 consecutive patients who switched from weekly IM IFNB-1a to daily SC glatiramer acetate (GA). We now report an extended prospective follow up of these patients for up to 42 months on GA after switching.

Methods: 85 consecutive treatment naïve RRMS patients who received weekly IM IFNB-1a 30 mcg for 18 to 24 months were evaluated. Baseline re-

lapse rate for 2 years prior to initiating therapy with IFNB-1a was obtained from charts. All 85 patients were switched to GA 20 mg SC daily and prospectively followed for 36 to 42 months. Patients were switched because of persistently active clinical disease or unacceptable toxicity. Six patients were lost to follow up at 42 months.

Results: Mean age and disease duration were 33.7 years and 5.7 years, respectively. Annual relapse rate (ARR) was 1.39 at the time of initiating therapy with IFNB-1a. Mean duration of therapy with IFNB-1a was 19.7 months. Mean ARR on IFNB-1a was reduced to 1.13 (1.39 vs 1.13, $p = 0.0005$) or by 19.8%. All 79 patients were switched to GA and followed for 36 to 42 months (mean 37.5 months). Mean ARR on GA was 0.47 with a mean reduction in the ARR of 0.66 (1.13 vs 0.47, $p = 0.0001$) or by 58% from the relapse rate on IFNB-1a. Mean EDSS was 3.50 at the time of initiating therapy with GA and 3.29 at the last visit ($p = 0.0007$). 19 of 79 (24%) switched to GA because of persistent toxicity due to IFNB-1a and 60 (76%) because of lack of efficacy. Subgroup analysis showed that mean ARR in patients receiving IFBB-1a who switched because of toxicity was 0.61 compared to 1.29 for those who switched because of lack of efficacy. There was no significant difference in the relapse rate reduction between IFNB-1a (0.61) and GA (0.45) for the subgroup of patients who switched treatment because of toxicity. However, for the subgroup of patients who switched because of lack of efficacy, the difference in the reduction in the relapse rate between IFNB-1a (1.29) and GA (0.48) therapies was highly significant ($p = 0.0001$).

Conclusion: Patients with RRMS receiving IMT and clinically active disease may benefit from switching IMT. Our long-term prospective follow up confirms that RRMS patients with clinically active disease despite therapy with weekly IM IFN beta-1a may benefit from switching to GA. Follow up beyond three years on GA indicates sustained reduction in the relapse rate without increase in neurologic disability suggesting that GA may be a more appropriate first choice therapy in RRMS than weekly IM IFN beta-1a. Criteria based on clinical indications need to be developed for switching IMT.

191

**Cerebral axonal recovery in relapsing-remitting multiple sclerosis patients treated with glatiramer acetate.** O. Khan, Y. Shen, C. Caon, W. Ching, E. Sonenvirth, Z. Latif, V. Segal, J. Hu, Wayne State University (Detroit, USA)

Objective: To determine the effect of glatiramer acetate (GA) on cerebral axonal damage in relapsing-remitting MS (RRMS) patients.

Background: With no significant effect at the blood brain-barrier (BBB), it is postulated that GA induces bystander suppression of inflammation inside the CNS by way of centrally mediated Th2 responses. Thus, it is of interest to examine the effect of GA on cerebral axonal integrity in understanding and validating the mechanism of action of GA. N-acetylaspartate (NAA) is a reliable marker of neuronal integrity and can be measured invivo using magnetic resonance spectroscopy (MRS). This technique has been validated to demonstrate axonal injury and loss in MS.

Methods: We performed combined MRI and MRS on 18 RRMS patients before starting treatment with GA and one year later. Four patients receiving no treatment were also studied with baseline and annual MRI/MRS scans. Neurologic examination including EDSS scores were also obtained on patients. MRS intensities of NAA relative to creatine (Cr) were measured in a volume-of-interest (VOI) centered on the corpus callosum that predominantly contained white matter. We looked at the entire VOI as well as white matter appearing normal (NAWM) on conventional MRI.

Results: At the time of submission, 14 patients in the treated group had completed one year follow up MRI/MRS scans. Mean age ($n = 14$), disease duration, and EDSS were 36.7 years, 8.7 years, and 2.82, respectively. Mean NAA/Cr in the entire VOI at baseline (before therapy with GA) was 1.95 (+0.22) and increased by 10.2% to 2.15 (+0.15) after one year of therapy ($p = 0.02$). Similarly, mean NAA/Cr values in the NAWM increased from 2.06 (+0.07) to 2.25 (+0.03) (9.22%, $p = 0.04$). In contrast, longitudinal brain MRS studies in untreated MS patients have shown an average annual decline of 5 to 7% in NAA/Cr values. There was no change in individual or mean EDSS. MRS data on untreated patients ($n = 4$, mean age 43, mean EDSS 2.87) as well as conventional MRI measures on all patients after year of follow up will be presented.

Conclusion: Our data suggest that therapy with GA can reverse cerebral axonal injury in RRMS. In the absence of significant effect at the BBB level, increase in the NAA/CR values in abnormal as well as NAWM seen in GA-treated patients provides further insight into GA's ability to restore axonal function. We speculate that GA may have significant anti-inflammatory and neuroprotective effects inside the CNS.

192

**Efficacy and safety of modafinil for the treatment of fatigue in multiple sclerosis: a randomized, placebo-controlled, double blind multicentre study.** B. Stankoff, E. Waubant, C. Confavreux, G. Edan, M. Debouverie, L. Rumbach, T. Moreau, J. Pelletier, C. Lubetzki, M. Clanet, Centre d'Investigation Clinique, University of California, Service de Neurologie, Fédération de Neurologie (Paris, F; San Francisco, USA; Lyon, Rennes, Nancy, Besançon, Dijon, Marseille, Toulouse, F)

Fatigue is a frequent and incapacitating symptom in multiple sclerosis (MS). It has both physical and cognitive components and its pathophysiology remains poorly understood. Up to now, available treatments are unsatisfactory. Modafinil is an alpha-1 adrenergic wake-promoting agent whose exact mechanism of action remains undetermined. It is effective for the treatment of excessive daytime sleepiness in patients with narcolepsy. Preliminary reports have suggested that this agent could be useful for the treatment of fatigue in MS patients, but these previous studies had significant methodological limitations.

Objective: to assess the safety and efficacy of modafinil for the treatment of MS fatigue.

Methods: Patients aged 18–65 years with a diagnosis of MS, a stable disability level < 6.5 on the Kurtzke extended disability status scale, and a mean score > 45 on the modified fatigue impact scale (MFIS) were eligible for the 5 weeks randomized, double blind, placebo-controlled study. Patients complaining of anxiety or depressive disorder were excluded. The initial daily dose of modafinil was 200 mg, and, depending on the tolerance, the dose was increased of 100 mg every week up to 400 mg/day. No modification of the treatment was tolerated between day 21 and day 35. The primary outcome variable was the reduction of the MFIS score at day 35. Secondary outcome variables comprised the evolution of the fatigue impact scale (FIS), Epworth scale, physical and cognitive components of the FIS and MFIS, visual analogue scale for fatigue.

Results: One hundred and fifteen patients were included and participated in the intention to treat analysis. Whereas there was a strong improvement of MFIS scores between day 0 and day 35 in both placebo-treated and modafinil-treated groups, no difference was detected between the two groups. There was even a trend to a deterioration of the cognitive component of fatigue among modafinil-treated patients. In the post-hoc analysis, the patient population was stratified according to the existence of excessive daytime sleepiness (discriminative value of the Epworth score = 10) at inclusion. Half of the patients had excessive sleepiness. In this latter subgroup, modafinil was equivalent to placebo on the cognitive component of fatigue, but trended to be superior to placebo on the physical component of fatigue. By contrast, among patients without excessive sleepiness, modafinil was not superior to placebo, and was even inferior to placebo on the cognitive component of fatigue.

There was no major safety concern in this study.

Conclusions: Among the whole population of MS patients with fatigue, modafinil is not superior to placebo in this study. However, subgroup analysis suggests that the use of modafinil may improve the physical component of fatigue among patients with excessive daytime sleepiness, whereas it does not provide any benefit for patients without excessive daytime sleepiness.

193

**Prognosis and disease progression in relapsing neuromyelitis optica: an analysis of 27 French Caribbean patients.** P. Cabre, A. Signaté, S. Olindo, D. Caparros-Lefebvre, D. Smadja, University Hospital Fort-de-France, University Hospital Pointe-à-Pitre (Fort-de-France, Pointe-à-Pitre, F)

Background: Relapsing neuromyelitis optica (RNMO) a unique demyelinating disease characterized by recurrent optic neuritis (ON) and myelitis (MY) is supposed to carry a very poor prognosis. However, series of RNMO, involving very small numbers of patients, were hospital-based and diagnostic criteria were not uniform; thus risk factors for progression and prognosis such as age of onset, early natural history have not been evaluated. In addition, the influence of immunosuppressive therapies on disease progression of RNMO is yet unknown.

Methods: As part of a population-based multiple sclerosis survey in French Caribbean, we investigated the disease progression and survival in 27 Afro-caribbean patients with RNMO (26 women, one man; mean age at onset, 30.4 years). We evaluated these patients based on the recently proposed RNMO diagnostic criteria (Wingerchuk et al. 1999) and excluded NMO patients with proven infectious or systemic disease. We also excluded patients with disease duration < 5 years.

Results: The median time from initial symptom to combined ON and spinal cord involvement was 1.8 years (range 0–7). Median intervals from onset to aid-requiring walking, confinement to a wheelchair, a bedridden state and death were 5.6 (SD = 0.8), 9 (SD = 1.2), 9.2 (SD = 1.2) and 12.8

(SD = 1.4) years, respectively. After a mean disease duration of 8.3 years, mean EDSS score was 7.7 (range 3–10). Age of onset and time from initial symptom to combined ON and spinal cord involvement were not associated with differences in worsening of function or survival. Treated patients (N = 13) with immunosuppressive therapies (cyclophosphamide and/or mitoxantrone) had slower functional deterioration than non treated patients (aid-requiring walking: 8.8 vs 2.6 years, $p < 0.001$; bedridden state: 10.8 vs 6.8 years, $p = 0.04$) and showed longer survival (15 vs 10 years: $p = 0.01$). Likewise, mean EDSS score was higher in non treated patients compared to that observed in treated patients: 8.8 vs 6.3 ($p = 0.001$).

Conclusion: The present study suggests that no factors are involved in the progression of RNMO but most importantly, early aggressive immunosuppression seems to be efficient to alter natural history of this disabling inflammatory disorder of the central nervous system.

194

**The impact of change in interferon beta-1a dose regimen (30mcg qw to 44mcg tiw) in patients with relapsing MS – cross-over results from the EVIDENCE study.** M. K. Sharief for the EVIDENCE Study Group

Introduction: The EVIDENCE study demonstrated that interferon (IFN) beta-1a (Rebif®), 44mcg SC tiw, was more effective in reducing relapses and MRI lesion activity in patients with multiple sclerosis (MS) compared with IFN beta-1a (Avonex®), 30mcg IM qw. It remains unknown whether patients who change from weekly to thrice weekly will experience enhanced benefit.

Objective: To examine whether additional benefit is seen in patients with MS changing from low dose qw, to high dose, tiw IFN therapy.

Methods: At the conclusion of EVIDENCE, consenting patients in the 30mcg qw arm crossed over to 44mcg tiw and were followed for an average of 31 weeks. The time-point at the end of the comparative phase of EVIDENCE is referred to as the 'transition'. Pre-planned analyses for the post-transition phase included change in relapse counts and change in T2 active lesion counts, within the same patients, comparing the post-transition phase with the entire pre-transition phase and the 6-month interval immediately preceding transition.

Results: For patients continuing on 44mcg tiw (272/299 completing EVIDENCE), relapse count was 0.23 prior to transition and 0.21 post-transition ($p = 0.549$). For patients changing from 30mcg qw to 44mcg tiw (223/306 completing EVIDENCE), relapse count decreased from 0.32 pre-transition to 0.21 post-transition ($p = 0.015$). Furthermore, the number of MRI T2 active lesions, proportion of active scans per patient and proportion of patients with no active scans significantly improved ($p < 0.001$) in patients changing from 30mcg qw to 44mcg tiw. No new safety concerns were noted but treatment discontinuations during the post-transition phase were higher in patients increasing dose (n = 30) than those on 44mcg tiw since study start (n = 15).

Conclusions: Patients crossing over from 30mcg qw to 44mcg tiw experienced significant reductions in disease activity to a level in the post-transition stage that is comparable with that of patients who have been on 44mcg tiw from study start. Lack of blinding at this stage could affect clinical outcomes but the blinded MRI results support the improvement seen on relapse count. The magnitude of change in continuous 6-monthly intervals exceeds any meaningful contribution of regression to the mean. The results are consistent with the findings of EVIDENCE and support the concept of increased dose/frequency of IFN as important for maximal treatment effect.

# Session 32

## Peripheral neuropathy – 3

195

**Mutations in MTMR13, a new pseudo-phosphatase homologue of MTMR2 and Sbf1, in two families with an autosomal recessive demyelinating form of Charcot-Marie-Tooth disease associated with early-onset glaucoma.** H. Azzedine, A. Bolino, N. Birouk, A. Bouhouche, R. Gouider, A. Brice, J. Laporte, E. LeGuern, INSERM U289, Dulbecco Telethon Institute, Hôpital des Spécialités Rabat, Hôpital Razi, IGBMC, CNRS/INSERM/ULP (Paris, F; Genoa, I; Rabat, MA; Tunis, Strasbourg, F)

Charcot-Marie-Tooth disease (CMT) with autosomal recessive (AR) inheritance is a heterogeneous group of inherited motor and sensory neuropathies. In some families from Japan and Brazil, a demyelinating CMT, mainly characterized by the presence of myelin outfoldings on nerve biopsies, co-segregated as an autosomal recessive trait with early-onset glaucoma. We identified two such large consanguineous families from Tunisia and Morocco with ages at onset ranging from 2 to 15. We mapped this syndrome to chromosome 11p15 in a 4.6 cM region overlapping the locus for an isolated demyelinating ARCMT (CMT4B2). In these two families, we identified two different nonsense mutations in the Myotubularin-related 13 gene, MTMR13. The MTMR family includes proteins with a phosphoinositide phosphatase activity, and proteins in which key catalytic residues are missing, and that are thus called pseudo-phosphatases. MTM1, the first identified member of this family, and MTMR2 are responsible for X-linked myotubular myopathy and Charcot-Marie-Tooth type 4B1, an isolated peripheral neuropathy with myelin outfoldings, respectively. Both encode active phosphatases. It is striking to note that mutations in MTMR13 also cause peripheral neuropathy with myelin outfoldings, although it belongs to a pseudophosphatase subgroup, as its closest homologue MTMR5/Sbf1. This is the first human disease due to mutation in a pseudo-phosphatase, emphasizing the important function of these putatively inactive enzymes.

196

**Detailed morphometric and immunological analysis of early disease stages in the CMT rat.** M. W. Sereda, N. Uzma, U. Suter, K. A. Nave, Max Planck Institute, ETH (Germany, D; Zurich, CH)

Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy in humans and has been associated with a partial duplication of chromosome 17 (CMT type1A). We have previously generated a transgenic rat model of this disease by moderately overexpressing the peripheral myelin protein 22 (PMP22, gas-3) gene. Heterozygous transgenic rats develop gait abnormalities caused by a peripheral hypomyelination, Schwann cell hypertrophy ('onion bulb' formation), and muscle weakness at the age of approximately 4 weeks. Reduced nerve conduction velocities closely resemble recordings in human patients with CMT1A. When heterozygous animals are bred to homozygosity, Schwann cells are not able to produce any myelin (Sereda et al. (1996) Neuron 16:1049–1060).

We have used this animal model to investigate early postnatal morphological disease stages, as human biopsy material is not available for these time points due to ethical reasons. Electron microscopy of sciatic nerve sections was performed of PMP22 transgenic and wildtype animals aged postnatal day 1 (P1), 6 (P6) and 16 (P16). In the detailed morphometric analysis we found that the g-ratio, an indicator of myelin thickness, was unchanged at the time points examined showing neither demyelination nor hypermyelination. However, the amount of myelinated axons in comparison to non-myelinated axons was significantly reduced in the transgenic animals. Furthermore, we found a decreased total amount of axons in the transgenic animals, when compared to wildtype controls. In an attempt to analyse immunological markers which may influence the disease progression, we performed Western-blot analysis of full protein preparations from sciatic nerves. Hereby, we could find auto-reactive PMP-22 antibodies in the serum of transgenic animals, which proportionally correlated with the transgene dosage. To examine whether immune-competent cells play a role as an additional epigenetic factor, we performed immunhistochemistry of sciatic nerves from wildtype, heterozygous and homozygous animals. No immune competent cells, e. g. macrophages, were found. Taken together, our data suggest that PMP22 overexpression may influence the myelination of axons at early developmental stages and that immunological parameters may influence the disease course of the CMT rat, and possibly of human CMT1A.

197

**X-linked Charcot-Marie-Tooth disease: involvement of peripheral and central nervous system.** D. Pareyson, F. Taroni, C. Ciano, M. Milani, I. Moroni, M. Morbin, G. Lauria, A. Sghirlanzoni, A. Erbetta, L. Chiapparini, V. Scaioli, C.Besta National Neurological Institute (Milan, I)

The X-linked form of Charcot-Marie-Tooth disease (CMTX) is associated with mutations in the Connexin 32 gene (Cx32), and is characterized by no male-to-male transmission, intermediate motor conduction velocities (MCV), and more severe disease in males. In our series of CMT patients, we found 9 different Cx32 mutations in 11 families (one novel mutation). Overall there were 26 patients (13 males), aged 11–76 yrs. Age at onset ranged considerably (1–60 yrs.), but symptoms began earlier in males (mean 15.4 yrs.) than females (25 yrs.). All patients were autonomous, but disease severity was greater in males, while 4 female carriers were asymptomatic. One patient had Babinski sign and another had rest tremor. Upper limb MCV ranged between 25 and 57 m/s, and were in the range of CMT1 (< 38 m/s) in 10/13 males but only in 3/11 females. Abnormalities were unevenly distrib-

uted within and among nerves. In some cases nerve conduction slowing was non-uniform within single nerves. In both genders, distal motor latency was significantly more prolonged in the median nerve than in the ulnar nerve. In females, amplitude of compound muscle action potential was significantly more decreased in the median than in the ulnar nerve. We also investigated the presence of subclinical abnormalities of the central nervous system (CNS) by multimodal evoked potentials (EPs), magnetic resonance imaging (MRI), and proton magnetic resonance spectroscopy (H-MRS). Abnormalities suggestive of CNS dysfunction were found in 10 out of 12 patients (7/8 males, 3/4 females). In detail, central component latencies or interpeak times were prolonged in 8/10 brainstem auditory EPs, 2/6 pattern-visual EPs, 3/5 flash-visual EPs, 3/9 somatosensory EPs, and 3/6 motor EPs. MRI showed cerebral atrophy in two young males and mild hyperintensity of the cortico-spinal tracts in a female patient. H-MRS evaluation of the peak amplitude for the dominant metabolites was performed in 5 patients. Expression of Cx32 in the brain is the likely explanation of these findings that confirm previous non-systematic observations. Subclinical evidence of CNS involvement is very common in CMTX, and may be of help in addressing molecular studies.

Partially supported by a grant from the Italian Ministry of Health to F.T and D. P.

198

**Anticipation in Charcot-Marie-Tooth type 1A (CMT1A)?** L. Schöls, F. Taroni, A. Sghirlanzoni, V. Scaioli, C. Ciano, M. Milani, U. Schara, W. Mortier, W. Klein, J. T. Epplen, S. Otto, H. Przuntek, M. Vorgerd, J.-P. Malin, M. Tegenthoff, D. Pareyson, Ruhr-University, C.Besta National Neurological Institute (Bochum, D; Milan, I)

Charcot-Marie-Tooth type 1A (CMT1A) is the most frequent autosomal dominantly inherited neuropathy. It is associated with a submicroscopic duplication of the 17p11.2 chromosomal region encompassing the peripheral myelin protein 22 gene (PMP22). Despite genotypic homogeneity, the phenotypic expression of the disease is highly variable.

We investigated clinical features and electrophysiological characteristics in 85 patients with genetically confirmed CMT1A. To study intergenerational variability, we focused on phenotypic variability in 35 parent-offspring pairs.

Age at onset of symptoms varied from 1 to 68 years (mean 11.6 ± 14.1). Ten patients ranging from age 10 to 74 years were asymptomatic. Disability varied from severe gait difficulties due to steppage gait at age 51 to no symptoms at age 74. In accordance with previous reports, we found generalized demyelinating neuropathy as the electrophysiological hallmark of CMT1A even in clinically unaffected individuals with motor nerve conduction velocities (MNCV) between 7 and 39 m/s in the median nerve. MNCV showed an inverse correlation with age at onset.

Age at onset was reduced from 17.1 ± 19.4 years in the parent generation to 9.8 ± 11.0 in the offspring. MNCV in the median nerve was 23.9 ± 4.5m/s in parents and 19.2 ± 6.2m/s in offspring ($p < 0.001$, T-test). MNCV decreased further in 28 of 35 parent-offspring pairs and was stable in another pair.

Electrophysiological data confirm our clinical impression that CMT1A sometimes tends to start earlier and get more severe in the offspring generation, a phenomenon called anticipation which is frequently observed in neurodegenerative disorders caused by unstable repeat expansions. Since anticipation is observed in clinical as well as in electrophysiological parameters, it is unlikely that our results only reflect a bias due to increased attention for symptoms in the younger generation. However, the molecular basis underlying this phenomenon remains to be elucidated.

199

**Hereditary motor neuropathy type II (HMN II) - clinical and electrophysiological findings from a unique, multigenerational Czech family linked to 12q24.** P. Seeman, R. Mazanec, J. Irobi, E. Nelis, P. De Jonghe, V. Timmerman, Charles University Prague, University of Antwerp (Prague, CZ; Antwerp, B)

Inherited peripheral neuropaties (IPN) are usually classified into hereditary motor and sensory (HMSN), motor (HMN) and sensory and autonomic (HSN/HSAN) neuropathies. Mutations in at least 17 genes can cause HMSN, but no responsible gene is known for HMN yet despite at least seven reported chromosomal loci.

HMN are characterised by distal muscle weakness and atrophies without sensory abnormalities. Linkage to 12q24 was reported by Timmerman et al. in a large Belgian autosomal dominant (AD) HMN family (Timmerman et al. (1996) Hum Mol Genet 5:1065–1069). The responsible gene, mutated in this family is not known yet.

We report here an extensive Czech family with autosomal dominant HMN with at least 33 known affected individuals within at least 6 generations.

At present, there are 20 living clinically affected members and 16 of them were studied clinically and 18 by DNA.

The disease started between the age of 12 to 28 years, by weakness of the extensor muscles of the big toes and the feet. Increased patellar tendon reflexes are probably the very first clinical sign of the disease. The disease progresses continuously and 3 persons we have examined were wheelchair bound before the age of 50 years. Sensory involvement was absent clinically and electrophysiologically until the age of 45. There is always a pronounced weakness to plegia on lower extremities with only mild distal weakness on the upper extremities. Earlier onset resulted usually in more severe handicap.

In total 55 DNA samples from this unique family were used for linkage studies with STR markers from the critical region, previously reported by a Belgian HMN II family. Linkage to 12q24 was confirmed in this family and LOD score of 10.6 was obtained with marker D12S76. Due to this family, the gene interval for dHMN II was refined from 5 Mb to 1.8 Mb.

Supported by IGA NF 6504–3 and by VZ 111300003

200

**Demyelinating Charcot-Marie-Tooth disease: clinicopathological and genetic features in a cohort of Turkish patients.** Y. Parman, M. Poyraz, E. Bataloglu, I. Baris, B. Bilir, N. Bissar-Tadmouri, P. Serdaroglu, F. Deymeer, Istanbul University, Bogazici University (Istanbul, TR)

Charcot-Marie-Tooth (CMT) disease is the most common inherited peripheral neuropathy. Demyelinating form includes CMT type 1 (CMT1), CMT type 4 (CMT4), and X-linked CMT (CMTX). We studied clinicopathological and genetic features of 81 Turkish patients with demyelinating CMT. Thirty-nine patients underwent sural nerve biopsy. CMT1A duplication was identified in 21. In this group, 3 patients had asymmetrical neuropathy starting from the upper extremities, 2 showed normal muscle testing, and all deep tendon reflexes were present in 3. Electrophysiological study showed conduction blocks in one patient. Six patients had a mutation in Cx32, 5 in MPZ, 3 in PMP22, and one in PRX genes. One CMTX patient was clinically better than his mother. In the other mutated patients, early-onset with severe phenotype was the prominent feature. No duplication or mutation were found in 45 patients. Six patients from this group showed autosomal dominant inheritance. A recessive inheritance was suspected on parental consanguinity and/or affected siblings in 27 patients. Most of these patients had a severe phenotype. Twelve remaining patients were isolated cases. Histopathologically, hypo/demyelinating neuropathy with onion bulbs was the prominent feature in all. Teased fiber preparations of myelinated fibers displayed extensive demyelination or myelin thickness irregularities with segmental demyelination. Our findings illustrate the clinical and genetic heterogeneity in patients with demyelinating CMT. Low frequency of the CMT1A duplication (26%) might be due to the high incidence of demyelinating autosomal recessive form (CMT4) in our cohort of patients.

# Session 33

## Peripheral neuropathy – 4

201

**Long-term IV immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy (CIDP).** P. Van Doorn, D. Dippel, M. Van Burken, A. Sterrenburg, M. Suntjens, M. Vermeulen, Erasmus Medical Center Rotterdam, Academic Medical Center (Rotterdam, Amsterdam, NL)

Objective: Chronic inflammatory demyelinating polyneuropathy (CIDP) can improve after intravenous immunoglobulin (IVIg) treatment. Most patients need intermittent IVIg to maintain improvement. Limited information is available on factors related to improvement. This study aims to analyse these factors especially in relation to longterm treatment and prognosis.

Methods: Data were collected from all CIDP patients known at the Erasmus Medical Centre in Rotterdam, treated with IVIg and followed for at least 2 months. 50 clinical and laboratory parameters were analysed for a possible relation with improvement (Rankin scale). Patients presently needing

longterm treatment received a questionnaire about their experiences with IVIg treatment.

Results: 64 males and 31 females were followed for a period ranging from 2.5 months to 19.5 years (median 4.0 years). 15 patients received additional treatment to reduce the amount of IVIg. 77/95 (81%) patients showed improvement after start of IVIg. 66/77 (86%) patients needed intermittent IVIg treatment for at least 2 months, suggesting that improvement was not due to a spontaneous remission. Improvement was related with a relapse in the past (all 12 patients improved), progressive weakness until treatment and no discrepancy in weakness between arms and legs. Mean time on IVIg treatment until remission was 3.5 years (median 2.1 years). Patients with sensory-motor disturbances ($p = 0.002$; HR 3.2) and a relative short duration of weakness ($p = 0.008$; HR 2.6) had a higher chance to reach remission after discontinuation of IVIg. 10% of patients needed IVIg for a period over 8.7 years (maximum 19.5 years). Severe side-effects were not seen. Most patients needing longterm IVIg treatment (once every 2–6 weeks) were satisfied and not eager to switch to another treatment.

Conclusion: Most patients need IVIg for a long period to maintain a good clinical condition. Especially due to high costs, it may be considered to switch or add another immunomodulatory drug in a rather early stage of disease. Since most patients were treated with IVIg only, this study may serve as a national history survey of CIDP during longterm IVIg treatment.

202

**Mycophenolate mofetil for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) – an open-label study.** K. Schweikert, A. Radziwill, P. Fuhr, A. Steck, University Hospital Basel (Basel, CH)

Background and goals: CIDP is an acquired progressive or relapsing peripheral neuropathy of motor and/or sensory fibres responding to immunotherapy. Mycophenolate mofetil (MMF), a new immunosuppressive drug, inhibits the de novo pathway of guanosine nucleotide synthesis and thus B- and T-lymphocyte proliferation. Clinical improvement was described in myasthenia gravis, inflammatory myopathy, and CIDP. The aim of this open-label study was to investigate efficacy and tolerability of MMF in CIDP patients refractory to previous immunotherapies.

Methods: Patients between the ages of 18 to 80 years with acquired CIDP, diagnosed according to published guidelines, were enrolled if not responding to intravenous immunoglobuline (IVIg), oral prednisone, azathioprine, cyclophosphamide or beta interferon. 'Not responding' was defined as progression of disability and/or dependency on IVIg at least every 4 weeks. MMF was given in an oral dose of 1 g every 12 hours with possible increase up to 3 g daily. Efficacy was defined as stability or improvement of disability after 6 months and/or reduction of IVIg dose within 12 months. Follow-up consisted in physical and neurological examination (Disability grade, Neurological Disability Score, Hammersmith motor ability score, timed 10 m walk) at entry, month 2, 4, 6 and 12. Safety was assessed by clinical status, monitoring of adverse events, and blood cell count.

Results: Seven patients were enrolled (5 men, 2 women; mean age 57.6 years) with duration of CIDP from 2 to 23 years (mean 9 years), progressive course in 3 and relapsing in 4 patients. MMF treatment period was 4 to 23 months. After 6 months 3 out of 6 patients improved; improvement was sustained for 9, 15, and 23 months, respectively. MMF was discontinued in 1 patient after 5 months despite improved neurological scores because of progressive dysesthesia and in 2 patients after 12 months because of progressive disability. No major side effects or laboratory abnormalities were observed.

Conclusions: MMF could improve neurological status in CIDP patients with disease progression under conventional immunotherapies. A large prospective randomised controlled trial is needed to assess long term efficacy of MMF in CIDP.

203

**Rituximab therapy in chronic inflammatory polyneuropathy with antibodies to neural antigens.** S. Simonetti, D. Bianchini, S. Ratto, E. O. Ospedali Galliera (Genoa, I)

Rituximab, a monoclonal antibody directed against the B-cell surface membrane marker CD20, has been shown to be effective in IgM antibody-related polyneuropathies, but, until now, only few treated cases have been reported. We treated four patients with chronic inflammatory demyelinating polyneuropathy (CIDP) associated with antibodies to neural antigens, with four weekly intravenous infusions of Rituximab (375 mg/m$^2$). One patient had anti-sulfatide antibodies and no monoclonal gammopathy of undetermined significance (MGUS), while the remaining three had antibodies to myelin-associated glycoprotein (MAG) and IgMK MGUS. All the 4 patients had progressive sensory-motor polyneuropathies and previous ineffective treatment with various combinations of intravenous immunoglobulin, plasma exchange, prednisone and cyclophosphamide. Quantitative assessment included modified Rankin disability score, MRC strength score, four limb sensory score, electrodiagnostic studies and autoantibody serum concentration. All the four patients noted a subjective improvement within 3 months, while an objective improvement was observed within 6 months after treatment. Improvement persisted during a one-year follow up. No significant side effects were reported. Our results seem to indicate that Rituximab treatment may be effective in CIDP with antibodies to neural antigens.

204

**Guillain-Barré syndrome: how well do patients recover?** C. Dornonville de la Cour, J. Jakobsen, A. Fuglsang-Frederiksen, H. Andersen, Aarhus University Hospital (Aarhus, DK)

Objective: To study motor, sensory and autonomic function in a long-term follow-up after Guillain-Barré syndrome (GBS).

Background: Recovery after GBS is not always complete. Weakness, axonal loss, sensory, and autonomic deficit after GBS have not been studied quantitatively. Impaired muscular endurance might lead to fatigue. We examined muscle strength and endurance, reinnervation plus sensory and autonomic function using quantitative methods in a population based study.

Material: We asked patients with a confirmed diagnosis of GBS in Aarhus County during 1989–99 to participate. Healthy controls were matched for age, sex, height and weight.

Methods: Symptoms and signs of neuropathy were scored. Ankle dorsal flexion peak torque and endurance was measured by isokinetic dynamometry. Detection threshold of vibration and cold was tested using computer assisted sensory examination (CASE), and autonomic function by studying R-R-variations. Reinnervation study by macro-EMG in the anterior tibial muscle was done on patients only.

Results: The study group consisted of 40 patients, and the same number of healthy controls was included. There were 26 men and 14 women in each group. Mean age of patients was 46.6 years and mean time since diagnosis 6.8 years [1,2–13,9]. There was no difference in daily physical activity between the groups. Patients had more symptoms and signs of neuropathy ($p < 0.001$). Mean peak torque in patients' ankle dorsal flexion was 27.7 NM ($\pm 8.68$) and in controls 32.1($\pm 7.55$), $p = 0.024$. This corresponds to an average loss in strength of 13.6% in the patient group. Endurance was not significantly different between the groups. 64.9% of patients had signs of reinnervation on macro-EMG. Relative peak torque correlated inversely to reinnervation, $r = -0.61$, $p < 0.001$.

Quantitative sensory testing showed a higher threshold for cold in feet and hands ($p = 0.004$ and 0.012 respectively) and a trend for higher vibratory threshold in the feet ($p = 0.062$). The tests of autonomic function revealed no difference between the groups.

Conclusion: Long-term follow up of GBS patients demonstrates reduced strength of dorsal ankle flexion correlated to motor axonal loss. Muscular endurance is normal. Cold threshold is affected whereas autonomic function is normal. The results indicate that permanent damage to the motor and sensory fibers is common in GBS.

205

**What is the ultimate fate of CIDP patients who respond to therapies?** M. Sabatelli, F. Madia, L. Quaranta, G. Lippi, A. Conte, M. Mereu, P. Tonali (Rome, I)

Nearly 50–60% of CIDP patients show a clinical remission with the administration of standard therapies. Unfortunately the achievement of this goal does not conclude patients' troubles but coincides with the beginning of another story whose course is often uncertain. In our study of CIDP patients we attempted to establish the fate of 36 patients showing an initial remission after treatments. Over a long-term period of follow-up, we observed that the disease in these patients can follow 3 different clinical courses. A first group of 11 patients had a monophasic disease. All of them achieved a complete remission after therapy with corticosteroids and no relapses were observed after drug interruption over a follow-up period of 6 months – 12 years; the duration of treatment varied between 3–24 months. A second group of 6 patients had a relapsing course; these patients became asymptomatic with corticosteroids but relapsed after a period of time of at least six months (range 6 months – 16 years) since therapy suspension. The number of relapses varied from 1 to 4. A third group was represented by patients (19 in our series) who needed continuous treatment over a long period of time to maintain improvement. The apparent relapsing-remitting course in these patients was always related to tapering of therapies. The duration of this chronic active form ranged from 2 to 17 years. 5 patients of the last group were able to stop therapies without evidence of worsening over a follow-up period of 2–12 years. This subgroup of patients had a prolonged monopha-

sic course rather than a relapsing-remitting form. Our findings show that CIDP is a heterogeneous disease encompassing cases with a single monophasic episode in which the term "chronic", with respect to GBS, is referred mainly to the prolonged phase of worsening and cases in which the chronic nature of the condition is outlined by the long duration (up to decades) of the "active phase", made-up of therapy-related relapses/remissions.

206
**Clinical and electrophysiological recovery after carpal tunnel release.** O. Hasegawa, S. Matsumoto, G. Gondo, Yokohama City University Medical Center, Daini-Kawanami Hospital, Shonan-Kamakura General Hospital (Yokohama, Fukuoka, Kamakura, JP)

We studied clinically and electrophysiologically 20 hands from 17 patients with carpal tunnel syndrome before and after decompressive surgery. Conventional motor or antidromic sensory nerve conduction studies using surface electrodes were performed in the distal segment of the median nerve. In the intrafascicular method a tungsten microelectrode was inserted into the median nerve trunk at the elbow. A compound nerve action potential (CNAP) was recorded with a supramaximal stimulation at the wrist. The electrode was situated where the greatest compound nerve action potential could be obtained. A sensory nerve action potential (SNAP) was also recorded with a supramaximal stimulation at an innervated finger. Ninety percent of patients recovered from paresthesia or pain within 3 months after surgery, together with the improvement in the sensory nerve conduction velocity between finger and elbow. The maximum conduction velocity (NCV) of the CNAP in the forearm segment was found to remain normal both before and after the operation. The peak-to-peak amplitude (C-Amp) was low with a value of $82 \pm 44$ (mean $\pm$ SD) microV before surgery. However, it significantly improved to $178 \pm 68$ microV twelve months after the release ($p < 0.01$). The sensory nerve conduction velocity (SCV) of SNAP was also low with an initial value of $36.8 \pm 7.9$ m/sec, which significantly improved within 1 month ($p < 0.01$) and then from 1 month to 3 months ($p < 0.05$) after the release. We failed to bring out a significant change in conventional nerve conduction studies. But the number of hands, whose sensory nerve action potential was obtainable by conventional method, gradually increased from 45% before surgery to 100% at 12 months after the release. Patients' subjective improvement closely paralleled the improvement of the conduction delay or block across the wrist. The retrograde axonal degeneration in the forearm segment of the median nerve slowly improved during the 12 post-operative months, and this improvement also closely corresponded to the recovery from Wallerian degeneration, which was considered to be responsible for the atrophy of abductor pollicis brevis muscle.

In conclusion carpal tunnel release operation immediately improves conduction disturbance across the compression site, but distal and proximal axonal degeneration recovers gradually after six months.

207
**Electrically induced neurogenic axon-reflex in the evaluation of diabetic small fibre neuropathies.** H. H. Krämer, M. Schmelz, F. Birklein, A. Bickel, University of Mainz, University of Heidelberg, University of Erlangen (Mainz, Mannheim, Erlangen, D)

The neurogenic axon-reflex mediated flare reaction depends on density and function of C-fiber nociceptors and could possibly be used as a parameter in the evaluation of small-fiber-neuropathies (SFN).

Methods: We induced neurogenic flare vasodilation by intracutaneous electrical stimulation with increasing current (1 Hz, 1 to 20 mA, total duration 21 min) and used a Laser Doppler Scanner (LDI, Moor-Instruments, Devon, UK) to analyze the size and intensity of the neurogenic flare on the foot dorsum and ventral thigh in 12 diabetic patients with suspected SFN and 11 age-matched controls.

Results: Flare size was significantly smaller at the thigh at all points of measurement in the patient group compared to the controls ($p < 0.007$) although no clinical symptoms were present within the tested area. At the foot, however, flare size in patients was reduced if current exceeded 15 mA ($p < 0.007$). Electrical threshold for the start of the flare reaction was higher in patients (8 to 12,5 mA) than in controls (6 mA).

Conclusions: Transcutaneous electrical stimulation allows reliable testing of the neurogenic flare reaction and in combination with LDI scanning, it might be a useful tool to diagnose SFN in diabetics at an early stage of the disease.

## Neurorehabilitation

208
**Outcome predictors in stroke patients with severe dysphagia.** G. Ickenstein, M. Horn, N. Rallis, R. Goldstein, U. Bogdahn, J. Stein, University of Regensburg, Spaulding Rehabilitation Hospital (Regensburg, D; Boston, USA)

Background and Purpose: Dysphagia is estimated to occur in 25–50% of the stroke rehabilitation population. Those patients with severe dysphagia may receive feeding gastrostomy/jejunostomy tubes (FGT) if noninvasive therapies prove ineffective in eliminating aspiration or sustaining adequate energy intake. Our aim was to quantify recovery of swallowing function, and to identify variables predictive of mortality after dysphagic stroke.

Methods: We identified all consecutive stroke cases with FGT placement admitted between May 1998 and October 2001. The medical records were reviewed, and demographic, clinical, videofluoroscopic (VSS) and neuroimaging information were abstracted. A follow-up telephone interview was performed to determine whether the tube is still in use or taken out or if the patient died in the meantime. In addition all death certificates were reviewed for time of death. Univariate and multivariate analyses were performed.

Results: 77 of all 664 (11.1%) stroke patients admitted had FGT insertion for dysphagia (mean age 69.8 years (+ 13.1 years, range 20–92), 52% male, 63 ischemic (81.8%), 14 hemorrhagic stroke (18.2%)). 31.2% (24/77) had FGT removal before discharge and they resumed oral diets. On univariate analysis patients who had the FGT still in at discharge from the rehabilitation hospital were more likely to die in the first two follow-up years (5 vs. 50%) compared to those who had the FGT out before discharge from stroke rehabilitation ($p < 0.006$). On multivariate analysis we found a model with FGT removal ($p < 0.011$) and aspiration during VSS ($p < 0.040$) that was significantly associated with death during follow-up.

Conclusions: Dysphagia requiring FGT is common in patients with stroke referred for rehabilitation. Patients who had a FGT still in place at discharge or aspirated during VSS were substantially more likely to have died by the time of follow-up compared to those who had the FGT removed before discharge from the stroke rehabilitation unit.

209
**Limits and predictability of the functional benefit of botulinum toxin injection in the inferior limb of stroke patients.** M. Rousseaux, M. J. Launay, O. Kozlowski, CHRU de Lille, Hopital Swynghedauw (Lille, F)

Background: Botulinum toxin injection reduces spasticity level. However, the effect on gait parameters and daily living activities remains controversial, and the prediction of results remains poorly evaluated.

Objective: To investigate these effects and the predictability of results.

Methods: Forty seven injections were performed in 43 patients with stroke (M: 23; mean age: 51.3), in the soleus, gastrocnemius, tibialis posterior and anterior, and flexor digitorum longus, with a global dose of 300 U (Botox). Each was evaluated at day 0 (D0), day 14 (D14), month 2 (M2) and month 5 (M5).

Results: We observed a significant but moderate reduction in spasticity (Ashworth) of foot flexor (0.72/4) and extensor muscles, with an increase in the range of active and passive movement, and improvement in gait parameters, especially for distal positioning. Gait velocity (10 metres) with usual aids was not affected in the global population, but improved in patients who were able to walk without any aid. The Rivermead Motor Assessment showed a discrete improvement in gross functions (sitting to standing, walking without aid, walking up and down four steps) and leg and trunk assessment (sitting to standing, half-crook lying, step unaffected leg, tap ground, dorsiflex). In daily living, patients reported better foot positioning, facilitation in limb propulsion, and better static and dynamic balance. Improvement was best predicted – for gross functions (RMA) by the age (negative relation) and severity of foot varus in standing position (positive) – for leg and trunk assessment by the age and active dorsiflexion movement (positive) – for subjective improvement by the sex (better in men), delay (negative) and distal spasticity of dorsiflexors (positive).

Conclusion: This study confirmed a moderate benefit of botulinum toxin injection on spasticity, and showed a discrete improvement in gait parameters is possible, especially in patients less affected. Furthermore, improvement can be partially predicted by the initial analysis of distal disorders.

211
**Evaluation of grip force control and handwriting after head trauma.** S. Buhmann, J. Hermsdörfer, C. Marquardt, Städtisches Krankenhaus München Bogenhausen (Munich, D)

Introduction: From clinical scores it is experienced that after head trauma following car accident most patients show deficits concerning motor coordination and sensory-motor control even if gross force remains. Although deficits in fine motor control are one of the most frequent and long-lasting effects of head trauma, correlation with trauma severity and the development of posttraumatic deficits has yet not been described in more detail. Therefore the aim of our study was to analyse and quantify sensory-motor deficits using the following methods:

Methods:Elementary finger force and movement control: For the examination of basic deficits of finger force control we measured maximum grip force and fast force changes in a precision grip (system FCA); sensory function was assessed by resistance to perturbation (system FS).

Handwriting: To analyse more complex capabilities we evaluated handwriting movements using a digitizing tablet (system CS).

Functional Force control: To analyse grip force control during daily object manipulation we use an instrumented hand-held object supplied with force and acceleration sensors (system GF).

Results: In an ongoing study we analysed four patients (three male and one female, 18–60 years old, right handed, right hand affected) suffering from head trauma after car accident and four healthy sex- and age matched control subjects.

On the elementary control level (FCA) the patients showed normal maximum finger force, but during fast force changes all showed slowness and dys-coordinated force production with the affected hand. Sensory function was not affected.

In handwriting (CS) the patients were slowed writing a test sentence and also showed clear deficits during simpler sub-movements underlying handwriting.

When the patients held the instrumented object (GF) stationary and subsequently performed cyclic movements with increasing speed they produced greater grip forces than the control subjects during both tasks (up to 100% increased) and grip force was not well coordinated with movement-induced loads.

To examine the effort of rehabilitation we did a follow-up on three patients approximately after four months. Almost no difference in the FCA and FS-tasks were obvious, the grip force level was now only slightly elevated compared to control results. Concerning the handwriting tasks, all patients still showed deficits in writing velocity and also in sub-movements.

Conclusion: The patients yielded preserved capabilities in basic tasks but seemed to be increasingly impaired in more complex tasks.

The patients produced uneconomically elevated grip forces during both stationary and dynamic tasks. This might represent a more general control strategy when the output of motor commands is suboptimal.

The follow-up of three patients suggested that it takes more effort and time to improve deficits especially in complex aspects of fine motor control such as handwriting.

212
**A re-analysis of the efficacy of trunk control retraining (Bon Saint Côme orthesis) in patients with spatial neglect.** J. Rousseaux, T. Bernati, J. C. Millis, C. Rossignon, O. Kozlowski, CHRU de Lille, Hôpital Swynghedauw (Lille, F)

Background: It has been suggested (Wiart et al. 1997) that in neglect patients, specific training of trunk rotation coupled with mobilisation of spatial attention to the side contralateral to the brain lesion, using the Bon Saint Côme (BSC) orthesis is able to significantly improve neglect signs and independence in daily living. Positive effects have also been reported in stroke patients with balance disturbances (De Sèze et al. 2001). However, these results have not been replicated.

Objective: To re-evaluate this favourable influence of axial postural rehabilitation using the BSC orthesis.

Methods: Twenty two patients who had left spatial neglect after a stroke were prospectively included. Neglect was assessed using the Albert and the Bells cancellation tests (AT and BT), and the Schenkenberg line bisection task (ST). Patients were randomly included in the BSC or in the conventional treatment (CT) groups. They received this treatment in addition to classical physiotherapy, for 20 hours during 1 month. They were assessed at day 0 (D0), month 1 (M1), 6 (M6), and 12 (M12), using four measures of neglect (AT, BT, ST and the behavioural Catherine Bergego Scale (CBS)), the modified Motricity Index (MI: 2–300), evaluation of gait [5–0], and the Functional Independence Measure (FIM: 18–126). Investigators were blind of the inclusion in either groups.

Results: Age, sex, delay since stroke, hemianopia, and lesion volume were comparable in both groups. Deficits (MI) and disability (FIM) were initially more severe in the BSC group, then the initial severity was introduced as a covariance factor in each ANOVA of group and examination (from D0 to M12). Improvement was discretely more important in the BSC than the CT group for the MI (trunk, global) and FIM was similar for the CBS (patient questionnaire), MI (upper and lower limbs) and gait, and less important for the percentage of deviation and number of lines omitted (ST) and the number of left minus right omissions (AT, BT). Furthermore, main effects of treatment and interactions were not significant.

Conclusions: We did not replicate results of both previous studies, and failed to demonstrate more important effect of Bon Saint Côme orthesis training than of conventional rehabilitation of trunk and standing balance.

# POSTER SESSIONS

## Poster Session 1

### Cerebrovascular disorders

P213
**Stroke in patients with heart failure: aetiology and outcome.** E. Manios, A. Dimitriou, V. Kotsis, G. Tsivgoulis, P. Konstantopoulou, K. Spengos, K. Vemmos, University of Athens (Athens, GR)

Background: Congestive heart failure is the second most frequent cardiac disorder after atrial fibrillation associated with stroke. Aim of our study was the evaluation of stroke etiology and clinical outcome in acute stroke patients with history of heart failure.

Methods: We studied a consecutive series of first-ever stroke patients confirmed with standardized clinical and CT criteria admitted to our hospital within 24 hours after symptom onset from June 1992 to June 2002. Patients were classified according to etiopathogenic mechanisms into stroke subtypes and were followed up to 10 years. The clinical severity of heart failure was based on the New York Heart Association (NYHA) classification. Survival was determined using Cox regression analysis.

Results: From 1594 study patients (mean age 70.3 ± 12.6) 126 (7.9%) had signs of heart failure. The most common stroke etiology among them was cardioembolism (78 patients, 61.9%) followed by cryptogenic ischemic infarcts (20 patients, 15.8%) and large vessel atherothrombotic disease (12 patients, 9.5%). In 8 patients (6.3%) stroke was due to decreased systemic perfusion. Lacunar stroke was evident in 3 (2.4%) patients and intracerebral hemorrhage in 5 (3.9%). Patients with heart failure had a significantly ($p = 0.001$) higher stroke mortality rate at 1 month (31%) and a significantly ($p = 0.002$) lower cumulative long term survival (24.2 ± 2.82 months) than stroke patients without heart failure (16% and 64.5 ± 1.51 months respectively).

Conclusions: In 40% of stroke patients with concomitant heart failure the cause of stroke is not cardioembolism. Early and long-term survival in these patients is poor.

P214
**Isolated pontine infarction: aetiology, outcome and recurrence.** K. Spengos, G. Tsivgoulis, P. Konstantopoulou, E. Manios, G. Bozas, M. Panas, K. Vemmos, University of Athens (Athens, GR)

Background: There have been limited reports of isolated pontine infarction with sufficient number of patients. Aim of our study was the evaluation of stroke etiology, clinical outcome and recurrence in patients with pontine infarcts.

Methods: From a series of 1550 consecutive patients with acute first-ever ischemic stroke, 100 (6.5%) had isolated pontine infarction. They all had an initial CT-scan on admission and a second CT, MRI or MRA. Stroke was classified based on etiopathogenic mechanisms. They were followed up to 5 years. Statistical analysis was performed by using Kaplan-Meier estimates and log rank test.

Results: The most common stroke etiology was basilar artery branch disease (BABD) in 43 (43%) patients, followed by small vessel disease (SVD) in 34 (34%) and large vessel disease (LVD) of vertebrobasilar arteries in 21

(21%). The cumulative risk of survival free of stroke recurrence after 5 years was 70.6% for patients with SVD, 85.7% for patients with LVD and 97.6% in patients with BABD ($p = 0.023$). Patients with LVD had a significantly ($p = 0.031$) higher mortality rate at 1 month (14.3%) than patients with other etiology of pontine infarction. Cumulative long-term (5 year) survival did not differ significantly ($p = 0.7$) among stroke subtypes.

Conclusions: BABD is the most common cause of isolated pontine infarction, followed by SVD and LVD. Early mortality rate is higher in patients with LVD, while stroke recurrence is more frequent in patients with SVD.

## P215

**Hypoxia-induced angiogenesis or death pathways in endothelial cells: the role of matrix metalloproteinase (MMP)-2.** Y. Ben-Yosef, S. Shapiro, N. Lahat, A. Miller, Carmel Medical Center (Haifa, IL)

Background & Objectives: Exposure of endothelial cells (ECs) to hypoxia, occurring in ischemic brain injury, promotes tissue remodeling and angiogenesis or results in death of the ECs. The fate of the ECs depends on the severity and duration of the hypoxic insult, which together with hypoxia-induced factors in the injured brain, such as VEGF and TNF-a, may determine divergent intracellular signaling pathways. The matrix metalloproteinases (MMPs) enzymes, amongst them MMP-2, are important in extra-cellular matrix (ECM) re-modeling, crucial for both survival and death of ECs. Our previous study, employing an EC line, demonstrated hypoxia-mediated modulation of MMP-2 expression. Presently, primary human umbilical vein endothelial cells (HUVEC) are being studied to determine a possible role for MMP-2 in hypoxia-mediated proliferation, migration leading to angiogenesis as opposed to cell death either by apoptosis or necrosis.

Methods: HUVEC, are exposed to hypoxic (3% oxygen) or normoxic conditions (21% oxygen) for 6–72h and MMP-2 protein and mRNA levels are detected by zymography and commercial gene-array kit, respectively. XTT assay and 3 [H]thymidine incorporation are used to assess ECs viability and proliferation. The levels of apoptosis and necrosis are determined by commercial ELISA and TUNEL assay. An in-vitro wound assay is used to evaluate ECs migratory capacity.

Results: Hypoxia of human ECs enhances MMP-2 expression and activity while inducing cell death by apoptosis but not necrosis of these cells. Both MMP-2 activity and apoptosis are further enhanced by TNF-a during normoxic and furthermore during hypoxic conditions. The wound assay demonstrated hypoxia-mediated enhanced migration of ECs that was suppressed by the addition of a specific MMP-2 inhibitor. In addition the MMP-2 inhibitor partially suppressed the combined hypoxia/TNF induced apoptosis.

Conclusions: The present study points to the involvement of MMP-2 in both ECs migration as well as apoptotic processes following hypoxia. Understanding the role of hypoxia-induced MMP-2 in angiogenesis and death pathways of ECs may contribute to the development of future therapeutic interventions for ischemic CNS injury.

## P216

**Alterations of SO2 with position in acute stroke patients.** A. P. Titiz, S. Ozturk, S. Ozbakir, Ankara Numune Education and Research Hospital (Ankara, TR)

Hypoxemia is an important factor that increases cerebral damage in acute stroke patients. This study was planned to evaluate relations between oxygen saturation and position in acute stroke patients. The SO2, pulse and blood pressure values of the subjects initially, at the 15th, 30th and at the 60th minutes were recorded from the patients lying on both paretic and healthy sides in the lateral decubitus position on the 1st, 3rd and the 7th days. The lesion properties were determined on CT. Clinical parameters were also recorded. Fifty patients (19 male, 31 female) were taken into this study with the diagnosis of the acute stroke a mean age of 68.3. In 19 of the patients haematoma, in 30 of them infarct and in only 1 of them hemorrhagic infarct were proved on CT. The initial, at the 15th, 30th and 60th minutes' SO2 values of the subjects recorded from the healthy sides dependent the lateral decubitus position in the first day of stroke were found to be high ($p = 0.000$ initially, $p = 0.002$ at the 15th minute, $p = 0.013$ at the 30th minute, $p = 0.024$ at the 60th minute). In female patients the levels of SO2 were found to be low in both of the recumbent positions ($p = 0.017$, $p = 0.020$). The levels of SO2 in the group of haematoma were lower than of the patients in the group of infarct ($p = 0.038$). The values of SO2 of patients who died were lower than those of patients alive on the 3rd day ($p = 0.013$ initially, $p = 0.012$ at the 30th minute, $p = 0.020$ at the 60th minute). The SO2 levels in the same recumbent positions demonstrated improvement in the course of time ($p = 0.042$). There was no relation between the positions and the values of pulse, whereas, values of the systolic blood pressure were found to be higher in the patients lying on the healthy side ($p = 0.013$ initially, $p = 0.009$ at the 15th minute, $p = 0.017$ at the 30th minute). Finally; when taken into attention the importance of providing the sufficient oxygen perfusion in the acute period treatment of stroke patients, additional to the medical treatment, in order to provide optimal oxygen saturation, the most proper position for such patients was decided to be lateral decubitus position on the healthy side.

## P217

**Movement disorders following acute stroke: a clinico-radiological study.** D. Karakostas, D. Parissis, T. Doskas, A. Drevelegas, A. Charitandi, N. Artemis, I. Milonas, Ahepa Hospital (Thessaloniki, GR)

Involuntary hyperkinetic movement disorders are uncommon manifestations of stroke, either due to ischemic infarction or due to cerebral hemorrhage.

The aim of our study was to identify patients with acute or delayed movement disorders among patients who had hospitalized in the Stroke Unit of our Department. We retrospectively studied the clinical and neuroimaging profile of 6 patients with hyperkinetic syndrome (HS), developed at the acute or chronic stage among 565 stroke patients (1.06%) admitted to the hospital from 1998–2000. The HS was related to cerebral hemorrhage in two and cerebral infarction in four patients, the responsible lesion was solitary in four and multiple in two, while hyperkinesia developed acutely in two patients and was delayed (24 hours–2 months) in four. The HS consisted of limb choreo-athetosis with head tremor in two patients, athetosis with dystonia in one, tremor with upper limb myoclonus in one, palatal myoclonus in one and arm-head choreic movements in one. On neuroimaging, the lesions responsible were located in thalamic nuclei (ventral oral, ventral intermediate, ventral caudal) in three patients, the pons or midbrain-pontine junction in two, an extensive temporo-parietal area in one and a similar temporo-occipital area associated with bilateral involvement of the lenticular nuclei in one.

Based on these findings, as well as on recent literature data, we conclude that:

1. Hyperkinesias develop in 1% of stroke patients either acutely or in the delayed phase, with frequent involvement of thalamic nuclei, in vascular territories of the deep perforating arteries (most commonly thalamogeniculate).
2. Largely unilateral, they most frequently develop in the upper limb and present favorable outcome regardless of any pharmaceutical intervention.
3. The clinical manifestations of these phenomena are not directly related to any specific anatomic location, but rather correspond to a global derangement of the complex network, with parallel processing of the thalamus, the basal ganglia and the upper brainstem.

## P218

**Glucosylated haemoglobin (HbA1c) in patients with cerebral stroke.** J. Staszewski, J. Kotowicz, A. Dmoch, G. Stypula, J. Kamieniowski, P. Grieb, M. Sadowska, M. Kopka, P. Poltora, Military Medical Institute, Dept. of Neurology, Polish Academy of Science (Warsaw, Kielce, Wroclaw, PL)

The fraction of glycosylated hemoglobin (HbA1c) is a measure of mean glycemia over preceding 3 months. Several reports have been published indicating that stroke patients frequently display HbA1c > 7% indicating either undiagnosed, or diagnosed but inadequately controled diabetes mellitus. However, these studies were based on a rather small number of cases. On the other hand, a recent epidemiological study (Khaw et al. BMJ 2001, 322:15) encompassing data for almost 6,000 persons followed over a 3 year period indicated that HbA1c constitutes one of the most potent risk factors of cardiovascular death (including that caused by stroke) which increases linearly over a whole HbA1c range. To confirm this conclusion we have started a multicenter trial in which the HbA1c fraction will be determined in at least 1,000 consecutive stroke patients. At the time of writing the present abstract our material comprises 252 stroke cases including 206 (82%) ischemic stroke, 27 (11%) hemorrhagic stroke, and 19 (7%) TIA cases.

The preliminary analysis of our data leads to the following conclusions:

1. 48/206 (23%) of ischemic stroke patients (but only 1/19 TIA patients and none of 27 patients with hemorrhagic stroke) had a history of diagnosed diabetes mellitus. Futhermore, the average HbA1c in the ischemic stroke patients with diabetes was 7.1 (1.9 SD)%, markedly higher than the average HbA1c in the ischemic stroke patients with no diabetes record, 5.5 (0.8 SD)%. Our data indicate that both diabetes mellitus and inadequate control of glycemia in diabetic patients are strong risk factors of ischemic stroke, but not hemorrhagic stroke or TIA.
2. Although only 3/158 (2%) patients with ischemic stroke and no prior

history of diabetes mellitus could be qualified as having undiagnosed diabetes mellitus (defined as HbA1c > 7%), the majority of them, i.e., 120/156 (76%), displayed HbA1c between 5 and 7% which could be interpreted as a sign of impaired glucose tolerance. Therefore, our data confirm the results of the aforementioned study of Khaw et al. and indicate that HbA1c level between 5 and 7% (indicating pre-stroke impaired glucose tolerance) is a significant risk of ischemic stroke.

Acknowledgement: The study is supported by the Fundation of Diagnostics and Therapy, Warsaw, Poland.

P219

**Hypercoagulopathy in patients with non-valvular atrial fibrillation: haematologic and cardiologic investigation.** N. Turgut, O. Akdemir, B. Turgut, M. Demir, G. Ekuklu, Ö. Vural, G. Özbay, U. Utku, Trakya University (Edirne, TR)

Introduction: Coagulation system is activated and coagulation activation markers elevated in acute ischemic stroke patients with nonvalvular atrial fibrillation (NVAF). Etiology, severity and prognosis of the ischemic stroke can be estimated with the level of the activation of coagulation system. The treatment procedure and the duration of treatment is important for patients with NVAF. In this study, we measured prothrombin F1 + 2 (F1 + 2), Di-dimer and fibrinogen levels for demonstrating hypercoagulability in acute ischemic stroke patients, and we correlated these parameters with echocardiographic findings.

Methods: From January 2001 to August 2002, 55 patients with acute ischemic stroke were included in the study. 29 of them had sinus rhythm (group I, age: 64,86 ± 10.58), 26 of them had NVAF (group II, age: 67.42 ± 11.28). The control group consisted of 20 sex and age matched healthy subjects (group III, age: 65.61 ± 10.41). We excluded patients taking oral anticoagulation. Patients in group I and II underwent neurological examination, ECG, transthoracic echocardiography (TTE), CT scan, cervical duplex ultrasonography and hematological parameters. Patients in group III underwent only hematological parameters. We evaluated left atrial dimensions and left atrial thrombus by TTE.

Results: Age, gender, hypertension, diabetes mellitus, hypercholesterolemia, hematocrit, D-dimer were not found significantly different between groups. In group I, PACI + TACI was 27.5%, LACI was 41.3%, POCI was 31%. In group II, PACI + TACI was 73%, LACI was 19.2%, POCI was 7.6%. 3.4% of the group I patients and 11.5% of group II patients had silent infarction. In group III fibrinogen level (251.64 ± 60.96) was significantly lower than in group I (347.97 ± 111.49) and II. (364.04 ± 86.20) ($p = 0.001$). In group II; F1 + 2 level (2.83 ± 0.89) was significantly higher than in group I (2.33 ± 0.80) and III (1.94 ± 0.64) (p values: group I–II = 0.036, group II–III = 0.001, group I–III = 0.104). In group II left atrial diameter (45.92 ± 10.19) was significantly larger than in group I (36.67 ± 5.10) ($p = 0.001$). Left atrial thrombus was detected in 3.4% of the group I and 11.5% of the group II patients.

Discussion: This study showed that coagulation system is activated especially in patients with acute ischemic stroke who had NVAF and significantly elevated F1 + 2 levels. Our results suggested that F1 + 2 may reflect a hypercoagulable state in patients with NVAF.

P220

**Initial clinical features of the recurrent aneurysmal subarachnoid haemorrhage.** I. A. Gontschar, G. K. Nedzvedz, N. I. Nechipurenko, A. A. Gontschar, Belarussian Research Institute of Neurology (Minsk, RUS)

Background: Rebleeding from ruptured aneurysms is usually associated with sudden episode of clinical deterioration, such as apnoea and coma.

Objectives: The aim of this study was to examine the relationship of the initial clinical features between first and recurrent aneurysmal subarachnoid haemorrhage (SAH).

Method: Prospective observational study has been performed in the 5th State Hospital in Minsk (Belarus). The cohort for data analysis was formed by consecutive series of 89 patients with aneurysmal SAH who have been admitted and treated in hospital between January 1, 1998, and December 31, 2001. Lumbar puncture, brain scanning (CT and MTI) was performed for detecting of the recurrent aneurysmal rupture. A source of bleeding has been identified with the help of the catheter angiography or/and autopsy.

Results: Of the 89 patients (range, 25 to 78 years), 35 (39,3%) patients suffered from 39 episodes of rebleeding at the neurological hospital before neurosurgical management. Mean age at the initial SAH was 47 ± 9.8 years, and at the recurrent haemorrhage 55 ± 13.6 years ($p = 0.004$). Mean GCS on admission at the first SAH was 12.5 ± 3.1 (range, 3 to 15) and at the second haemorrhage 8.6 ± 4.1 (range, 3 to 14). 64% patients were allocated to operative clipping of the aneurysm, mostly in the post-acute period of the SAH. Overall 30-day mortality was in patients with first SAH 16,7% (9 from 54) and with rebleeding – 25,7% (9 from 35). Aneurysmal locations noted as ACoA in 44,9%; ICA at PCoA origin in 28,3%; MCA in 21,3%; basilar tip in 3,3%, posterior fossa in 2,2%. Multiple aneurysms occurred in 9,6% of the patients. Risk of rebleeding was totally 39,3%: ACoA – 42,5%; ICA at PCoA origin – 40,0%; MCA – 36,8%; posterior fossa – 20%.

Complete information about initial clinical features of the first SAH were available in 87 patients: headache (90,8%), nausea (41,4%), vomiting (51,7%), neck stiffness (78,2%), (46,0%), epileptic seizures (20,6%), acute confusional state (11,5%), third nerve palsy (20,7%), hemiparesis (23,0%) and coma (43,1%).

The difference of these symptoms by the second aneurysm rupture were the following:

- headache (61,8%), nausea (23,5%), vomiting (23,5%), neck stiffness (61,8%), epileptic seizures (47,1%), acute confusional state (14,7%), third nerve palsy (23,7%), hemiparesis (23,5%), coma (74,5%).

Conclusion: Rebleeding from arterial aneurysm before neurosurgical management occurred in 39,3% patients. Frequency of recurrent SAH did not differ reliably at different localizations of the aneurysm. Our data showed that with recurrent haemorrhage more often as at the first SAH appear respiratory arrest and such clinical symptoms as epileptic seizures ($p < 0,001$) and coma ($p < 0,001$). It is necessary to consider the particulates of the detected clinical features at neurological examination of the patients with suspected rebleeding before brain scanning.

P221

**A new approach in early treatment of stroke.** R. P. Hofmann, B. Schmidt, J. J. Schwarze, S. Bocklisch, J. Klingelhöfer, Chemnitz Medical Centre, Technical University (Chemnitz, D)

After acute stroke the patient's outcome may be positively influenced by early therapeutic measures within a certain time frame. Necessary evaluations of patient's history and complex diagnosis are therefore limited in time. A computerized medical decision support system (MDSS) might be an assistance to optimize and accelerate diagnostic trials and to recommend therapeutic procedures.

The MDSS uses a computer-implemented concept of fuzzy-pattern-classificator networks, which models the processes of human decision-making. Fuzzy pattern classification has been investigated at TU Chemnitz and applied to industrial process control as well as to non-invasive assessment of ICP.

In the present study a MDSS is introduced in the early treatment of a stroke. Decision rules are derived from patient's reference data or from explicit expert knowledge. The MDSS is structured as a network of linked local fuzzy-pattern-classification knots. These knots represent a well-defined stage in patients' clinical, diagnostic and treatment process. They are concerned with a stage-related task of decision-making. The modular structure reduces complexity, increases transparency and facilitates testing of the system. In addition it simplifies a possible extension of the system.

In view of the complex stroke conditions we introduced the design of a MDSS prototype using data of representative stroke patients to demonstrate the decision-making strategies in principle.

The introduced MDSS constitutes a self-learning system and promises to be beneficial in processing huge amounts of data for decision-making in the therapy of acute stroke.

P222

**Diffusion-weighted imaging on low-tesla MRI unit – the method and diagnostic value in early stroke.** R. Krawczyk, M. Chahwan, A. Bochynska, A. Kobayashi, J. Ryterski, R. Poniatowska, Z. Lysiak, R. Boguslawska, Institute of Psychiatry and Neurology (Warsaw, PL)

Purpose: The aim of the study was to assess the usefulness of diffusion-weighted imaging in early stroke diagnose on low-tesla magnetic resonance unit.

Material and Method: 34 patients (20 men and 14 women, mean age 73 ± 11 years) with clinical symptoms of early stroke and normal CT image were studied using 0.23T magnetic resonance unit. The time between the onset of the symptoms and MR study varied from 1 to 22 hours. The MR study consisted of axial Fluid Attenuated Inversion Recovery (FLAIR) and diffusion-weighted (DWI) scans. The slice thickness on FLAIR scan was 6 mm, on DWI 10 mm. The total time of the study was less than 10 minutes. The DWI scan was performed 2 times – with b-value 0 and 600 to perform Apparent Coefficient Map (ADC map). On ADC map the standardized diffusion coefficient was measured, as the ratio between the value of the elliptic ROI of the lesion and ROI set on the opposite hemisphere corresponding to the lesion.

The volume of the lesion was manually calculated as the sum of the areas of the foci on all slices multiplied by slice thickness.

Results: The total number of hyperintense lesions found on diffusion MR studies was 41. The volume of the foci varied from 0.3 to 233.2 $cm^3$. The patients with the biggest lesion volume had very poor clinical outcome. In 14 cases the lesion was not yet seen on FLAIR images indicating acute stroke. In 12 cases the hyperintense focus was seen in the hypointense area of previous stroke. The standardized diffusion coefficient in new stroke area varied from 0.867 to 0.987, in old stroke it was elevated – from 1.056 to 1.148. In 2 cases it was not possible to calculate ADC map due to patients movement between DWI scans.

Conclusion: The diffusion-weighted imaging on low-tesla unit is a very useful diagnostic tool in early stroke stage. This method allows to assess not only the localization, but also the size of the lesion, which can be a valuable clinical predictive factor.

**P223**

**Five cases of locked-in syndrome.** S. Markoula, G. Lagos, S. Giannopoulos, M. Kosmidou, S. Tzavidi, A. P. Kyritsis, Medical School of Ioannina, Medical School of Thessaloniki (Ioannina, Thessaloniki, GR)

Purpose: To evaluate the natural history and prognosis of patients with locked in syndrome.

Background: The term locked-in syndrome describes the clinical state where the active mind is locked in a paralyzed body, most frequently occurring after basilar artery occlusion. Such an infarction may spare the somatosensory pathways and the ascending neuronal system that are responsible for arousal and wakefulness, but affecting the corticospinal and the corticobulbar pathways, depriving the patients of speech and the capacity to respond in any way except vertical gaze and blinking.

Cases presentation: There were five patients, two female and three male, with age range from 53 years old to 76 years old and mean age of 65 years old. All patients had a history of hypertension, and in addition two of them had atrial fibrillation and one had a history of a metallic cardiac valve replacement. Among them, two patients were brought to the hospital in a comatose state, and the rest with severe hemiplegia or tetraplegia and altered consciousness and became comatose in a few hours. All patients were initially managed in the intensive care unit (ICU) with mean ICU stay period of 30 days.

An emergency CT scan was performed in all patients. In three of them the imaging was normal (except of some older infarcts), but in two patients it disclosed low signal in the basis pontis. The imaging (CT or MRI) was repeated in 3–5 days and was abnormal in all patients. One patient had a single infarct in the basis pontis, two patients had infarcts in both pons and midbrain, the fourth patient had several infarcts in the brainstem and internal capsule, and the fifth patient had infarcts in the brainstem, right cerebellar hemisphere and white matter. Only one patient died, two months after the infarct, secondary to sepsis.

Conclusion: Locked-in syndrome should be differentiated from coma, vegetative state and akinetic mutism. Clinical examination as well as imaging characteristics are helpful in differential diagnosis. Survival could be long if there is aggressive supportive care.

**P224**

**Role of initial CT findings in prognosis of ischaemic stroke clinical course.** P. Nowacki, L. Cyrylowski, A. Bajer-Czajkowska, D. Nocoń, J. Podbielski, Pomeranian Medical University (Szczecin, PL)

Background: surprisingly, there is a small number of papers on comparison of ischemic stroke course within its acute phase with initial stage of nervous tissue, assessed with neuroimaging.

The aim of the study was to determine whether the dynamic of acute phase of ischemic stroke depends on earlier lesion of nervous tissue, including ischemic foci probably developed before present stroke, evaluated by CT examination, and especially, whether the early CT changes might be a prognostic factor of the course of stroke in its acute phase.

Material and methods: the prospective study was done on 126 patients with ischemic stroke, divided in two groups: I – 69 individuals with reversible ischemic neurological deficit (RIND) and II – 57 patients with complete stroke (CS) or progressive stroke (PS). The type and location of ischemic foci were determined according to Oxfordshire Community Stroke Project: total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), posterior circulation infarct (POCI) and lacunar stroke (LACI).

Results: the number of ischemic foci within the brain, visible on CT scans, was significantly higher than the number of stroke episodes: the ratio was 0.83 in RIND and 0.81 in CS/PS group. The mean minimal and mean maximal dimensions of ischemic foci were found to be much greater in CS/PS than in RIND patients ($p < 0.01$). In both analysed groups also the number of LACI on CT was higher than the number of other types of ischemic lesions – territorial strokes ($p < 0.01$). There were no differences in location of ischemic foci between RIND and CS/PS group.

Conclusions: it was found that probability of worse clinical course of stroke becomes involved per se by prior vascular lesion of the brain, visible on CT scans, including ischemic foci probably developed before the actual stroke. It also turned out that the size of ischemic foci on CT, and first of all, their longest dimension appears to be the important risk factor of worse course of acute phase of ischemic stroke. The number of ischemic foci on CT scans does not involve the clinical course of stroke.

**P225**

**Effect of aspirin, ticlopidin and clopidogrel: microembolic signals in posterior circulation strokes by atheromatous vessel disease.** S. Hallmeyer-Elgner, G. Gahn, H. Reichmann, University of Technology (Dresden, D)

Objective: To evaluate whether therapy with different antiplatelet agents in patients with acute ischemic strokes in the posterior circulation due to vertebrobasilar atheromatous disease could be monitored with embolus detection (ED).

Material and Methods: In 47 patients (34 males, 13 females, mean age 57,6 ± 12,7) and 50 controls (31 males, 19 females, 53,4 ± 11,4) we monitored microembolic signals (MES) in both posterior cerebral arteries (PCA's) for 30 min using multi-gated simultaneous bilateral TCD (DWL Multi-Dop X4, 9dB). All patients had ischemic stroke in the posterior circulation and evidence for atheromatous vertebrobasilar disease by ultrasound and neuroradiological imaging studies. No patient had potential cardiac source of embolism. 21/47 patients were treated with Aspirin (ASA), 11/47 with Ticlopidin (TIC) and later switched to Clopidogrel (CLO), 10/47 were treated with CLO only. 4/47 were treated with ASA and switched to CLO, of whom one received later combined ASA and CLO. One patient was treated with ASA initially and later switched to TIC followed by CLO. All patients were monitored after hospital admission before initiation of therapy and then every 3 months for 2 years.

Results: In 6 patients (12,8%) we detected 88 MES/h without symptoms. In 4 patients we detected MES directly after the acute event.

No MES could be detected in the other patients and in the control group. During follow up 1 MES-negative patient on ASA suffered stroke in anterior circulation.

Conclusion: In patients with symptomatic vertebrobasilar disease, who are treated with antiplatelet agents, cerebral embolism appears to be a very rare event. In selected cases, however, ED may help to monitor antiplatelet therapy.

**P226**

**Ischaemic stroke with previous TIA: different stroke patients?** R. Pires, S. Pires-Barata, L. Rebocho, I. Henriques, Hospital Espírito Santo Évora (Evora, P)

Background and purpose: TIA is strongly related with the occurrence of stroke and it is in some cases a missing opportunity for improving stroke prevention. Since patient's characteristics with previous TIA might differ from patients without previous TIA, we compared the two groups in order to find any possible differences.

Methods: We studied a hospital sample of 430 consecutive first ever stroke patients (347 ischemic) with median age 65 [19–88]. We considered previous TIA any TIA occurring before the stroke. All patients were studied according to a protocol that includes at least one CTscan or MRI and demographic and clinical data including risk factors and stroke etiology according to TOAST criteria. Statistical methods included Chi-square test and logistic regression analysis.

Results: Previous TIA was present in 49 ischemic patients (14.04%). There was no correlation between history of TIA and stroke etiology (cardioembolism $p = 0.516$; large artery disease $p = 0.8773$; lacunar infarct $p = 0.193$), location (anterior circulation $p = 0.7459$), risk factors (hypertension $p = 0.5598$; diabetes $p = 0.2377$; dislipidemia $p = 0.915$; smoking $p = 0.5967$, non-valvular atrial fibrillation $p = 0.2665$) or Rankin at discharge ($p = 0.5715$). No relation was found with age or sex. Time of arriving at emergency room within 3 hours of stroke onset was related with previous TIA ($p = 0.0432$; O. R. = 1.92; 95% Confidence Interval:1.01–3.6).

Discussion: In our stroke data bank, previous TIA was not related with a specific etiology, age, sex or risk factor subgroup. Arriving at emergency room within 3 hours of stroke was related with previous TIA which can be associated with previous level of information about the disease and may encourage public information campaigns.

P227
**Correlation of size and location of haematoma with prognosis in intracerebral haemorrhage patients.** K. Khosravi, J. Lotfi, N. Sedighi, S. Nafisi, Shariati Hospital (Tehran, IR)

Background: Intracerebral hemorrhage (ICH) is the third most common cause of stroke and has a significant mortality and disability. In this study we attempted to determine the correlation between size and location of hematomas and mortality and morbidity. We also studied epidemiology, clinical manifestations and risk factors of this disorder.

Materials & methods: Patients with clinical diagnosis of ICH confirmed by computerized tomography (CT) were included in this study. In patients who died, time of death was recorded, in others after six months, the degree of disability was determined using Barthel scale.

Results: 66 patients, 35 men and 31 women were studied; the mean age was 62.5 years. Two thirds of patients were in the age group of 50–70. History of hypertension was present in 80 % of patients. Putamen (41 %), lobe (24 %), and thalamus (20 %) were the most common sites of hematoma.

Mean hematoma volume was 30 ml, 64 % of patients had less than 30 ml and 36 % of patients had a hematoma ≥ 30 ml.

There was a statistically significant difference in mortality between patients with hematoma volume ≥ 30 ml and patients with volume < 30 ml (P value < 0.01).

Also statistically significant difference was seen in mean Barthel scale between two groups (P value < 0.01).

There was no statistically significant difference between the location of hemorrhage and the prognosis in this study (P value < 0.01).

Conclusion: We found a clear correlation between the size of hematoma and prognosis (mortality and/or disability) but we could not show any correlation between the location of hemorrhage and prognosis.

P228
**The prevalence of silent brain infarcts among patients with coronary heart disease.** N. Tasdemir, Y. Tamam, Dicle University Faculty of Medicine (Diyarbakir, TR)

The aim of this study was to determine the prevalence of silent brain infarcts (SBI) by cranial magnetic resonance imaging (MRI) among patients elder than 40 years of age with a definite diagnosis of coronary artery disease confirmed by coronary angiography. Another aim was to evaluate the relationships between several risk factors, sociodemographic features and SBI. Seventy-one patients (48 male, 23 female) aged ranging from 43 to 76 years (mean 57.9 ± 9.1) were included in this study. All cases had been hospitalized to Cardiology inpatient unit of Dicle University Faculty of Medicine with a preliminary diagnosis of myocardial infarction or angina pectoris and received a definite diagnosis of coronary heart disease after coronary angiography. All patients underwent detailed systemic and neurological examination and detailed form including information about sociodemographic features and, vascular and non-vascular risk factors was completed for each patient. Detailed cardiac evaluation, routine laboratory examinations including lipid blood levels and other parameters, electrocardiography, chest radiography and echocardiography were also performed. All patients were scanned with cranial MRG and carotid doppler ultrasonography one week after coronary angiography. MRG results were evaluated to find out the presence, localization and amount of SBIs. The results of this study showed that 28 of 71 patients (39.6 %) had at least one SBI. All patients with SBI had white matter hyperintensities in T2 weighted MRI whereas 50 % of them had additional gray matter hyperintensities in basal ganglia. Average age, involved number of coronary arteries, the prevalence of carotid artery stenosis was significantly higher among patients with SBI than patients without SBI. As a conclusion, this study has showed that SBI prevalence increase parallel to the increase in involved coronary arteries, and age and presences of carotid artery stenosis, and that SBI is a common complication encountered in patients with coronary arterial disease. The most important factors in development of SBI were determined to be advanced age and atherosclerosis, and SBI could be a manifestation of the result of the atherosclerotic impact on small vessels. As some studies reported SBI as a predisposing condition or a risk factor to stroke development, all necessary actions should be taken and appropriate treatment should be given to avoid the development of stroke or other severe vascular pathologies.

## Child neurology

P229
**A new mitochondrial mutation in ND3 gene causing Leigh syndrome.** M. Crimi, A. Papadimitriou, P. Palamidou, A. Bordoni, U. Papandreu, D. Papadimitriou, E. Drogari, N. Bresolin, G. Comi, University of Milan, University of Thessaly, University of Athens (Milan, I; Larissa, Athens, GR; Greece, I)

We describe a new ND3 gene mutation in a 9-month-old male infant presenting clinical and MRI features of Leigh Syndrome. After an uneventful pregnancy and normal delivery, the baby presented in the first months of life a progressive neurological disorder. At six months of age, he showed hypertonia of upper and lower extremities, was unable to hold up his head, and had nystagmus and no eye contact. Laboratory examination showed increased lactic acid: 82.5 mg/dl (5.7–22). The lactic acid of CSF was also increased 61.9 mg/dl (10.8–18.9) while hormone investigation and the metabolites of neurotransmitters of CSF (5HIAA, 5MTHF) were normal. Blood amino-gram showed a slight increased of Alanine but the amino-gram of urine was normal. The serum long chain fatty acids were also normal. His MRI-scan showed multiple necrotic lesions to the basal ganglia, brain stem and thalamus. The muscle biopsy was normal with no RRF and no COX deficient fibers. The analysis of two NARP mutations was negative, so we introduced this case in a complete genome-wide mitochondrial sequencing program. We disclosed a new T10158C mutation in the mitochondrial DNA. The mutation was heteroplasmic in blood-derived DNA, with a very high mutated aliquot of mitochondrial genomes (the heteroplasmic level was assessed at 78 %). This transition changes a hydrophilic amino-acid (Serine-34) to a hydrophobic (Proline) one, in a highly conserved region of a structural gene of the complex I, the ND3 (NADH Dehydrogenase subunit 3). This missense mutation was undetectable in blood of his unaffected parents, suggesting that it was a sporadic, germline mutation in the proband. As observed in this case, mutations in Complex I subunits are an emerging causative entity in COX-normal Leigh Syndrome.

P230
**Neurodevelopmental outcome in 50 pre-term infants.** F. Okan, Z. Yapici, O. Erkan, A. Nuhoglu, Sisli State Hospital, Ist Med Faculty (Istanbul, TR)

Background: Improved survival of preterm infants is a result of advances in obstetric and neonatal care, though some of them may have various degrees of impairment or disability.

Objective: The aim of this study was to investigate the mental-motor development of premature infants, and find out the perinatal events which contribute to the neurodevelopmental outcome.

Methods: Fifty premature babies, discharged from the Neonatal Intensive Care Unit (NICU) were followed-up by a complete physical and neurological examination and Denver Developmental Scale at the time of expected birth and 3, 6, 9, 12th months of corrected age. The degree of impairment was graded as none, mild to moderate, or severe. First ophthalmologic examination was performed by an ophthalmologist at the 4th postnatal age and then weekly.

Results: The gestational ages (GA) of babies were less than 34 weeks and the birth weights (BW) were under 2000 g. Among the babies, 25 (50 %) were very-low-birthweight infants (VLBW) and 32 (64 %) ≤ 32 GA. Mean (SD) GA was 31.5 (2.1) weeks and BW was 1538 [293] g. The degree of severity of initial illness was moderate, with a mean (SD) CRIB (clinical risk index for babies) score of 2.6 (2.5). They were discharged at a mean (SD) age of 22 [15] days. Two (4 %) of the babies had intraventricular haemorrhage Papilegrade 3 and 1 (2 %) periventricular leucomalacia on cranial ultrasonography and 2 (4 %) had ROP of stage 3. Blindness and deafness was not detected. In the first neurological examination 29 % of babies were found normal, 67 % had mild impairments, and 4 % had severe abnormalities. Nevertheless, at the 12th month of corrected age neurological deviations were found to be different: 40 % of the babies had normal neurological findings, 40 % had mild impairments and 20 % had major abnormalities. According to the analysis of risk factors, the presence of neurological sequelae was significantly related to lower gestational age, duration of oxygen therapy, and length of NICU stay. However, birthweight, five-minutes Apgar Score, and CRIB score wasn't related with neurological impairments.

Conclusions: In this preliminary study, it is suggested that gestational age and the severity of respiratory disease are the major factors that adversely affect the neurodevelopmental outcome of the preterm babies. We conclude that the neurodevelopmental status of preterm infants at 1 year can not predict the exact long-term outcome. The long-term follow-up should be necessary, as the preterm infant matures, mild abnormalities may resolve, however some learning disabilities may become evident.

P231
**Neurological and systemic aspects of tuberous sclerosis in relation to MRI/MRS findings in 11 children.** Z. Yapici, N. Dortcan, A. Dincer, A. Gokyigit, C. Baykal, M. Eraksoy, Istanbul University, PTT State Hospital, Radyomar Imaging Centre (Istanbul, TR)

Background: Tuberous sclerosis (TS) is an autosomal dominant disease characterised by mental retardation, epilepsy, skin lesions, and congenital hamartomas involving many organs.

Goal: Our purpose was to assess the relation between the clinical severity of the disease and cranial MRI/MRS findings.

Methods: We analysed 11 children, whose diagnosis of TS was made according to established criteria. The follow-up period was 2 months to 6 years (mean 3 years), during which time dermatological, cardiological, and fundoscopic examinations, ECG, echocardiogram, and abdominal US/CT were made. Neurological examination, age at onset of first seizure, seizure type, outcome of epilepsy, and interictal EEG were studied in all cases. The epileptic outcome was defined either as unfavourable or favourable according to the frequency of the seizures despite the appropriate antiepileptic medication. CT [8] and MRI [11] were reviewed for cortical tuber location, tuber count, subependymal nodule, and subcortical heterotopias. MRS was studied [8] prospectively, and N-acetylaspartate (NAA), creatine (Cr), choline (Cho), lactate ratios were measured in cortical hamartomas and in the lesions, particularly those showing prominent epileptic discharges on EEG.

Results: There were 6 girls and 5 boys and current age of the cases ranged from 3 to 16 years (mean 8.4). The presenting symptom was seizure in all of the cases with ages, at onset between neonatal period and 4 years (mean 1.7 years). Various seizure types were identified; generalized convulsions [10], complex partial seizures [5], infantile spasms [4], focal motor seizures [4], myoclonic seizures [3], and drop attacks [2]. Mental retardation was severe in 4 patients (associated with multiple seizure types in all, early onset in 3), moderate in 3 (early onset in all), and mild in 4. There were hypomelanotic macules [7], angiofibromas [4], shagreen patches [4], and ungual fibromas [1] on the skin. In systemic evaluations, renal cortical cysts and angiomyolipomas [2], cardiac rhabdomyoma [2], and retinal hamartoma [2] were detected. MRI revealed multiple bilateral tubers and subependymal nodules [11], subcortical linear heterotopias [4], and giant cell astrocytoma [1]. MRS findings of tubers were characterized by decreased NAA/Cr and increased Cho/Cr ratios in all. Lactate peak was detected in the regions showing epileptic focus on EEG in 4 patients, and multiorgan involvement in 3.

Conclusions: Unfavourable outcome in TS seems to be related with more than 2 types and early age at onset of seizures, great number of tubers with subependymal nodules with atrophy in MRI, significantly decreased NAA ratio with lactate peak in MRS, and multiorgan involvement. MRS could serve as a valuable investigational tool in evaluating the metabolites of epileptogenic foci of tubers. Further studies are needed to understand the underlying pathophysiological mechanisms and the possible clinical role of this technique.

P232
**Pseudotumour cerebri in childhood.** R. Talab, N. Jiraskova, M. Talabova, L. Serclova, J. Jakubec, M. Valis, Faculty Hospital (Hradec Kralove, CZ)

Background: Pseudotumor cerebri (PTC) or idiopathic intracranial hypertension (IIH) is characterized by an elevation of the intracranial pressure not associated with an intracranial process or ventricular enlargement, and with normal cerebrospinal fluid (CSF) contents. The elevation of the intracranial pressure is isolated. PTC is consistent with the theory that PTC is caused by reduced absorption of CSF, either from increased outflow resistance at the arachnoid villi or from obstruction of the dural venous sinuses.

Objectives: To describe the symptoms and neurological signs of PTC, diagnostic criteria for PTC, the several treatment modalities, incl. indications for surgical treatment and differences of the age, sex, and overweight.

Methods: A case note review of children with PTC, diagnosed at Faculty Hospital, Hradec Kralove, Czech Republic between 2001 and 2002. Diagnostic criteria, clinical features, mode of treatment, and outcomes.

Results: A total 5 children (2 girls and 3 boys) between ages of 6 and 13 years.Girls and boys shared similar clinical features (headache, slowly progressive visual loss or transient visual obscurations, nausea, vomiting) and underwent complete neuro-ophthalmologic evaluation and lumbar puncture. Neurological examination found no abnormalities except bilateral papilledema. Neuroimaging demonstrated no abnormalities. Four children were treated by acetazolamide, in one girl was performed optic nerve sheath fenestration.

Conclusions: Pseudotumor cerebri in children is relatively rare. The clinical features are similar in children and in adults. Children with PTC can have a normal body weight, incl. girls. Diagnostic criteria emphasize the need for the diagnosis of secondary and idiopathic intracranial hypertension. Acetazolamide treatment with eventually repeated lumbar punctures and weight loss in adults are usually efficient enough. Indications for surgical treatment of PTC include significant visual loss, progressive visual loss, or severe headache. Fenestration of the sheath of one optic nerve is the preferred surgical procedure more than lumboperitoneal shunts.

P233
**Leukodystrophy, primary ovarian failure and neurological regression: further evidence of a syndrome.** A. Ryan, D. Webb, R. Murphy, Adelaide & Meath Hospital, Our Lady's Hospital for Sick Children (Dublin, IRL)

Background: Several of the leukodystrophies have been well characterised at the metabolic and genetic level but in many cases an underlying cause is yet to be determined. These leukodystrophies of unknown aetiology are a heterogeneous group and represent a significant diagnostic challenge. Schiffmann et al. 1997 (Arch Neurol 41:654–651) described a subgroup of four patients with leukodystrophy in association with ovarian dysgenesis where all known causes of either leukodystrophy or ovarian malfunction had been excluded. To date, this is the only report of such cases in the literature. We now describe the clinical, biochemical and radiological features of a patient with leukodystrophy, ovarian dysfunction and neurological regression in the second and third decade thereby providing further evidence for the delineation of this phenotype.

Methods: A detailed history and clinical examination were carried out. A lumbar puncture was performed and analysed for standard biochemical and microbiological parameters including oligoclonal bands. Endocrine evaluation included assessment of anterior pituitary function before and after the administration of leutenising hormone releasing hormone (LHRH). Magnetic resonance imaging (MRI) of brain was performed. Extensive investigations for the known causes of leukodystrophy were undertaken. A karyotype was performed.

Results: The patient is a 25 year old woman with a normal birth history. Her initial developmental milestones were normal but she was noted to have learning difficulties in school. She had mild dysmorphic features, was of short stature and the onset of puberty was delayed. The result of her LHRH test was consistent with hypogonadotrophic hypogonadism and a pelvic ultrasound revealed the presence of an infantile uterus. Her karyotype was normal. She was diagnosed with primary ovarian failure and treated with oestrogen replacement therapy. At the age of 17 years she began to develop progressive ataxia and spasticity. There was no evidence of a peripheral neuropathy. An MRI brain revealed the presence of diffuse white matter abnormalities in the cerebral hemispheres bilaterally. Visual evoked responses and cerebrospinal fluid oligoclonal bands were normal. Extensive investigations for the known leukodystrophies, other metabolic conditions and for causes of ovarian failure revealed no abnormality.

Conclusions: This is only the second report of ovarian dysgenesis in conjunction with leukodystrophy of unknown cause. Primary ovarian failure should be sought in such cases as they appear to represent a discrete subgroup. Better description of these patients should aid classification and ultimately provide clues as to the underlying pathogenesis of this condition.

P234
**The assessment of sulcal development and neuronal migration in foetal brain with MRI.** U. Aksoy, Z. Yazici, Bursa State Hospital, Uludag University School of Medicine (Bursa, TR)

Purpose: The aim of this study is to evaluate the sulcal development and neuronal migration in fetuses with normal and abnormal central nervous system (CNS) development with prenatal MRI.

Methods and materials: Thirty patients with gestational ages between 18 and 36 weeks (mean 25 weeks) were imaged in a 1.5 T MR unit with HASTE sequence (TR: 4.4, TE: 64, flip angle: 150). Fourteen fetuses had CNS abnormalities (Group I), and 16 had normal CNS (Group II). Two radiologists analysed the development of sulcation and cerebral parenchymal layering.

Results: Ninenty-one percent of sulci and fissures were evaluated confidently, but 5% could not be assessed because of non-orthogonal imaging planes, and 3% because of "blurring" artifact. In 3 cases with overt hydrocephaly, calcarine, parietooccipital and cingular sulci could not be observed. In 2 cases in group I neuronal migration was delayed. In group II, all cases had normal cerebral layering pattern.

Conclusion: As markers of cortical maturation, sulcal development and migration disorders can be effectively evaluated in the antenatal period with MRI. MR imaging may be used as a complementary modality for patients who are at risk for abnormal cortical development and a positive family history.

P235
**Relationship of cranial computed tomography to clinical status in childhood traumatic brain injury.** D. Aygun, T. Yardan, Ondokuz Mayis University (Samsun, TR)

Background: In children with traumatic brain injury (TBI), the presence of a significant relationship between cranial computed tomography (CT-scan) and their clinical status is still controversial. We would like to investigate the relationship of CT-scan to clinical status in childhood TBI.

Methods: Twenty-nine consecutive child patients (age less than 17 years) were included in this study. A detailed history was taken from each patient or their general practitioner. Each patient underwent extensive neurological examination including the Glasgow Coma Scale (GCS) on several occasions. The severity of trauma was determined according to GCS scores. The CT-scans were obtained within the first 6 hours of trauma. In our study, cranial lesions included skull fractures, subarachnoid hemorrhage, subdural and epidural hematomas, intraparenchymal contusions, cerebral edema, and obliteration of the basal cisterns. Chi-square and independent t-test were used for statistical analysis.

Results: Mean age was 6.58 ± 4.1 years (from 3 to 15). There were 18 (62.06 %) boys and 11 (37.94 %) girls, with boy: girl ratio being 1:1.2. Mean GCS score was 12.58 ± 3.05 (from 3 to 15). Of our patients, 68.9 % displayed a CT-scan lesion, and 31.01 % had a normal CT-scan. However, only 34.4 % of all patients had a serious CT-scan lesion. The most common mechanism of injury was falling (n = 14; 48.27 %). There was no difference between boys and girls for both pathologic CT-scan findings (P = 0.628) and GCS scores (P = 0.505). There was no difference between patients without and with CT-scan lesion for both GCS scores (P = 0.051) and the severity of clinical status (P = 0.209); however, while all (100 %) of 3 patients with severe trauma had a cranial lesion, only 9 (56 %) of 16 patients with mild trauma had a cranial lesion. On the other hand, while in patients (n = 20) with CT-scan lesion, mean GCS score was 11,85 ± 3.4, it was 14,22 ± 0.83 in those (n = 9) without CT-lesion. The patients (19 of 21) with any symptom or finding had significantly CT-scan lesion more than those (2 of 8) without a symptom or finding (P = 0.002). The most important limitation of our study was a small number of patients.

Conclusions: Present results can suggest that there is a relationship between CT-scan and clinical status in childhood TBI. However, further prospective controlled studies are needed to confirm the present results.

P236
**Septo-optic dysplasia associated with hypoplasia of the right cerebellar hemisphere and schizoencephaly.** Z. Yapici, A. Dincer, M. Eraksoy, Istanbul University, Radyomar Imaging Centre (Istanbul, TR)

Background: The syndrome of septo-optic dysplasia (SOD) consists of hypoplasia of the optic nerves and hypoplasia or absence of the septum pellucidum. Mainly, the clinical presentation is variable degrees of ophthalmological problems. The diagnosis of SOD is made by ophthalmologic examination in conjunction with MRI.

Goal: In this report, a case with hypoplasia of the cerebellar hemisphere as an added component of this syndrome has been discussed.

Method: We studied a child with SOD together with right cerebellar hypoplasia and schizencephaly by means of clinical, neuroimaging, and metabolic examinations.

Results: A 5-year-old boy was presented with mental-motor retardation and absence of bulbi. There was no consanguinity marriage and pregnancy was uneventful. Systemic examination was normal except phitizis bulbi. Neurologic examination revealed severe mental retardation, axial hypotonia and spastic tetraparesis. The case had generalized convulsions 4–6 times a year under antiepileptic medication. MR images showed phitizis bulbi, hypoplasia of optic nerves and chiasm, agenesis of septum pellucidum, bilateral schizencephaly (open lip cleft on the right and closed lip on the left hemisphere), and hypoplasia of the right cerebellar hemisphere. The metabolic screening for inborn errors was unremarkable.

Conclusions: Since marked developmental delay and severe mental retardation with epilepsy are present in patients with bilateral schizencephaly, clinical severity can be explained by cortical malformation in our case. Although such MR appearances as lobar holoprosencephaly, hypoplasia of the corpus callosum or cortical maldevelopment (especially schizencephaly and gray matter heterotopia) are identified in patients with this syndrome, aplasia/hypoplasia of the cerebellar hemisphere has so far not been reported. The findings suggest that these abnormalities have different embryogenesis, or that they represent the rare association of SOD with cerebellar hemispheral hypoplasia.

P237
**Cerebrotendinous xanthomatosis: a study of a Tunisian consanguineous family.** N. Miladi, M. F. Vincent, F. Van Hoof, L. Hoefsloot, A. Verrips, R. A. Wevers, Institut National de Neurologie (Tunis, TN)

The authors report a Tunisian consanguinit family. The index case was the eldest. The onset of the disease was at 4 year-old with learning difficulties and ataxia. At 6 year-old, he had surgery for bilateral cataracts. When he was 18, he was mentally retarded, had ataxia and bilateral Achilles tendon xanthomas. Cerebrotendinous xanthomatosis was considered. Elevated plasma cholestanol (219 micromole/l) and cholesterol (3,7 mmol) levels were found as well as considerable amount of glucuronidated bile alcools in urine. Cerebral MRI showed increased T2 signal in the white matter and cerebellar atrophy. Genetic studies showed an exon 3 homozygous mutation of the G667C gene. He was treated by chenodeoxycholic acid for 8 years. He improved and showed reversal of his neurologic disability. A systematic biochemical and genetic screening performed for all his family, showed that his sister was affected. She was treated when she was 11 and improved much better than he did.

## Clinical neurophysiology

P238
**Electrophysiological findings in clinically pure sensory chronic demyelinating polyneuropathies.** P. Lozeron, A. Ferreira, C. Lacroix, D. Adams, G. Said, Hôpital de Bicêtre (Le Kremlin Bicêtre, F)

We retrospectively reviewed the electrophysiological data (EBX) of 28 consecutive patients with biopsy proven chronic inflammatory demyelinating polyneuropathy with pure sensory clinical signs (Sensory-CIDP).

The EBX were classified as demyelinating (D-EBX) according to AAN criteria (1991)or axonal (A-EBX) or intermediate when some EBX were in the demyelinating range (I-EBX). Three patients were studied with near nerve technique.

Patients (18 men) had a mean age of 57 years and a mean delay between onset of symptoms and electrophysiology of 3.5 years. A mean of 5 segmental motor conduction velocities (MCV), 4.5 distal latencies, 2.5 F waves and 3 segmental sensory conduction velocities (SCV) were determined for each patient. The most frequent EBX abnormalities were first abnormal electromyographic pattern (EMG) then prolonged distal motor latencies, reduced MCV or SCV and prolonged F waves in the demyelinating range in 22, 12, 4 and 2 occasions respectively.

Two patients had normal motor EBX including one with normal EMG. Thirteen patients had axonal motor EBX. Thirteen patients had at least one motor EBX parameter in the demyelinating range including a single prolonged distal motor latency as only abnormality in 5. No patient had conduction block. Dispersion of motor responses was found in four patients. EMG was normal in four patients and showed neurogenic pattern in the others.

Four patients had SCV in the demyelinating range which was the only abnormality in one. Four patients had dispersion of sensory potentials including one patient with motor dispersed responses and the three patients studied with near nerve method. Out of the 18 patients where it could be determined abnormal median and normal sural sensory potential pattern (AMNS) was found in three.

Sixteen patients were classified as A-EBX (57 %), one as D-EBX and eleven as I-EBX including one very close to D-EBX. Sensory examination, delay between onset of symptoms and electrophysiology and number of nerve segments investigated were not related to the EBX pattern. Patients with A-EBX were slighly older, presented less frequently paresthesiae and symetrical symptoms and more frequently numbness and affection of the lower limbs at the beginning of the disease.

During follow up, weakness finally developped in three patients who were classified as D-EBX and I-EBX in one and two cases respectively. SCV and MCV in the demyelinating range were found in 3 and 2 cases respectively. All had AMNS pattern.

Thus, our data suggested:

- Biopsy proven Sensory-CIDP most often have A-EBX pattern and AAN criteria are not sensitive in their diagnosis.
- Motor EBX parameters are more often in the demyelinating range than sensory ones in Sensory-CIDP.
- In spite of its absence of specificity, dispersion of sensory potential with near nerve method seems useful in suggesting a Sensory-CIDP.
- Motor and sensory conduction abnormalities in the demyelinating range were found in patients who developed weakness.

P239
**Direction-dependent visual cortex activation during horizontal optokinetic stimulation (fMRI study).** M. Dieterich, S. Bense, B. Janusch, G. Vucurevic, T. Bauermann, T. Brandt, P. Stoeter, Johannes Gutenberg-University, Ludwig-Maximilians University (Mainz, Munich, D)

Optokinetic nystagmus (OKN) is a reflexive ocular motor response that holds the images of the environment steady on the retina by driving the eyes in the direction of motion stimulation and then resetting the eyes in the opposite direction. An earlier fMRI study using FLASH sequences during horizontal OKN found bilateral activations in a complex cerebral sensorimotor network (esp. visual cortex, motion-sensitive area MT/V5, and ocular motor structures) without significant differences in individual comparisons of the extent of the activated areas in both stimulation directions. Due to earlier technical and statistical limitations, a new fMRI study using echo planar imaging (EPI) and group analysis was conducted to answer the question of direction-dependent differences and hemispheric dominances for processing of right and left OKN.

Fifteen healthy volunteers were examined using a 1.5-T scanner (Siemens, Germany) and a T2*-weighted EPI sequence. The protocol included 320 brain volumes of forty transversal slices each in alternating blocks of ten images at rest (looking at the stationary target) and ten during horizontal small-field OKN (computer generated pattern of 14 vertical black/white stripes, no self-motion perception). Subjects lay supine, their view being corrected by a mirror to a projection surface in front of the scanner bore. Volumes were realigned, spatially normalized, and smoothed prior to statistical random effects group analysis (SPM99b).

Besides bilateral, widespread activations of the visual cortex including the lateral occipito-temporal cortex (MT/V5) and adjacent occipitoparietal areas, OKN caused significant bilateral activations of the ocular motor structures such as the superior and inferior frontal eye field, the supplementary eye field, the prefrontal cortex, and the parietal eye field. The contrast rightward vs. leftward OKN showed statistical differences only in the right visual cortex (GF, GL, Cu, GOm, BA 19/18/17/31, x/y/z = 16/–82/9, 1007 voxels, T = 12.85); the contrast leftward vs. rightward OKN in the left visual cortex (GF, GL, Cu, GOm, BA 19/18/17/31, x/y/z = –10/–86/6, 1803 voxels, T = 19.34). No statistical differences were found for the ocular motor area activations even at lower thresholds.

This study shows that direction-dependent differences during horizontal OKN occur only in visual cortex areas in the hemisphere ipsilateral to the fast OKN phase. This can be explained by the direction dependent gaze shift ("Schlagfeldverlagerung") toward the fast phase which causes asymmetry of the total field of stimulation of both hemispheres. The absence of any difference in the activation pattern of the eye fields supports the concept that processing of both horizontal directions is mediated in the same rather than separate areas.

P240
**Transcranial magnetic stimulation demonstrates excessive inhibitory activity in the motor cortex of cerebellar patients.** S. Tamburin, G. Zanette, P. Manganotti, A. Andreoli, S. Marani, A. Fiaschi, University of Verona (Verona, I)

Objective: To examine the excitatory and inhibitory properties of corticospinal projections in cerebellar patients.

Methods: We investigated the motor evoked potential (MEP) and the cutaneous silent period (CSP) recruitment curves to increasing intensities of transcranial magnetic stimulation (TMS), as well as early and late intracortical inhibition (ICI) and facilitation (ICF) to paired-TMS, in the right abductor pollicis brevis muscle of 8 patients with 'pure' cerebellar syndromes and 14 age-matched controls. Peripheral silent period (PSP) to peripheral nerve stimulation was recorded to better define the anatomical level of the phenomena.

Results: No statistical difference was found between patients and controls in MEP recruitment curves for both resting and active conditions. CSP was significantly longer in patients at TMS intensities ranging from 90% to 130% of resting motor threshold. No abnormalities of PSP were found. Paired-TMS showed abnormally long duration of late ICI in cerebellar patients, with a normal balance between early ICI and ICF.

Conclusions: Our data show a prevalence of inhibitory circuitry in the motor cortex of cerebellar patients, both at rest and during voluntary contraction. This abnormality may depend on the hyperactivity of subtypes of GABA interneurones in the motor cortex of cerebellar patients. Our findings in cerebellar patients seem to be the opposite of those in movement disorders such as dystonia and Parkinson's disease. Cerebellum may counteract the basal ganglia in modulating motor system excitability.

P241
**Age and sex dependency of cerebrovascular reserve.** K. Hochauf, G. Gahn, H. Reichmann, University Hospital Carl Gustav Carus (Dresden, D)

Background and Purpose: Cerebrovascular reserve (CVR) is deemed to be an important parameter of an intact cerebral homeostatic system. The influence of biological factors such as age and sex on the mean blood flow velocity and the CVR have been reported in several studies, but mainly the age dependency of CVR is controversial. The aim of this work was to assess the age and sex dependency of CVR in all possibly cooperative age groups including children. This is the first study evaluating CVR in an age group ranging from 4 to 89 years.

Methods: Mean blood flow velocity of the middle cerebral artery (MCA) was recorded simultaneously with a bilateral transcranial Doppler device (2 MHz, DWL MultiDopX4) during randomized sequential breath-holding periods, typically for 10, 15, 20, 25 and 30 seconds and often with one maximum breath-holding capacity in a supine position in 81 volunteers. We subdivied them in four age groups, fourteen subjects between 0 and 16 years old (Group 1), 32 between 17 and 40 years old (Group 2), 15 between 41 and 60 years old (Group 3) and 20 between 61 and 90 years old (Group 4). Slope values, representing percent increase from baseline velocity to maximal velocity in the MCA during sequential breath-holding trials, Breath-Holding-Index (BHI) and the angle alpha, which describe the acceleration of the mean blood flow velocity, were determined and used to estimate the CVR.

Results: No significant changes in CVR between the four age groups could be observed, but age group 1 showed the highest vasodilatory response to $CO_2$ for all CVR descriptive parameters. If we consider men and women separately, no significant differences were found in CVR with increasing age. Women have significantly higher slope values compared with their male counterparts ($p < 0.05$). No significant change could be seen in BHI and angle alpha (BHI: $p = 0.49$, angle alpha: $p = 0.07$).

Conclusion: Our data suggest a trend for increased CVR in children and teenagers (Group 1) compared to adults (Groups 2, 3, 4). This may be related to structural changes and declining levels of cGMP with increasing age. We also found higher CVR in women, possibly due to hormonal status. Further evaluation of these identified subgroups is necessary.

P242
**Assessment of the motor pathway to the diaphragm using cortical and cervical magnetic stimulation in myotonic dystrophy.** H. Takada, S. Kon, S. Yamada, National Aomori Hospital (Namioka, JP)

The aim of this study was to evaluate the dysfunction of the central or peripheral respiratory pathway by means of magnetically evoked potentials (MEP) techniques in patients with myotonic dystrophy (MD), and to examine whether MEP parameters were useful for clinical application as a dysfunction scale. The study population comprised ten patients with MD aged from 37 to 65 years. The diaphragmatic MEPs were recorded from the xiphoid process with the reference of the seventh intercostal space using surface electrodes. Transcortical magnetic stimulation was performed to the vertex at least 20% above the threshold, on maximal deep inspiration during diaphragmatic facilitation. Cervical magnetic stimuli were applied on the posterior spinous processes between C2 and T2 at 65% of maximal magnetic output (Magstim 200, Magstim Co, UK; 2.0 Tesla maximum magnetic flux density). The threshold was judged as the lowest intensity level that evoked clearly discernable diaphragmatic MEPs. The onset latencies of the first negative potentials and the amplitudes from baseline to negative peak of elicited MEPs were measured. The central conduction time (CCT) that subtracted the peripheral latencies by cervical stimulation from the total latencies by cortical stimulation were calculated. MEP measurements were compared with those from ten healthy volunteers as normal controls, and values out of 90% confidential intervals were defined as abnormal. MEP results were checked up with the outcome of following respiratory functional tests. For conventional pulmonary function tests, total lung capacity, forced inspiratory volume, forced expiratory volume, tidal volume, and ventilatory volume per minute were measured in patients. Blood gases and nocturnal hypoxia index (NHI) that was calculated from transcutaneous recording of $O_2$ saturation during night were also analysed. All the patients showed abnormality in at least one of pulmonary function tests, blood gases, or NHIs. Regarding MEP parameters, the thresholds for cortical (mean ± S.D.; 66.7 ± 5%) and cervical (61.4 ± 2.5%) stimulations were increased in all patients. The amplitudes of MEPs by cortical stimulations (28.7 ± 15.0γV) were decreased in eight patients, and those by cervical stimulations (41.1 ± 21.6γV) were small in three patients. The central (14.1 ± 1.9ms) and peripheral (5.9 ± 0.7ms) MEP latencies were within normal limit in all patients. CCTs (8.2 ± 1.6ms) were prolonged in three patients. The MEP amplitude by cortical stimulation was negatively correlated with the value of NHI ($p < 0.05$). There was no correlation between MEP measurements and

the results of the pulmonary function tests or the blood gases. Our results suggested that MEP technique was effective for the detection of the dysfunction of the central or peripheral respiratory pathway, whereas the utility of MEP parameters for a clinical scale of the respiratory dysfunction was ambiguous.

P243
**Clinical neurophysiology of the stiff-man syndrome.** H. M. Meinck, University of Heidelberg (Heidelberg, D)

The clinical hallmarks of the stiff-man syndrome (SMS) comprise intense stiffness of the trunk and proximal limb muscles superimposed by painful spasms, exaggerated startle, and absence of firm neurological signs. In such patients, nerve conduction and cortical evoked potential studies are normal, but EMG reveals continuous firing of normal motor units in stiff muscles. However, such firing is also seen in muscle stiffness due to e.g. syringomyelia, myelitis, spinal arteriovenous malformations, or even Parkinson's disease and is therefore regarded by no means specific for SMS. In neurophysiological reflex testing, abnormalities comprise augmentation of mono- or polysynaptic brainstem (e.g. masseter or blink) reflexes, and attenuation or even loss of reflex inhibition (e.g. masseter inhibitory reflex). In about 50% of patients, moreover, electrical and particularly tactile stimulation of the face elicits abnormal short-latency reflex responses in the dorsal and ventral neck muscles (head retraction reflex). This vestigial withdrawal reflex of the face is not observed in normal subjects, but occasionally seen in other neurological disorders such as Parkinson's disease or ALS, and regularly positive in familial hyperekplexia, a rare genetic disorder of the inhibitory glycine receptor. More than 80% of SMS patients have abnormal reflex responses in the trunk and leg muscles (which are most frequently involved in stiffness and spasms) to remote electrical stimulation. After median nerve stimulation, responses consist of one or a few hypersynchronous EMG bursts which – with a short latency (50–80 ms) and at short intervals (80–120 ms) – simultaneously occur in the abdominal and paraspinal muscles. Hypersynchronous bursts are followed by a tonic decrescendo EMG activity over 0.5–2 s. These abnormal responses, termed myoclonic reflex spasms habituate rapidly and may disappear on benzodiazepine treatment. They have never been observed in other disorders with stiffness and spasms and may thus be regarded the most specific electrodiagnostic test for SMS.

P244
**Analysis of frontal sharp transients in neonatal polysomnography.** A. Crippa, R. Scola, C. Silvado, L. Paola, L. Werneck, R. Fernandes, UFPR (Curitiba, BR)

Objective: To identify and quantify frontal sharp transients found in neonatal polysomnography of healthy term newborn babies throughout different sleep stages.

Background: Frontal sharp transients (encoches frontales) usually appear bilaterally and synchronously, but may be asymmetric in amplitude. Sometimes they appear unilaterally, can be interpreted as either normal.

Design/Methods: Thirty-two neonatal polysomnographic studies of term babies from the Hospital de Clinicas da Universidade Federal do Paran (UFPR) were reviewed. The babies were chosen in a randomized way, with Gestational Age (GA) term, legal age of two days and all of them were considered healthy with adequate monitoring during pregnancy. Polygraphic studies were performed in a 21 channels EEG machine. The montages used followed internationally accepted standards for the neonatal period and the recording was performed without sedation. Frontal sharp transients (FST) are usually biphasic sharp waves, although sometimes they can be monophasic, with an initial surface negative component of low amplitude, followed by a positive component of higher amplitude and longer duration, with maximal amplitude in the frontal areas. When unilateral, with consistent morphology and occurring over Fz they were considered true frontal sharp transients. On the other hand, if they were monophasic, predominantly negative and occurred at the same time as eye movements were registered, they were considered as eye movement artifacts. All bilaterally synchronous and unilateral frontal sharps were counted and analyzed.

Results: The mean duration of the polygraphic studies was of 57 minutes. The total number of FST was 778 (25.6 per exam), of which 133 (16.75%) were unilateral (4.3 per exam). Distribution according to circadian cycle was as follows: 295 (37.9%) during quiet sleep, 273 (35.1%) in active sleep, 128 (6.5%) in transitional sleep and 82 (10.5%) in wakefulness. In quiet sleep there were 225 bilateral and synchronous (BS) and 70 unilateral (UL) sharps, whereas active sleep harbored 243 BS and 30 UL. Transitional sleep showed a total of 103 BS and 8 UL and in wakefulness 74 BS and 8 UL could be found. Using statistical tests (Kruscal-Wallis) Lateralized FST are found mostly during quiet sleep, whereas active sleep had more BS FST.

Conclusions: With the method used to define FST there was a low risk of misdiagnosis. One must remember that they can sometimes be unilateral, with an electric field over the temporal areas and, in some instances, that they can present with a hard to define morphology.

P245
**Electrophysiology tests in patients with Wilson's disease: an approach for demonstration of latent involvement of brain structures, apart from basal ganglia and cerebellum.** M. Klissurski, N. Muradyan, S. Novachkova, E. Vasileva, B. Ishpekova, Queen Joanna University Hospital (Sofia, BG)

Background: Wilson's disease (WD) is a rare autosomal-recessive hereditary disorder of copper metabolism that affects liver, eye cornea and brain. Spectrum of clinical symptomatic varies from behavioural and psychiatric manifestations to different movement disorder syndromes, mainly due to involvement of basal ganglia and cerebellum, as well as liver dysfunctions and cirrhosis. In the current literature there is little information concerning involvement of pyramidal tracts, brain stem pathways and nuclei in patients with WD and their functional assessment.

The aim of our presentation is to show the results of electrophysiological studies of four patients with WD and to discuss their clinical relevance.

Subjects and methods: Diagnosis was made on the basis of copper metabolism examination with significant copper levels in 24 h urine, neurological status, CT of the head, involvement of liver and presence of Kayser-Fleischer ring. All patients underwent complex EMG examination: Blink – reflex (BR), cranial and spinal motor Magnetic Evoked Potentials (MEP) and Nerve Conductive Velocities (NCV) of the limbs. First 3 patients (2 women and 1 man; mean age of 37,5 years; and mean duration of disease between 2–6 years), were examined before starting of d-penicillamine therapy and the last patient – 25 year-old woman, with 12-year-evolution of WD, after 2 years of Cuprenil treatment.

Results: All patients had normal NCV and spinal MEP. BR and cranial MEP were abnormal in 3 untreated patients. The patient with permanent treatment showed nearly normal parameters, except for reduced amplitudes of cranial MEP.

Conclusion: Although our results are preliminary and insufficient for steady conclusion and statistical analysis they do suggest that, by applying specific electrophysiological methods, we could determine clinically latent dysfunctions of central motor neuron, pyramidal tracts and brain stem in WD. There is evidence that some parameters of these tests could be additionally useful in verifying the efficacy of the treatment.

P246
**Diagnostic value of repetitive nerve stimulation and F-wave measurements in experimental autoimmune myasthenia gravis.** E. Tuzun, P. Christadoss, University of Texas (Galveston, USA)

Background: Repetitive nerve stimulation (RNS) is an important electromyography method for the diagnosis of experimental autoimmune myasthenia gravis (EAMG). At least a 10% decrease in amplitude from the first to fourth or fifth action potential is expected in mice with EAMG. However, with a 3–6 Hz stimulation frequency, generally used for MG patients, only 8–9% of mice with clinical findings of EAMG show a decremental response with this method.

Materials and Methods: To find the optimum method with best diagnostic sensitivity and an acceptable specificity, we performed RNS and tetanic stimulation methods and F-wave measurements. Consecutive action potentials were elicited with RNS on the sciatic nerve of anesthetized AChR-immunized B6 mice (2 months after first immunization) with clinical grades between 0–3 and compared the differences from the first to fourth and first to fifth amplitudes for several frequencies ranging between 3 and 96 Hz.

Results: With 48 Hz stimulation frequency, best sensitivity (54%) and specificity (96%) values were obtained. The correlation between clinical grades and amount of decremental response was best at 12 Hz and with the comparison of fifth response to the first one. We could not have better sensitivity values with neither tetanic stimulation method nor F-wave measurements. Repeating the same methods on the sixth month of disease did not improve the sensitivity or specificity values.

Discussion: We conclude that even with modified parameters, RNS cannot exceed the diagnostic value of clinical observation and serum anti-AChR antibody measurements.

P247
**Temporal variability of neurography.** O. Bouquiaux, A. Horward, F. C. Wang, CHU Sart Tilman (Liège, B)

Serial changes of repeated nerve conduction studies in healthy subjects have to be taken into account when effects of treatment on peripheral neuropathies are monitored.

To document variability of repeated neurography, motor and sensory nerve conduction measurements were collected from 30 healthy subjects (mean age 22 ± 2 years) twice (T1 and T2) at a time interval of 3 months by the same examiner. At T1 and T2, the protocol consisted of: 1) motor nerve conduction studies of median, ulnar, peroneal and tibial nerves from dominant side for measurement of amplitude (d-Amp), terminal latency (d-Lat), minimal F-wave latency (F-Lat), F-wave amplitude (F-Amp), and calculation of motor conduction velocity (MCV); 2) sensory nerve conduction studies of median, ulnar, radial, lateral and medial cutaneous, sural and superficial peroneal nerves from dominant side for measurement of amplitude (Amp) and calculation of sensory conduction velocity (SCV).

F-Amp varied most (on average 34–46%), followed by motor and sensory Amp (on average 6–19%), d-Lat (on average 5–8%), SCV and MCV (on average 4–5%) and F-Lat (on average 2–3%). A highly significant correlation ($r^2 \geq 0.8$) between data recorded at T1 and T2 with a coefficient of variation less than 10% were calculated only for: F-Lat from each motor nerve, cubital and tibial d-Amp, sensory Amp from radial, lateral and medial cutaneous nerves. Therefore, these parameters and nerves are the most suitable for a neurophysiological follow-up of patients with a diffuse peripheral neuropathy.

The limits of temporal variability were determined (95th percentile) for each variable from distinct nerves.

## Dementia/Higher function disorders

P248
**The Q7R polymorphism in the Saitohin gene is associated with late-onset Alzheimer's disease.** J. Infante, O. Combarros, L. Rodero, E. Palacio, J. Berciano, J. Llorca, University Hospital Marques de Valdecilla, School of Medicine (Santander, E)

Background: It is not well established whether genetic variation in tau is associated with Alzheimer's disease (AD). A recently identified novel protein, named Saitohin (STH), shares tissue expression pattern to tau, and preliminary evidence in a North American population indicates that homozygosity for the R allele of Q7R STH polymorphism might be a risk factor for AD, perhaps through modulating neurofibrillary tangle formation.

Objective: We investigated the association of this polymorphism and AD in a Spanish case-control study.

Methods: The study included 315 AD patients (70% women; mean age 75.6 years; SD 9.0; range 50–98 years) who met NINCDS/ADRDA criteria for probable AD. Control subjects were 307 unrelated individuals (72% women; mean age 80.5 years; SD 7.7; range 63–100 years) with Mini Mental State Examination scores of 28 or more, which were verified by at least one subsequent annual follow-up assessment. According to the median age at onset or sampling (72 years), cases and controls were stratified into two age groups (early-onset: less than 72 years; late-onset: more than 72 years).

Results: When compared to QQ genotype, the odds ratio (OR) for the RR genotype was 1.17 (95% CI = 0.33–4.14, P = 0.82) in the early-onset group, and 2.17 (95% CI = 1.04–4.54, P = 0.04) in the late-onset group. In the presence of the APOE e4 allele, the risk of STH RR genotype carriers was not different to the risk of STH QQ genotype carriers.

Conclusions: Increased risk of AD is associated with the STH RR genotype, but is limited to late-onset (after age 72 years) AD cases.

P249
**Donepezil maintains activity in a specific neural network in patients with Alzheimer's disease.** J. Moeller, H. Rusinek, M. Casanova, J. Hoffman, J. Votaw, L. Tune, C. Perdomo, R. Pratt, J. Ieni, R. Jewart, Columbia University, New York University School of Medicine, The Medical College of Georgia, Emory University, Eisai Inc (New York, Augusta, Atlanta, Teaneck, USA)

Objective: To quantify the brain response to donepezil therapy in a double-blind, 24-week neuroimaging study of Alzheimer's patients using positron emission tomography with [18F]-fluorodeoxyglucose (FDG/PET) and a new multivariate analysis that enhances regional statistical sensitivity.

Methods: In the double-blind, 24-week pilot study, patients with mild to moderate, probable AD (mean MMSE score 21 ± 3.9) were randomized to donepezil (10 mg/day) or placebo (n = 14 per group). FDG/PET scans were acquired to quantify grey matter glucose metabolism in 27 regions-of-interest (ROIs) per hemisphere at baseline, Week 12, and Week 24. ROI metabolism was corrected for patient regional brain atrophy based on structural MRI data, and normalized by global metabolic rate. A new multivariate statistical method was applied to the corrected data to quantify the ROI responsiveness to donepezil therapy compared with placebo. Cognitive function was monitored concurrently at 6-week intervals using the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog).

Results: At baseline, regional metabolic activity and ADAS-cog scores were not significantly different in the two treatment groups. The primary outcome measure was the change in regional metabolic activity from baseline to Week 24. Global normalization and atrophy correction markedly improved the statistical sensitivity in all ROIs, giving rise to a uniform level of measurement precision. Donepezil treatment had a significant effect in the following ROIs: the temporo-occipital, parieto-occipital, visual associative, and calcarine areas in the posterior cortex; and, Broca's area, superior and middle frontal, and premotor areas in the anterior cortex. Metabolic activity in this brain network was maintained in donepezil treated patients and was associated with enhanced cognition, whereas the placebo group showed a post-baseline reduction in metabolic activity at Weeks 12 and 24 ($P < 0.005$). In the donepezil group, the network's metabolic response also predicted the change in clinical response ($r = 0.75$; $P < 0.01$); however, network activity did not correlate with clinical outcome in the placebo group.

Conclusion: Donepezil treatment had a beneficial effect on activity in a brain network known to be affected in AD, correlating with enhanced cognition. This analysis confirms and extends previous findings from the study, and demonstrates a positive correlation between the metabolic effects of donepezil therapy and clinical outcome.

P250
**How reversible is the 'reversible' dementia in normal pressure hydrocephalus?** J. A. L. Vanneste, E. Geiger, R. Walchenbach, H. A. M. Middelkoop, Sint Lucas Andreas Ziekenhuis, Westeinde Ziekenhuis, LUMC (Amsterdam, den Haag, Leiden, NL)

Background. One of the main symptoms in normal pressure hydrocephalus (NPH) is "subcortical" dementia, which may be reversible after ventricular shunting. Very few studies have provided details on the type of mental impairment, the criteria for considering mental improvement as clinically meaningful, the profile of post-shunt cognitive improvement, and the number of patients with post-surgical mental amelioration.

Methods. Potential NPH patients referred to one of our centres for further diagnosis and therapy were included in this study. Mental deterioration was assessed with the MMSE and a series of neuropsychological (NPS) tests assessing cortical functions, memory, mental speed, concept shifting, and abstract reasoning. Quantitative psychometric tests used for post-surgical follow-up were the 10 Word test (recall & recognition), the trail making tasks A & B, the symbol digit memory test, and the Stroop tests I-III. Improvement of mental symptoms after a shunt was ranked on arbitrarily pre-established percentages of amelioration on each of the psychometric tests. The results of post-surgical psychometry were ranked into 3 categories of global cognitive improvement: [1] marked, [2] slight, [3] none. Mental amelioration was also evaluated by interviewing the family, the medical personnel and people involved in the care of the patients. Psychometry was carried out 2, 6 and 12 months after shunting, and at irregular intervals during several years.

Results. Between July 1994 and July 2002, 57 potential NPH patients were included. The median age was 73 years (range 55–87). Longitudinal post-shunt NPS assessment was possible in 53 patients. The pre-surgical results of some complex frontal tasks were in the range of percentile < 10 in 48/53 (90%) patients, even in patients who appeared mentally "normal" at clinical examination and on the MMSE. 58% showed global post-surgical improvement (including gait), but only 28% had substantial mental amelioration. Only 1 of the 10 patients with severe dementia (MMSE < 18) improved. Among the 14 patients with either no or only mild mental impairment (MMSE 25–30), frontal tests showed substantial improvement in 7.

Conclusions. Frontal tests are much more sensitive than the MMSE for detecting cognitive impairment in NPH; substantial mental improvement occurs in only a minority of patients shunted for presumed NPH; frontal tests are more reliable than the MMSE and clinical examination for showing improvement after surgery.

P251
**Apathy in Alzheimer's disease and mild cognitive impairment: evaluation using the apathy inventory.** P. Bedoucha, S. Clairet, P. H. Robert, P. Brocker, M. Benoit, M. Chatel, CHU Nice (Nice, F)

Apathy is commonly defined as a lack of interest, emotion and motivation. Apathy is reported to be frequent in patients with stroke, Parkinson's disease, traumatic brain injury, Alzheimer's disease and depression. In comparison with the neuropsychiatric Inventory (NPI) and other existing scales the Apathy Inventory (IA) was designed to provide a separate assessment of the emotional (Emotional blunting), behavioral (lack of initiative) and cognitive (lack of interest) aspects of apathy. The IA is based on the NPI model, and information can be obtained from the spouse or another person intimately familiar with the patient's behavior. Furthermore, the patient him/herself can also be evaluated by direct questioning.

In this study, using the IA, we compared the apathy symptomatology in a population of 90 subjects with Alzheimer's disease (AD), 33 subjects with mild cognitive impairment (MCI) and 17 healthy elderly subjects.

AD subjects had significantly higher IA scores than controls. The MCI patients' scores fell between those of the AD patients and the controls. With the patient-based evaluations, no differences were found among the AD, MCI and control groups. The results also indicated that AD patients had poor awareness of their emotional blunting and lack of initiative. In summary, the Apathy Inventory is a rapid and reliable method for assessing several dimensions of the apathetic syndrome, and also the subject's awareness of these symptoms.

P252
**Behavioural and psychological correlates of low levels of vitamin B12 and folate in patients with dementia: a prospective study.** S. Engelborghs, J. Goeman, R. D'Hooge, P. Mariën, J. Saerens, A. Symons, F. Clement, B. A. Pickut, P. P. De Deyn, Middelheim General Hospital, University of Antwerp (Antwerp, B)

Objective: We set up a prospective study to test for possible correlations of decreased serum vitamin B12 and red cell folate levels with behavioural and psychological symptoms (BPSD) in a population of patients with Alzheimer's disease (AD) and frontotemporal dementia (FTD).

Background: Since associations between decreased folate and vitamin B12 levels and (worsening of) psychiatric symptoms and disorders have been described, an association with BPSD could be expected. However, studies in this domain are very sparse, produced conflicting results and only focused on AD and vitamin B12.

Design/methods: Patients with probable AD (N = 108) and probable FTD (N = 18) were included in this prospective study. Behaviour was assessed covering a period of 2 weeks prior to inclusion using a battery of behavioural assessment scales (Middelheim Frontality Score, Behavioural Pathology in Alzheimer's Disease Rating Scale (Behave-AD), Cohen-Mansfield Agitation Inventory (CMAI), Cornell Scale for Depression in Dementia). Blood sampling and determination of serum vitamin B12 and red cell folate levels were performed within a time frame of two weeks of inclusion.

Results: Besides a significant but weak negative correlation between levels of red cell folate and verbally agitated behaviours (CMAI verbally agitated behaviour cluster: $r = -0.231$, $P = 0.022$), no other significant correlations were found in the AD patient group. In FTD patients however, we found statistically significant negative correlations of levels of vitamin B12 with psychosis (Behave-AD psychosis cluster: $r = -0.696$, $P = 0.001$) and with the total score of the Behave-AD ($r = -0.616$, $P = 0.006$).

Conclusions: In AD patients, we only found a weak association of verbally agitated behaviour with low levels of red cell folate. Although psychotic symptoms are rather rare in patients with FTD, the significant negative correlation between serum levels of vitamin B12 and psychotic features could mean that FTD patients with low vitamin B12 are at risk for the development of psychosis.

P253
**CSF beta-trace protein in patients with NPH: diagnostic marker and indicator of meningeal dysfunction.** J. Brettschneider, M. Riepe, H. Petereit, H. Tumani, University of Ulm (Ulm, D)

Normal pressure hydrocephalus (NPH) is diagnosed clinically and by cranial scanning. Though the treatment of NPH is established, there may be still difficulties to distinguish it from other causes of dementia such as Alzheimer disease (AD). Furthermore, the pathomechanism leading to NPH remains to be clarified. According to histopathologic studies meningeal fibrosis may be involved in the pathogenesis of NPH.

Using a biochemical approach we aimed to study the diagnostic relevance of the mainly meninges-derived cerebrospinal fluid beta-trace protein in patients with NPH and disease controls such as AD. To assess the effects of ventricular/spinal pressure on beta-trace concentration we included as a further disease control patients with pseudotumor cerebri (PTC).

Data were acquired from 17 patients with NPH, 18 patients with AD, 12 patients with PTC and 26 (15 old and 11 young) age-matched normal control subjects (NC).

Beta-trace was measured by immunonephelometry in paired cerebrospinal fluid (CSF) and serum samples. Albumin CSF/serum quotient was determined in all groups to account for effects of blood-CSF barrier function.

To achieve a reliable comparison of all groups by allowing for effects of age CSF beta-trace concentration was plotted against age. Using this approach beta-trace levels were significantly lower in patients with NPH ($16.4 \pm 3.7$ mg/l; $p < 0.001$) if compared to age-matched NC ($25.3 \pm 4.9$ mg/l) and to patients with AD ($22.9 \pm 5.9$ mg/l). In patients with PTC ($16.3 \pm 3.9$ mg/l) beta-trace levels did not differ significantly from age-matched controls ($13.9 \pm 3.5$ mg/l).

These results indicate that meningeal dysfunction occurs in NPH and that a chronic meningiopathia may play an important role in the pathogenesis of NPH. Furthermore these data suggest that ventricular and spinal pressure as present in PTC does not affect the beta-trace producing cells within the meninges. In addition, significantly decreased levels of beta-trace may allow to improve the differential diagnosis by distinguishing patients with NPH from patients with other causes of dementia.

P254
**Patients with severe Alzheimer's disease derive significant global, cognitive, functional and behavioural benefits from donepezil therapy.** H. Feldman, B. Vellas, S. Gauthier, J. Hecker, Y. Xu, J. Ieni, E. Schwam, University of British Columbia, Hopital Purpan, McGill Centre for Studies in Aging, Memory Disorders Study Unit, Pfizer, Eisai (Vancouver, CAN; Toulouse, F; Verdun, CAN; Daw Park, AUS; New York, Teaneck, USA)

Objective: To investigate the efficacy and safety of donepezil in a subgroup of patients with severe Alzheimer's disease (AD).

Background: A double-blind trial has demonstrated donepezil's benefits in patients with moderate to severe AD. This analysis focuses on the subgroup of patients with severe AD.

Methods: 290 patients (standardized Mini-Mental State Examination [sMMSE] scores 5–17) were divided at the median baseline score; 145 were classified as having severe AD (sMMSE score 5–12). Patients were randomized to donepezil (n = 72) or placebo (n = 73) for 24 weeks, and were assessed at Weeks 4, 8, 12, 18 and 24. The primary outcome measure was the Clinician's Interview-Based Impression of Change with caregiver input (CIBIC-plus) at Week 24, using a last observation carried forward (LOCF) analysis.

Results: Baseline patient demographics were similar between treatment groups. Mean sMMSE scores (± SD) at baseline were $9.0 \pm 2.19$ for the donepezil group and $8.9 \pm 2.19$ for the placebo group. Mean CIBIC-plus scores for donepezil-treated patients showed improvement or minimal change from baseline throughout the study, while mean scores for the placebo group declined steadily. Treatment differences were significant in favor of donepezil at each assessment and at Week 24 LOCF (mean difference = 0.7, $P = 0.0002$). Significant benefits in favor of donepezil were also observed at Week 24 LOCF for all secondary outcome measures, including sMMSE (mean difference = 2.0, $P = 0.0022$), Severe Impairment Battery (SIB mean difference = 7.4, $P = 0.0017$), Disability Assessment for Dementia (DAD mean difference = 7.2, $P = 0.0082$), and Neuropsychiatric Inventory 12-item total (NPI 12-item total mean difference = 6.9, $P = 0.0062$). 90% of donepezil- and 86% of placebo-treated patients completed the trial, with 7% and 5%, respectively, discontinuing due to adverse events. 82% of donepezil- and 78% of placebo-treated patients experienced adverse events, the majority of which were rated as mild or moderate in severity.

Conclusions: The large treatment responses observed with donepezil in this subgroup of patients with severe AD, across global, cognitive, functional and behavioural measures, suggest that donepezil has important benefits in advanced AD.

P255
**Neuropsychological findings in frontotemporal dementia, semantic dementia and Alzheimer's disease.** J. Diehl, T. Grimmer, A. Kurz, Technical University Munich (Munich, D)

Background: Frontotemporal Dementia (FTD) and Semantic Dementia (SD) are clinical phenotypes of frontotemporal lobar degeneration associ-

ated with predominantly frontal (FTD) or temporal (SD) brain atrophy. In Alzheimer's Disease (AD) brain atrophy involves particularly temporoparietal brain regions. FTD, SD and AD differ not only in terms of behaviour, but also in cognitive ability, linguistic performance, and semantic knowledge. Overlap of symptoms can make differential diagnosis difficult.

Objective: (i) to determine the cognitive profiles in patients with FTD and SD compared to AD using the CERAD neuropsychological battery; (ii) to identify neuropsychological tests which discriminate between FTD and SD.

Methods: The study refers to 34 outpatients who were consecutively examined in a university hospital memory clinic. 25 patients (mean age 62.2 years) were diagnosed with FTD, 9 patients (mean age 62.2 years) had SD according to Lund-Manchester consensus criteria. Dementia was mild in all patients. Cognitive ability was examined using the CERAD-NP (The Consortium to Establish a Registry for Alzheimer's Disease – neuropsychological battery), German version. The instrument includes animal category fluency, object naming (modified 15 item Boston Naming Test, mBNT), word list learning and recall, word list recognition, and constructional praxis. It also incorporates the Mini Mental State Examination (MMSE). To compare CERAD-NP results between patients with FTD/SD and AD, 30 patients with AD were matched for age, sex, and global severity of cognitive impairment. The ability of the CERAD-NP subtests to discriminate between FTD and SD was examined using receiver-operator characteristic (ROC) analysis.

Results: On the CERAD-NP patients with FTD and SD performed significantly better than patients with AD on word list learning, delayed verbal recall and visuoconstruction ($p < 0.05$). There were no significant differences between FTD/SD and AD on verbal fluency tasks. Although they showed the same overall severity of dementia, patients with SD performed significantly worse on the mBNT than patients with FTD and AD. ROC analysis demonstrated that at a cut-off value of 7 the mBNT distinguished SD from FTD with a sensitivity of 0.96 and a specificity of 0.73. There were no other CERAD-subtests, which showed a difference between patients with FTD and SD.

Conclusion: The comparison of the profiles of cognitive impairment between FTD/SD and AD on the CERAD-NP reveals that this clinical battery can be valuable for the differential diagnosis. The modified version of Boston naming Test, which is a measure of semantic knowledge is helpful to distinguish between SD and FTD/AD.

P256

**Midlife blood pressure and the risk of hippocampal atrophy. The Honolulu Asia Aging Study.** E. Korf, L. White, P. Scheltens, L. Launer, VUmc, Pacific Research Institute of Health, NIH (Amsterdam, NL; Honolulu, Bethesda, USA)

Introduction: In Alzheimer's disease, hippocampal atrophy (HA) is usually attributed to the deposition of neurofibrillary tangles and neuritic plaques. However, the hippocampus, particularly the Ca1 area is vulnerable to global ischemia which may lead to atrophy as well. Hence, we investigated the association of high blood pressure (BP) and HA in a sub-sample of a cohort of Japanese American men participating in the Honolulu Aging Asia Study (HAAS).

Methods: The HAAS, an ongoing longitudinal community based study, is an extension of the Honolulu Heart Program, that started in 1965. At midlife, blood pressure was measured on 3 subsequent exams. Data on the use of anti-hypertensives, education, smoking, alcohol-use, APOE-genotype and cognitive functioning were collected. About 29 years later, 563 subjects underwent MRI, on which we assessed cortical and subcortical infarcts (large vessel damage), lacunes and white matter hyperintensities (small vessel damage) and hippocampal volume.

Results: Mean age of the sub-sample was 81.6 years (SD 5). Systolic and diastolic blood pressure (SBP and DBP) was not significantly associated with hippocampal volume. Those not treated with anti-hypertensives had a significantly increased risk for HA (OR 1.63 (CI = 1.06; 2.51). The treated normal SBP group had a marginally significantly reduced risk for HA (OR = 0.59; CI = 0.35; 1.01) and the non-treated high DBP group had a (non-significantly) higher OR for a HA compared to the non-treated normal DBP group (OR = 2.66; CI = 0.97; 7.31). Adjusting for any vascular damage resulted in a higher estimated risk for HA in this group (OR = 3.48; CI = 1.20; 10.08). This change in the OR resulted from adjustment for small vessel damage (OR = 3.56; CI = 1.23; 10.29), not large vessel damage (OR = 2.46; CI = 0.88; 6.87).

Conclusion: Treatment with anti-hypertensive treatment seems to modify the association of BP and reduced hippocampal volume, such that high levels of DBP adversely affect the hippocampus.

P257

**Efficacy of rivastigmine in patients with severe Alzheimer's disease.** R. Blesa, G. Karlsson, R. Spiegel, Hospital Clinic Universitari, Novartis Pharma AG (Barcelona, E; Basel, CH)

Rivastigmine, an inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), is indicated for the treatment of mild-to-moderate Alzheimer's disease (AD). Evidence that rivastigmine may be effective in more advanced AD has become available from a meta-analysis of three placebo-controlled studies. Within this dataset, 165 patients had baseline Mini-Mental State Examination (MMSE) scores of 10–12. Within this subgroup, rivastigmine-treated patients showed significantly less cognitive decline over 26 weeks than patients on placebo, as assessed using the Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog) (intention-to-treat [ITT] analysis, $p < 0.001$).

A multicentre, randomized, double-blind, placebo-controlled, 6-month study of rivastigmine in 218 patients with moderate to severe AD (MMSE 5–12; GDS 5–6) was completed in October 2002. Cognitive changes were evaluated using the Severe Impairment Battery (SIB) and the MMSE. Global responses were assessed using the Clinician's Global Impression of Change (CGIC). The ITT population comprised 104 and 106 rivastigmine- and placebo-treated patients, respectively. Mean baseline SIB scores were 74.4 in the rivastigmine group and 70.3 in the placebo group; at endpoint a difference between the changes from baseline was 4.0 ($p = 0.026$). Mean baseline MMSE scores were 9.0 in the rivastigmine group and 8.7 in the placebo group; at endpoint scores had improved by 0.6 and declined by 1.0, respectively (treatment difference 1.6, $p < 0.001$). CGIC results demonstrated significant benefits with rivastigmine over placebo ($p = 0.024$).

Rivastigmine provided significant benefits over placebo in patients with moderate to severe AD. This may have important implications for patients with severe AD, for whom there are currently no approved symptomatic treatments.

Study supported by Novartis Farmaceutica SA, Spain, and Novartis Pharma AG, Switzerland.

## Epilepsy

P258

**A novel missense mutation in KCNQ2 K+ channel in a family with BFNC, West syndrome and mental retardation.** R. Borgatti, A. Cavallini, M. Ferrario, C. Panzeri, C. Zucca, E. Fontana, M. Taglialatela, N. Bresolin, M. T. Bassi, IRCCS E. Medea, University of Verona, Federico II University of Naples, IRCCS Ospedale Maggiore Policlinico (Bosisio Parini-Lecco, Verona, Naples, Milan, I)

Background: KCNQ2 and KCNQ3 are two K+ channel subunits which combine to form heterotetrameric channels with properties of neuronal M channels. Loss of function mutations in either subunits can lead to benign familial neonatal convulsion (BFNC), a rare autosomal disorder first classified as generalised idiopathic epilepsy. BFNC is characterized by frequent brief seizures starting on or after the second day of life and disappearing spontaneously within a few (about 4) weeks. However 16% of BFNC-affected individuals have seizure episodes later in life.

Results: here we describe a family with four affected members in two generations. The proband is a 7 year old female who had benign neonatal convulsions later followed by West syndrome. Now she has a severe drug-resistant epilepsy associated to a profound mental retardation. The younger sister of the proband and the mother had seizures at the age of three days which spontaneously resolved. In the younger sister of the mother, seizures had started at the age of three days and later developed as focal seizures. Epilepsy continued until the age of 5 years; she is now mildly mentally retarded with a moderate motor impairment and nystagmus. Brain CT scan and MRI examinations for all the affected members of this family are normal. Cariotype analysis of the affected individuals is normal. We analysed KCNQ2 and KCNQ3 coding sequence and exon-intron junctions in the affected members of the family and the only mutation found is a nucleotide change at position 1620 of the cDNA sequence (g1620 > t) leading to a new missense mutation falling into the C-terminal portion of KCNQ2 protein. The mutated amino acid residue is highly conserved among all human members of the KCNQ family of K+ channel and is also part of the minimum KCNQ2 sequence necessary for CaM binding. Functional assays to test the effect of this new mutation in KCNQ2 are in progress. This mutation segregates in all affected members of the family while it is not present in the healthy father. Conclusion: the complex clinical picture observed in this family, due to the co-occurrence of BFNC with infantile spasm, focal epilepsy and mental retardation all segregating in

different individuals of the same family has never been reported to our knowledge. Indeed in the literature, families are described with BFNC and West syndrome, or BFNC later developing generalized and focal seizures associated to a mildly retarded psychomotor development. Overall, previous findings and our data together with the recent report of a family with BFNC and myokimia strongly indicate that the phenotype presented by patients who experienced neonatal convulsions (BFNC) might be quite complex and heterogeneous. On the other hand, the molecular finding in our family suggests an apparently common genetic origin for all the features observed, challenging in some way the clinical classification of BFNC.

P259
**Focal EEG findings in adult patients with absence epilepsy.** Z. Matur, C. Gurses, B. Baykan, A. Gokyigit, Istanbul University (Istanbul, TR)

Background and Purpose: It is well known that, in absence epilepsy (AE), EEG typically shows symmetrical 3 Hz generalized spike and wave discharges. However, a few authors have reported to interictal focal EEG features in about 1/3 of the cases, but without video-EEG documentation. The purpose of the current study was to examine focal EEG features in adult patients with AE and their clinical correlates.

Method: We investigated a total of 476 EEG examinations of 98 consecutive patients, who are older than 18 years diagnosed as having AE according to the ILAE criteria. Fifty patients who had typical absence seizures recorded during EEG were followed by our epilepsy clinic. We included 50 patients with ictal discharge recordings, 23 of whom also had video EEG monitoring.

Results: There were 23 males and 27 females, followed for an average period of 8 years [1–22], aged between 9 and 91 years (average: 29). Focal EEG features were observed in 17/50 (34%) patients, classified as "Group I", whereas the remaining patients classified as "Group II" had no focal discharges.

The observed focal abnormalities consisted of spike/spike and wave discharges in 13 patients and paroxysmal slow waves of theta and/or delta frequency in 4 patients. Focal abnormalities were mostly seen in frontotemporal (7 patients, 41%), and frontal (5 patients, 29%) regions.

There was no significant difference with respect to age and sex between the groups. The average ages at onset of absence seizures were 15 [3–67] in "Group I" and 11 [1–29] in "Group II".

Semiological data showed that the absence seizures accompanied by automatisms were significantly higher in "Group I" (24%) when compared with "Group II" (6%) ($p < 0.072$). In Group II, however, eye deviation during absence seizures (21%) was higher than in Group I (none of the patients) ($p < 0.041$).

From the EEG point of view, in Group I, the frequency of asymmetry (47%), asynchrony (29%) and presence of associated "fast rhythms" (24%) during the ictal discharges and frontal lobe predominance (65%) was significantly higher with respect to Group II (18%, 9%, 3%, 27%) ($p < 0.031$, $p < 0.063$, $p < 0.022$, $p < 0.010$).

Conclusion: Seventeen of 50 adult patients with AE in our series exhibited focal EEG features. Thus, such focal findings do not preclude the possibility of AE. In such patients, video-EEG documentation is invaluable in reaching a correct diagnosis and choosing an appropriate AED regimen.

P260
**Safe administration of Olanzapine in psychotic epileptic patients.** A. Thomas, D. Iacono, A. Luciano, K. Armellino, C. Romagnoli, B. Perfetti, M. Onofrj, Neurophysiopathology (Pescara, I)

Introduction: Certain forms of epilepsy may develop chronic interictal psychosis (schizophrenic-like psychosis of epilepsy – SLPE), 3–7% of the epileptic population. In these patients the psychotropic medication is not commonly used because of the potential risk to lower seizure threshold.

Patients and Methods: We selected 16 patients affected by SLPE followed in our Center of epilepsy. The psychotic disturbance was diagnosed according to DSMIV criteria. All patients gave their informed consent to participate in a 12 months double-blind cross-over study with olanzapine (10 mg/day) vs haloperidol (12 mg/day). All patients were on a stable AED dosage for more than three months. During the study all patients were evaluated with EEG recordings and the Brief Psychiatric Rating Scale (BPRS) at baseline and after 1, 3 and 6 months in every treatment branch. During the 12 months study patients were asked to report seizures into a diary. Each branch consisted of the evaluation of the drug efficacy, recurrence of seizures and side effects for 6 months. The first drug was then discontinued and the other drug introduced in a 15 days titration period and then evaluated for the following 6 months.

Results: In the patient population there was no statistical significance in age, sex, recurrence of seizures ($p = 0.9$) and BPRS score ($p = 0.8$). They were randomized with a computer system into the treatment groups. 13 of the 16 patients completed the study; 1 patient dropped out because of severe EPS during haloperidol treatment, 1 patient was not compliant in the diary completion and one patient was withdrawn from the study because of temporal lobe surgery. Patients during olanzapine treatment period had a statistically significant reduction in BPRS scores compared to the haloperidol treatment period ($t = -4.1$, $p = 0.02$). The recurrence of epileptic attacks was minimally reduced or unchanged during olanzapine treatment, increment of seizures during haloperidol treatment compared to baseline values was statistically significant at $p = 0.04$ level. Olanzapine induced slowing of EEG but without any effect on epileptiform activity. In conclusion, it seems that olanzapine can be safety administered in patients with SLPE without modifying the recurrence of seizures and improving the psychotic symptoms.

P261
**Epidemiology and aetiology of intractable epilepsy in Qatar.** H. Al Hail, O. S. Tag-Eldin, A. Hamad, Hamad General Hospital (Doha, QA)

Study aim: This a hospital-based retrospective study to find out the incidence and causes of intractable epilepsy among native Qatari patients.

Methods: Total of 1217 epileptic patients above the age of 13 years and from all nationalities admitted to the hospital between the years 1992 and 2000 were included in the study. Intractable epilepsy was defined as uncontrolled epileptic seizures occurring 2–3 times per month for the last 2–3 years despite adequate anti-epileptic mono- or polytherapy.

Results and Conclusion: A total number of 219 patients (15%) had intractable epilepsy. Fifty-one (23%) were Qatari and 168 (77%) were non-Qatari patients. F: M ratio 2:1. The age in Qatari patients ranged between 13 and 45 years (mean 29 years) and between 13 and 48 years (mean 30 years) in non-Qatari. The incidence of epilepsy in the whole population was calculated as 174:100000 per year, 96 in male and 78 in female with M:F ratio of 1.2:1. The incidence rate of hospital admission with uncontrolled epilepsy was 25 in 100000 person per year and the incidence of intractable epilepsy as 4.5:100000 per year. Among native Qatari the incidence was 1:100000 per year whereas in non-Qatari 3.5:100000 per year.

The most common type of intractable epilepsy was idiopathic generalized epilepsy (26%), followed by symptomatic partial epilepsy (25%). In the latter, stroke constituted 11%, head injury 8%, hippocampal sclerosis 4% and temporal lobe epilepsy 2% of the underlying causes.

P262
**The assessment of emotional memory, learning and behavioural parameters in immature rats after pentylenetetrazole-induced status epilepticus.** F. Erdogan, A. Gölgeli, F. Arman, A. Ersoy, Erciyes University (Kayseri, TR)

Status epilepticus can be harmful on developing brain. Our knowledge about the behavioral consequences of status epilepticus in developing animals remains limited. For this reason we investigated short and long term effects of pentylenetetrazol (PTZ) induced status epilepticus on emotional memory, learning and behavioral parameters in immature rats.

SE was induced in 16–20 day-old rats (P16–20) using intraperitoneal PTZ while controls received intraperitoneal saline. All animals were tested postseizure behavioral battery, elevated T maze and open field test at one, 14, 30 and 180 days after SE for evaluated emotional memory, learning and behaviors. Open field test was used to check the behavioral responses to a novel environment, motor abilities, and habituation. Numbers of squares crossing and rearing increased significantly in open field test at one and 14 days after SE. There was a significantly decreasing number of defecation one day after SE whereas increasing 14 days after SE. Postseizure behavioral battery was simple, reliable, and also quantitative battery of tests for studying behavioral changes after SE or seizures. PTZ treated animals score significantly higher on two of the behavioral tests one day after SE: finger snap and pick up tests. There was also significantly high score on touch-response test at 180 days after SE. Elevated T-Maze was used in assessment of drug effects on memory and the relationships between neural subsystems involved in emotionally related behaviors and in processes underlying learning. There weren't any differences between SE and control group on elevated T maze test parameters.

These results indicated that behavioral changes were mostly transient and no emotional memory and learning deficits persisted for a long time period after PTZ-induced SE in immature rats.

P263
**Clinical, demographic and laboratory data in patients with late onset non-lesional focal epilepsy.** A. Mavioglu, N. Dericioglu, P. Özdemir Geyik, S. Saygi, A. Ciger, Hacettepe University Medical Faculty (Ankara, TR)

Introduction: Epileptic seizures usually start during childhood and early adulthood. When focal epileptic seizures occur later in life, they usually tend to be symptomatic. However in some patients with late onset focal seizures, no lesions can be detected in cranial MRI. We designed a retrospective study where the clinical, demographic and laboratory data of patients with late (> 30 years, Group I) and early (< 30 years, Group II) onset focal seizures were compared.

Patients and Method: Patients with non-lesional focal epilepsy who admitted to the Neurology Department of our hospital and were evaluated by one of the authors (SS) within the last 10 years were included in the study. They were divided into two groups according to age of onset (before or after 30 years) of their seizures. Gender, presence of nocturnal seizures, personal and family history, type of seizures, EEG, cranial MRI, blood tests, treatment regimens and prognosis were compared statistically between the two groups.

Results: There were 64 patients (32 M, 32 F; age of onset of seizures: 30–73, mean 41.72) in Group I and 113 patients (53 M, 60 F; age of onset of seizures: 1–29, mean 8.53) in Group II. In Group I secondary generalized seizures were more common. Most of the patients were seizure free without medication or consumed a single antiepileptic drug and had a lower seizure frequency. Hypertension was more common as were a family history of endocrinological and oncological diseases. Febrile convulsions, head trauma, perinatal injury and parental consanguinity were more common in Group II.

Conclusion: Patients with late onset of partial seizures and normal cranial MRI tend to have a good prognosis in terms of seizure control. The presence of various endocrinological and oncological (some of which might be immune mediated) diseases in family history may indicate that at least in some patients immune system disorders may play a role. This hypothesis needs to be investigated with further studies.

P264
**Parietal lobe epilepsy: clinical, electroencephalographic and neurodiagnostic findings in 45 patients.** H. Karatas, S. Saygi, N. Dericioglu, A. Ciger, Hacettepe University (Ankara, TR)

Introduction: We aimed to identify the clinical, EEG and MRI characteristics of patients with drug resistant symptomatic parietal lobe (PL) epilepsy. These findings might help us localise the seizure focus in some patients with cryptogenic partial epilepsy (CPE) and tell us when the PL should be sampled with intracranial electrodes prior to epilepsy surgery.

Method and Results: Clinical files, routine scalp EEGs and neurodiagnostic images of 45 epileptic patients (age: 18–80 years; 21F, 24 M) with PL lesions were investigated, retrospectively. Auras were reported in 77 % (35/45) of the patients and included sensory symptoms (57 %), headache (23 %), nausea-vomiting (23 %), psychic symptoms (23 %) and visual symptoms (14 %) among others. The most common ictal behavioural changes were paresthesia (70 %) and focal clonic activity (39 %). Tonic posture, various automatisms, head deviation, staring, sensation of pain and speech disturbances occurred to a lesser extent. Simple partial seizures were present in 70 % of the patients. Complex partial seizures occurred in 21 % and secondary generalised tonic clonic seizures were reported in 51 % of the patients. Medical history revealed febrile convulsions in 20 %, family history of epilepsy in 18 % and parental consanguinity in 9 % of the patients. Presumed etiologic factors were as follows: post-traumatic encephalomalacia (22 %), cerebrovascular disease (20 %), tumour (15 %), cortical developmental abnormality (15 %), atrophy (11 %), cavernoma (9 %) and arteriovenous malformation (6 %). Lesion localisations were as follows: right hemisphere in 47 %, left hemisphere in 44 % and bilateral in 9 % of the patients. Interictal EEG disclosed abnormal background activity in almost 1/3 of the patients. Epileptiform abnormalities were found in 34 % and lateralized paroxysmal slow, sharp-slow waves were detected in 57 % of the patients. EEG findings were normal in 34 % of the patients. In none of the EEGs isolated parietal foci were detected.

Conclusion: Clinical and laboratory findings in patients with PL epilepsy are scarce in the literature. Our results indicate that PL seizures may have different symptomatologies and interictal scalp EEGs contain epileptiform abnormalities in only 1/3 of the patients. We conclude that drug-resistant CPE patients who have various sensory symptoms should undergo long term video-EEG monitoring (with intracranial electrodes placed over the PL when needed) and cranial MRI should be obtained according to a certain protocol.

P265
**Investigation of phenotypic similarities and differences in familial epilepsies.** M. Yaman, N. S. Yeni, I. U. Cerrahpasa Medical School (Istanbul, TR)

Introduction: Epilepsy has been known for centuries and since Hippocrates, it has been depicted that epilepsy may have a genetic base. In recent years significant advances in epilepsy genetics have developed. Idiopathic epilepsy, the most common group, is believed to be genetically transmitted. Nowadays the specific gene of five epilepsy syndromes and locus of many idiopathic epilepsies have been determined.

Objective: Epilepsy, either phenotypically or genotypically is a heterogeneous disorder. The aim of this study is to determine the extent of heterogeneity in familial epilepsies (FE).

Method: This study included patients with FE among epilepsy out patients who admitted to Istanbul University Cerrahpasa Medical Faculty Neurology Department between July, 2001 and September, 2002. The proportion of FE and sporadic epilepsy (SE) has been evaluated. The rate of risk factors has been compared between FE and SE. Clinical/laboratory data were documented and phenotypic features and variabilities of FE have been pointed out.

Results: The rate of epilepy in first degree relatives of patients is 10.3 %. History of febrile convulsion (FC) in FE is more commonly seen than SE. Furthermore, history of head trauma (HT) and perinatal problems (PP) do not seem to be more common in FE. Among the FE's, the families with homogeneous epilepsy syndrome mainly consist of focal epilepsies. Concordance rates of idiopathic familial epilepsies are not different from cryptogenic epilepsies. The concordance rates of syndromically homogeneous FE are not different than syndromically heterogeneous FE.

Discussion: There is strong correlation between FC and FE's. But HT and PP are not additional risk factors for FE. In FE, etiological concordance was found to be high among family members but there was significant discordance in terms of clinical symptoms and laboratory data. Clinical expression of epilepsy discloses important variabilities in family members.

P266
**Avicenna and epilepsy.** M. Moghaddasi, Iran University of Medical Sciences (Tehran, IR)

Avicenna is the Latinised form of Ibn-e Sina an abridgement of the full name Abu Ali al-Husain Ibn Sina (980–1037 AD) is the most famous scientist of Islam and Iran and also all races, places and time. He was born in the village Afshane in the province of Balkh, an area under rule of Iran in the past, now in Uzbekistan. His tomb is now in Hamedan in the west of Iran. He has many works in theology, metaphysics, astronomy, philosophy, physics and medical sciences. In medicine he wrote several books of which the Canon of Medicine (Al-Ganun) is the best that was regarded as the principal text up to 1650 at Montpellier, France great center of medical studies and some other universities in the world. In the third volume, he described epilepsy, provoked and unprovoked seizures, febrile convulsion, a primary classification of epilepsy, predisposing factors, nutrition and epilepsy, the rules of treatment and many other points.

In this article we review what he thought of epilepsy.

P267
**Left hemispheric localization of intracranial organic lesion and/or epileptic focus as a risk factor in the occurrence of status epilepticus.** T. Yoldas, R. Yigiter, B. Müngen, H. Ulvi, Firat University (Elazig, TR)

Several risk factors including acute organic brain damage, idiopathic epilepsy, metabolic disorders, irregular anti-epileptic drug usage, fever, partial seizures, infections of central nervous system etc. have been identified in development of status epilepticus (SE). Whether intracranial lesion and/or hemispherical lateralization of epileptic focus (localization in right or left hemisphere) are risk factors for development of SE has not been investigated to date. In present study we investigated these possible risk factors in a group of patients with SE (16 female, 25 male; mean age: 39,8 ± 21,9 years; min: 5, max: 87). Organic lesion and/or epileptic focus were in left hemisphere of 15 cases, whereas in 8 cases they were located in right hemisphere. Bilateral localization was observed in three cases since localization in left hemisphere was more frequent (p: 0.004) it was thought to be a risk factor for development of SE.

## General neurology

**P268**

**Risk factors and prediction of polyneuromyopathy.** J. Bednarik, P. Vondracek, L. Dusek, E. Moravcova, I. Cundrle, University Hospital, Masaryk University (Brno, CZ)

Introduction: The aetiology and mechanisms of neuromuscular involvement in critically ill patients are not completely understood. Numerous clinical, laboratory, and pharmacological variables have been reported as significantly associated with the development of critical polyneuromyopathy (CIPM).

Methods: We performed a prospective one month observational clinical and electrophysiological consecutive case study aimed at the identification of the risk factors of CIPM. The detection of the CIPM was based primarily on the electrophysiological criteria. One hundred and two critically ill patients were enrolled into the study and 61 (31 men, 30 women, median age 59, range 22–81 years) completed the 28-day follow-up. Primary endpoints were electro-physiological signs of new CIPM detected at the end of the follow-up 28-day period.

Results: Electrophysiological signs of the CIPM were detected in 35 critically ill patients, and were further classified as myopathy (14 patients), neuropathy [12] and mixed [9]. The CIPM development was significantly associated with the presence and duration of systemic inflammatory response syndrome (SIRS) and the severity of multiple organ failure (MOF) expressed as the Sequential Organ Failure Assessment score (SOFA). The mean 28-day total SOFA score reflecting the cumulative severity of MOF during the 1st month shows the closest correlation with the CIPM development. Respiratory, central nervous, and cardiovascular system displayed independent significant association with the CIPM. Myopathy was not significantly associated with the administration and dosage of corticosteroids or non-depolarising muscle blocking agents. Multivariate logistic regression model based on the initial SOFA score, summed 1st week SOFA score and duration of SIRS in the 1st week was able to correctly predict the development of CIPM in about 85 % of our cases.

Conclusions: The presence and duration of SIRS and the cumulative severity of multiple, respiratory, neurological and cardiovascular failures are associated with increased risk for the development of CIPM. These variables could be used for prediction of the CIPM development. SIRS is probably the most important etiological factor of the whole spectrum of neuromuscular involvement in critically ill patients. The parallel time course of MOF and that of neuromuscular involvement favourize the concept of CIPM as a part, not a consequence, of multiple organ failure (a neuromuscular failure).

**P269**

**Vestibular deactivation during imagined locomotion: a fMRI study.** K. Jahn, A. Deutschländer, T. Stephan, M. Strupp, T. Brandt, Ludwig-Maximilians University (Munich, D)

Locomotion is a motor performance based on spinal generators under the control of several distinct and separate supraspinal centers that initiate locomotion or modify locomotion speed. Sensory systems, such as visual and vestibular contribute to maintenance of direction and balance, particularly during locomotion at slow speed. We hypothesized that visual and vestibular sensory control might be inhibited during running, which is generated by highly automated spinal patterns. The aim of the present fMRI study was [1] to identify supra- and infratentorial brain regions involved in locomotor control in humans and [2] to compare activation and deactivation patterns during stance, walking, and running. Imagination of these conditions was chosen since actual locomotion was methodologically impossible with MRI in our setup.

Thirteen healthy subjects were trained to imagine lying, standing, walking, and running with eyes closed. Functional imaging was done using a Siemens MRI 1.5 T scanner and echo-planar imaging (EPI) sequences. A total of 34 slices covered the whole brain, large parts of the cerebellum, and the upper brainstem. Subjects were instructed to imagine the four different conditions on acoustic demand (20 sec). Data processing was done using statistical parametric mapping (SPM99) and MATLAB scripts. BOLD signal increases and decreases were tested for statistical significance for each subject and for the group ($p < 0.001$).

During the imagination of walking (compared to lying) midline cerebellar structures, bilateral fusiform gyrus, left basal ganglia, left anterior insula, and precentral areas on the right side showed activation. Deactivation was found in the bilateral postcentral gyrus and right superior temporal gyrus. During the imagination of running the cerebellar midline structures (spinocerebellum) and fusiform gyrus showed BOLD signal increases. Deactivation under this condition was found in the right postcentral gyrus, bilateral superior temporal gyrus, and posterior insula including Heschl's gyrus.

The main activation found during imagined locomotion was situated in the midline cerebellar structures, which corresponds best to the cerebellar locomotor center. The deactivations found in sensory systems (somatosensory and vestibular) during locomotion were most pronounced during running. This fits earlier findings about the inhibitory interaction between locomotion and sensory functions, which is differentially regulated for walking and running.

**P270**

**Reversible posterior leukoencephalopathy syndrome: clinico-radiological correlation and first necropsic study after improvement.** R. C. Ginestal, M. Garcia-Villanueva, P. Calleja, C. Sanchez-Bueno, J. C. Martinez-Castrillo, Hospital Ramon y Cajal (Madrid, E)

Introduction: The Reversible Posterior Leucoencephalopathy Syndrome (RPLS) is a condition clinically defined by an acute encephalopathy usually associated with visual symptoms and seizures. Many etiologies have been related to this syndrome, including arterial hypertension and glomerulonephritis. The neuroimaging is the clue for the diagnosis. With the etiological treatment, the rapid resolution of clinical and neuroradiologic abnormalities suggests that the pathogenic mechanism is a vasogenic edema. We report two cases of RPLS that demonstrate the perfect clinico-radiological correlation of this syndrome and the importance of an early diagnosis to avoid permanent damage to affected brain tissues. Moreover, we present the first clinical-radiological-pathological description of a RPLS with good response to prompt treatment.

Case report: Patient 1: A 38-year-old woman in her 31st week of pregnancy (with arterial hypertension during the whole gestation) was treated with a cesarean section because of uterus anatomic abnormalities. Four hours later, the patient developed generalized tonic-clonic seizures and status epilepticus. Blood biochemical studies found a mild hypocalcemia. Computed Tomography (CT) showed a diffuse hypodensity involving the brainstem and the basal ganglia. In the same regions the MRI presented areas of increased T2 signal. The successful control of hypertension, hypocalcemia and seizures attainted to resolve the clinical and radiological abnormalities. The patient was discharged with only a mild gait disturbance and without lesions in a new MRI study.

Patient 2: A 37-year-old woman was admitted suffering abdominal pain due to inferior vena cava thrombosis. She had peritonitis and massive ascites. She presented a nephrotic syndrome and long term hypertension. She was treated with intravenous infusion of albumin. On the 30th hospital day she developed an acute encephalopathy with coma. CT and MRI did the diagnosis of RPLS. With the control of arterial hypertension and electrolyte and osmolality disturbances, the patient improved on her neurologic problems as did the neuroimaging. On the 60th hospital day, she died because of multiple visceral thrombosis due to the nephrotic syndrome and to a membranous glomerulonephritis. The necropsic study of the brain set the confirmation of the reversibility of this syndrome with only a few microscopic infarctions in the pons and no signs of edema.

Discussion: The RPLS is an acute and severe encephalopathy with a tight correlation between clinical and radiological findings. Treatment can induce a complete clinical recovery with radiological resolution of the previous lesions. The first clinical, radiological and pathological description of a RPLS with good reponse to prompt treatment shows that an early therapy can solve the vasogenic edema and thereby afford an excellent prognosis to the patient by avoiding any permanent brain damage.

**P271**

**Lumbar spinal stenosis: correlation between the degree of narrowing and walking ability.** S. Vohanka, B. Adamova, L. Dusek, University Hospital, Masaryk University (Brno, CZ)

Background Data: There is general agreement that the degree of narrowing of the lumbar spinal canal does not correspond with the clinical severity of the disease.

Objectives: The aim of the study was to compare a history of neurogenic claudication, physical examination, and a ten-metre walking test with the radiologically established severity and extent of lumbar spinal stenosis (LSS).

Methods: A group of 68 patients suffering from LSS underwent physical examination and were given a simple ten-metre walking test. Mean age was 55 years [43–67], neurogenic claudication was present in 57 %, weakness of lower limbs in 32 %.

The clinical data, including walking time, were compared with an axial CT scan of the lumbar spine. The number of stenotic levels and the nar-

rowest transversal and sagittal values. as well as the presence of scoliosis, were evaluated. (Normal transversal value > 16.0 mm, normal sagital value > 11.7 mm).

Results: The number of stenotic levels was not significantly associated with decreased ability to walk (OR 1.58, CI (0.52; 4.79)*). The same result was found for the relation between ability to walk and narrowest sagital diameter (OR 1.28 (0.46; 3.55) or presence of scoliosis OR 0.93 (0.16; 5.3).

The ability to walk was significantly ($p < 0.05$) influenced by transverse reduction of the spinal canal at the narrowest level OR 2.89 (1.43; 4.13). (Odds ratio (OR) expressing risk of decreased walking ability (10-m test performed in > 15 s) associated with above mentioned radiological risk factor, univariate logistic models, RR expressed with 95% confidence interval in parenthesis.)

The number of stenotic levels and narrowest sagittal canal diameter did not correlate with the presence of neurogenic claudication or lower limb weakness. The transversal narrowing of the spinal canal correlated with pareses of lower limbs ($p < 0.05$). (Mann-Whitney test for quantitative parameters, binomial test for relative frequencies).

Conclusions: We were not able to document any association between the extent of lumbar spinal canal stenosis and restriction of walking capacity.

Only the severity of the transversal stenosis at the narrowest level predicts restriction of walking capacity and presence of lower limb weakness.

Supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic Grant Nos. NF/5938-3 and NK/7129-3

P272

**Relation between intracranial and intraocular pressures.** M. H. Harirchian, H. Sabery, M. H. Malekmadany, A. Sajady, Tehran University Of Medical Science (Tehran, IR)

Introduction: Different physical and chemical mechanisms that protect the central Nervous system (CNS) from injuries are the most important reasons that have made physicians look after indirect indexes in order to evaluate CNS function. One of important specifications of cranium, as the container of the brain, is its pressure, whose pathologic alterations will lead to major CNS dysfunctions. Nowadays, the most common method used for measuring intra-cranial pressure (ICP), is lumbar puncture (LP), which is a painful and invasive but sensitive procedure with some complications and contra-indications. In this study we wanted to know if there is any relationship between ICP and intra ocular pressure (IOP) whose measuring is very easy, noninvasive, cheap and safe.

Study method: According to the results of one previous study the number of cases were estimated 50 that were chosen from the patients of neurology wards of two big hospitals of Tehran University of Medical Sciences by simple randomization. All the patients have undergone LP because of other clinical work-ups and their ICPs were measured by a neurologist with the same instrument and in the same position. Patients' IOPs were estimated by one Schiotz tonometer and by measuring the pressure of each eye with two different scales and obtaining the mean pressure from these 4 measurements by an ophthalmologist who had not any information of the patient's ICPs. Our exclusion criteria were: 1-Glaucoma history 2-History of using IOP altering drugs 3-Abnormal fundoscopic exam 4-Age under 15. Findings: From total 50 patients, 29 were female and the range of their age was between 15 to 73 years old. According to the disease type the patients were categorized in 8 different groups. Twenty five of patients had raised ICP. Among the raised ICP cases 92% had raised IOP too; and all of the normal ICP patients had normal IOP. The statistical analysis by SPSS-10 software showed an adjusted R-square of 0.912 between the two main variables ($p < 0.001$) and on the basis of the regression model these formulas were obtained: ICP = 1.16 IOP-4.86 (ICP and IOP are in mmHg).

Result: Although LP is the best clinical method for ICP measurement, but according to the significant correlation between IOP and ICP and because of the non-invasive, safe, easy and cheap nature of measuring IOP in comparison to LP, it could be adviseable in some special cases.

P273

**Progressive encephalomyelitis with rigidity and myoclonus with atypical clinical presentation.** D. Diamantas, R. Divari, T. Avramidis, E. Agapitos, I. Anastasopoulos, A. Papadimitriou, Red Cross Hospital of Athens, University of Athens (Athens, GR)

Progressive Encephalomyelitis with Rigidity and Myoclonus (PERM) is a rare disorder, considered as the most severe form of the "stiff-man" syndrome, although it is also suggested that it represents a distinct entity of unclear pathogenesis. The course is usually of steady progression leading to death in 1–3 years. Rigidity and spasms are usually accompanied by brain stem signs. Immunomodulating therapy has been reported to be efficacious when antiGAD ab's are present.

A case of brain biopsy verified PERM is presented with atypical (remitting-relapsing) course.

A 70 year old woman presented with bilateral VI nerve palsies, a left VII nerve palsy, dysphagia, dysarthria, vertigo and nystagmus. The symptoms developed over a 10 days period and fully reversed after about a month. Four months later she presented generalised myoclonus and gradually painful spontaneous and reflex spasms leading to respiratory failure and tracheostomy. She also presented signs of autonomic dysfunction (hypersalivation, hypertensive crisis, sweating), but no signs of cranial nerve palsies. EMG studies revealed continuous motor unit activity with normal NCV. Muscle biopsy was normal and antiGAD ab's negative. The CSF showed mild pleocytosis and protein elevation and negative 14-3-3 protein. All possible focal or systematic causes of this clinical entity were excluded by the laboratory investigation and the diagnosis of PERM was considered. The patient was treated with muscle relaxants, antiepileptics and although no autoab's were detected, with steroids and IVIG. The symptoms remitted completely within two months, but two months later they reappeared with steady progression, not responding to any treatment, leading to death a year and a half after the onset of the disease. A postmortem examination of the brain and spinal cord revealed perivascular lymphocytic cuffing throughout the brainstem, with gliosis and neuronal loss, consistent with the diagnosis of PREM.

This is in our knowledge the first case of PREM with a) onset with brain stem involvement, which completely subsided before the spinal symptoms appeared; b) a full initial response to immunomodulating therapy with negative auto ab's.

Concluding, this rare condition should be considered in obscure cases of brain stem pathology and immunomodulating therapies should be tried early in the course of the disease, even if no obvious autoimmune factors are apparent.

P274

**Enzymatic, morphological and molecular genetic studies in adult polyglucosan body disease.** E. Sindern, F. Ziemsen, T. Ziemsen, Y. Shin, T. Podskarbi, F. Brasch, M.-J. Schröder, M. Vorgerd, BG-Kliniken Bergmannsheil, Max Planck Institute, University Childrens Hospital, University Hospital Aachen (Bochum, Munich, Aachen, D)

Adult polyglucosan body disease (APBD) is a rare autosomal recessive disorder presenting in adulthood with upper and lower motor neuron signs, dementia, and urinary dysfunction. Most patients with APBD have primary deficiency of glycogen branching enzyme (GBE) due to mutations in the GBE gene leading to the formation of spherical inclusion bodies (polyglucosan bodies).

A 50-year-old Geran woman presented with progressive spastic tetraparesis, urinary incontinence and dementia since age 43. She died unexpectedly of acute cardiac failure. Enzymatic, morphological and mRNA expression studies of autoptic material were performed. Mutational analysis in the patient revealed missense mutations (Arg515His, Arg524Gln) in the GBE gene. Autopsy showed accumulation of polyglucosan bodies in the cardiomyocytes, peripheral nerve and brain, but neither in skeletal muscle nor in liver. GBE actvity was decreased or absent in the morphological affected tissues, but was normal in unaffected tissues. Expression of GBE mRNA in the brain, nerve, heart, and skeletal muscle was similar to the mRNA expression pattern in normal controls.

Conclusion:

1. Severe cardiomyopathy may occur in APBD beside peripheral and central nervous system involvement.
2. Morphological and enzymatic alterations reflect the clinical phenotype of severe involvement of the heart, brain, and peripheral nerve.
3. The missense mutations within the GBE gene of the APBD patient may act on a posttranscriptional level.

P275

**Correcting incapacitating orthostatic hypotension with ambulatory noradrenaline pump in pure autonomic failure.** P. Michel, R. Chakour, D. Hayoz, C. Hasler, M. Burnier, J. Bogousslavsky, T. Kuntzer, Centre Hospitalier Universitaire Vaudois (Lausanne, CH)

Background: Orthostatic hypotension (OH) due to pure autonomic failure (PAF) is a potentially incapacitating symptom in otherwise functional individuals. We present a patient in whom treatment of recurrent orthostatic syncopies with an ambulatory noradrenaline pump allowed resumption of normal daily and professional activities.

Case Report: A 46 year old man with no past medical history developed

progressive OH due to PAF (Bradybury-Eggleston syndrome). Multiple system atrophy and peripheral neuropathies were excluded based on a 6-year follow-up, and several radiological, peripheral nerve and biochemical investigations. Maximal medical treatment, tight elastic trousers, and fractionated meals resulted in supine hypertension and did not prevent disabling orthostatic syncopies starting at age 52.

Methods: During tilt-table testing, neurological symptoms were recorded by video, arterial blood pressure (BP) and heart rate (HR) changes by continuous non-invasive measurements (Finapress ®), and noradrenaline and ADH-concentrations by repeat plasma measurements. 24-hour BP and HR-values were recorded by portable equipment. After exclusion of systemic arterial disease and determination of noradrenaline-requirements by noradrenaline-infusions in the antecubital vein, a subclavian port-a-cath was implanted and connected to a small portable medication pump (CADD-Legacy PLUS ®, Deltec Inc., St.Paul, Minnesota). Changes in predetermined infusion rates (off-sitting-upright) were initiated by the patient 3–4 minutes before changing positions (Oldenburg O et al. (2001) JACC).

Results: In upright position on a tilt-table, convulsive syncopy, unmeasurably low BPs, severely deficient HR adaptation, absent noradrenaline response and excessive ADH-response were documented (Kaufmann H et al. (1992) Neurology) before installation of the pump. In order to achieve physiological arterial BP values, noradrenaline infusion-rates of 0.2 mg/hours in the sitting and 0.8 mg/h in the standing position were needed. With the pump running, tilt-table testing resulted in the absence of BP drop and of syncopy, in mild elevation of HR (from 60 to 85bpm), in supraphysiological plasma noradrenaline levels, and in near-normalized ADH-values. 24-hour BP and HR-patterns were near-physiological.

All other medications could be discontinued, and supine hypertension disappeared. No more syncopies occurred, the patient regained complete independence and resumed professional activities without limitations. Transient mild chest tightness without ECG-changes was noted at the beginning of each activation of the pump.

Conclusions: Near normal physiological conditions, and functional and professional independence were re-established in a patient with disabling OH thanks to an ambulatory noradrenaline pump.

**P276**

**Andersen syndrome: a case report.** R. Gürer, N. Isik, A. Alpay, R. Kaval, D. Gökce, E. Akyüz, SSK Goztepe Educational Hospital (Istanbul, TR)

Andersen syndrome is a rare autosomal dominant potassium level disorder with prominent dysmorphic features and skeletal and cardiac involvement. We report the physical, radiological and biochemical findings of a patient diagnosed as Andersen syndrome.

A 45 year-old man with periodic muscular weakness since age 38 is presented.The episodes were of variable duration, from one day to three days, and were either spontaneous or induced by physical exercise or by stress. His initial evaluation showed mild proximal weakness with no clinical or electrical myotonia. Tendon reflexes were decreased, sensation was normal. He had no cardiac symptoms and the family history was negative. The serum potassium level was 2.7 mEq/lt. He showed dysmorphic features with short stature, a hypoplastic mandible, hypertelorism, low set ears, micrognathia, bilaterally short index fingers, broad forehead. His three children had almost the same dysmorphic features. His father died of heart failure at age 36. Dysmorphic features were recognized from his photograph.

His brain and spinal cord magnetic resonance imaging were unremarkable. EMG of the limb muscles showed no evidence of myotonia. His muscle biopsy from deltoid muscle showed mild myopathic findings with chronic denervation. His cardiological examinations including echocardiography, holter monitoring and electrocardiography were normal. Hypokalemic challenge test with intravenous glucose (2 mg/kg) followed 30 minutes later by insulin (0.1 unit/kg intravenously) resulted in no change in muscle strength despite a drop in serum potassium from 4.3 to 3.7 mEq/lt. Potassium challenge was not performed, because of danger of fatal cardiac complications. Acetazolamide was given to the patient, and no attacks were seen.

Recognition of the characteristic face in Andersen Syndrome permits an early diagnosis and the detection of the severe systemic manifestations associated with the syndrome.

**P277**

**Man in the barrel syndrome caused by cervical spinal cord lesion: two cases.** M. Martínez Ginés, M. Rodriguez Yañez, B. Castaño Garcia, A. Esquivel López, A. Gil Nuñez, C. De Andrés, Hospital Gregorio Marañón (Madrid, E)

Background: Acute brachial diplegia with normal findings of the legs, "man-in-the barrel" (MIB) syndrome, is generally thought to be caused by bilateral supratentorial brain lesions of the prerolandic cortical and subcortical area. Thus, MIB syndrome caused by a cervical spinal cord lesion is an unusual presentation.

Objective: To describe two patients with MIB syndrome due to different cervical spinal cord lesions: infarction and spondylitic compressive myelopathy.

Material and methods: Two cases report:

*Case 1:* A 24-year-old woman without any previous relevant medical history was admitted because of acute diplegia of the arms after feeling a severe cervical pain irradiating to both shoulders. On examination there was no paresis of the legs which showed normal muscle tone. Deep tendon reflexes of both arms were absent with the presence of a cervical C2 sensory level for pain and temperature, and a bladder retention.

The following serological tests were negative: lues, borrelia, brucella, Mycoplasma pneumoniae, HIV and varicella zoster. The cerebral spinal fluid (CSF) and the cervical-thoracic CT-scan were normal. A spinal angioMagnetic Resonance Imaging (MRI) demonstrated an anterior hyperintense lesion and moderate swelling of the cervical spine expanding from C3 to D1, like an anterior spinal artery infarction.

*Case 2:* A 72-year-old man with larynx cancer in 1995 that was treated with chemotherapy and radiation therapy in partial remission without surgical treatment. He was admitted because of acute diplegia of the arms after feeling a severe neck pain irradiating to both shoulders. On examination there was no paresis of the legs, all sensory modalities were normal in arms and legs. Bladder and bowel functions were normal, too. The serological tests were normal or negative excluding vaculitis and infection. The cerebral spinal fluid was negative. A cervical spinal MRI showed rachistenosis from C2 to C5 and spondylolisthesis C3-C4 with severe stenosis and signal change in the cervical spinal cord at that level.

Conclusion: In brachial diplegia with normal supracervical findings a cervical spinal cord lesion like that needs to be taken into consideration in emergency service.

**P278**

**Ataxia with isolated vitamin E deficiency: case report.** E. Karakoc, E. Demirci, S. Erdem, G. Nurlu, K. Varli, Hacettepe University (Ankara, TR)

Ataxia with isolated vitamin E deficiency (AVED) is an autosomal recessive neurodegenerative disorder associated with a phenotype similar to "mutation negative" Friedreich's ataxia. As replacement therapy may arrest deterioration or improve clinical findings, vitamin E deficiency should be excluded in all patients with progressive ataxia of unknown etiology. A 24-year-old male patient with diagnosis of AVED, but with no relevant family history, no parental consanguinity, and no gastrointestinal symptoms is presented in this report. On clinical examination, he had thoracic and lumbar rotoscoliosis, foot deformity, dysarthria, gait and limb ataxia, impaired joint position and vibration sense in all four limbs, and bilateral Babinski sign. Mild distal paresis in all extremities, dystonic posture in the neck, and amyotrophy in lower limbs were also noted. He fulfilled Harding's clinical criteria for diagnosis of Friedreich's ataxia with the exception of retained tendon reflexes and sensory action potentials that were close to the lower limits of normal. Somatosensory and motor evoked potentials were significantly involved in spinal cord. Sensory nerve biopsy was also performed. Although AVED is frequently reported in Mediterranean region, this is the first detailed international report from Turkey. We consider that increasing awareness of this clinical entity and usage of improved diagnostic tools, providing earlier recognition and treatment, will lead to a better outcome.

**P279**

**The role of MRI in early recognition of Wernicke's encephalopathy.** B. Petrovic, L. Hadnadjev, D. Kozic, A. Jovanovic, D. Hadnadjev, Institute of Oncology, Clinical Center, Institute of Neurology (Sremska Kamenica, Novi Sad, YU)

Purpose: Wernicke's encephalopathy (WE) is most frequently seen as severe complication of chronic alcoholism. The purpose of this report is to present the role of magnetic resonance imaging (MRI) for early recognition of WE in a patient in whom this disorder was induced by total parenteral nutrition.

Methods: Prompt MRI of the brain was indicated in a patient with a clinical history of acute pancreatitis and total parenteral nutrition who presented with relatively sudden and markedly progressive confusion and ophthalmoplegia.

Results: Symmetrical lesions were seen in medial thalami, along the walls of the third ventricle and within the periaqueductal region of the midbrain. Asymmetrical enlargement of the mammillary bodies with postcontrast enhancement was evident. Partial enhancement of the inferior colliculi was also noted. After thiamine therapy complete neurological recovery and regression of MR abnormalities were obvious.

Conclusion: Since bilateral basal ganglia involvement may be revealed in a wide spectrum of disorders, postcontrast enhancement of mammillary bodies can be a very important distinguishing sign, necessary for early recognition of WE on MR examination, that enables prompt treatment and better prognosis.

P280

**Immigrant population in Spanish neurological ambulatory assistance.** A. Miralles, E. Díez Tejedor, P. Barreiro, Hospital La Paz (Madrid, E)

Background: Immigration is a growing phenomenon in Spain and it increases sanitary assistance demand. We propose to evaluate the weight of the immigrant population in neurological ambulatory assistance, and their pathologies compared with Spanish population.

Material - Methods: Descriptive and prospective study of first visits attended in neurological ambulatory assistance during 3 consecutive months. We analyzed demographic variables (age, sex, nationality, stay time in Spain) and the different diagnostic reached, defined in the CIE-9 classification.

Results: Immigrant community older than 14 years comparises 4,2 % of the population of our sanitary area. We attended 709 patient: 645 (91 %) were Spanish (68 % women, mean age of 50,5 years); and 64 (9 %) immigrants (75 % women, mean age of 36,8 years). The mean stay in Spain was 75 months. The most frequent origin was Ecuador (28 %) and Colombia (22 %). The most prevalent pathologies in Spanish group were headache (31,6 %), syncope, neuropathies, cognitive disorders and cerebrovascular disease; and in immigrant group were headaches (53,1 %), epilepsy, neuropathies, traumatic brain injury and spondyloarthropathy.

Conclusions: Immigrants are 9 % of ambulatory neurological assistance. Neurological assistance demand is higher than expected for their demographic weight. The most frequent origin is South America. Headache is the most prevalent pathology in both populations. Immigrant population is younger, and there is a lower prevalence of advanced ages pathologies. These data should be considered in the organization of sanitary assistance.

P281

**Chondroitin and heparan sulfotransferases in the injured CNS: their role in the formation of the glial CCAR and in the inhibition of axon regrowth.** F. Properzi, R. A. Asher, L. M. Camargo, J. W. Fawcett, University of Cambridge (Cambridge, UK)

The CNS response to injury ends in the formation of the glial scar, an inhibitory substrate for axon regrowth. Both CSPGs and HSPGs are upregulated during the scarring process and CSPGs sugar chains are primarily involved in neuronal growth inhibition. We show that chondroitin (CSSTs) and heparan (HSSTs) sulfotransferase expression is differently regulated in the adult rat CNS after injury and in the different cell types involved in the scarring process. Semiquantitative RT-PCR showed that HS2ST, C6ST-1 and UST mRNAs are selectively up-regulated after a cortical stab lesion, whereas the other HSST and CSST mRNAs remain unchanged. On Neu7 astrocytes, a non-permissive substrate for axon growth, due to the production of inhibitory CSPGs, all the CSST mRNAs are expressed at high levels, as well as in oligodendrocytes precursors, an important cellular component of the glial scar. Interestingly, in A7 astrocytes, which are not inhibitory for axon elongation C6ST-1 mRNA is not detectable. These data suggest that CSPG sulfation pattern could play a role in the inhibition of axon regrowth in the CNS and that HSST expression can also have a role in the scarring process.

## Genetics

P283

**Genetic heterogeneity of intracranial aneurysms: elastin polymorphism haplotype and intracranial aneurysms are not associated in Central Europe.** A. Hofer, M. Hermans, N. Kubassek, M. Sitzer, H. Funke, F. Stögbauer, S. Ozkan, J. Oldenburg, A. Raabe, H. Steinmetz, G. Auburger, University of Frankfurt, Westfalische Wilhelms University Munster (Frankfurt, Munster, D)

Background and Purpose: The most important congenital predisposition leading to subarachnoid hemorrhage manifests in intracranial aneurysms. Recent genomic studies in Japan have achieved the probable localization of one IA gene close to the candidate gene elastin and have defined one haplotype of elastin polymorphisms as an important risk factor, both in affected sib pairs and sporadic patients (Onda et al. 2001).

Methods: The aim of this study was to test the relevance of this association in a Central European Sample. Therefore we have genotyped two single nucleotide polymorphisms in the elastin gene by fluorescent sequencing, dHPLC and MS-PCR technology and evaluated their allelic association with intracranial aneurysm in 30 familial and 175 sporadic patients, as well as 235 population controls.

Results: The genotyping data did not generate a significant allelic association between this elastin polymorphism haplotype and intracranial aneurysm.

Conclusion: Our data probably reflect the genetic heterogeneity of intracranial aneurysm.

P284

**Association study of GSK3-beta and CDK5 polymorphisms in progressive supranuclear palsy.** J. Campdelacreu, M. Ezquerra, E. Muñoz, E. Tolosa, Hospital Clinic (Barcelona, E)

Background: The extended tau haplotype H1 has previously been described associated to progressive supranuclear palsy (PSP) and is so far the only known genetic risk factor for this disease. However, it is neither necessary nor sufficient to cause the disease. Abnormal tau hyperphosphorylation is a key feature of PSP and other tauopathies. Therefore, kinases that phosphorylate tau, such as glycogen synthase kinase 3 beta (GSK3-beta) and cyclin-dependent kinase 5 (CDK5), could play a role in PSP pathophysiology. GSK3-beta is increased in tau sarcosyl-insoluble fractions, and its active phosphorylated form is expressed in tau-containing lesions in PSP and other tauopathies. CDK5 is overexpressed in affected brain regions in PSP, especially in neurons with tau accumulation.

Objectives: To investigate whether single nucleotide polymorphisms (SNPs) of GSK3-beta and CDK5 genes behave as genetic risk factors for PSP.

Methods: 76 PSP patients and 116 healthy controls were included. Genomic DNA was extracted from peripheral blood using standard procedures. The genetic analysis of the GSK3-beta polymorphism -50T/C located in the promoter region was carried out through PCR amplification and subsequent endonuclease restriction with AluI as previously described. The SNP rs2069459 (NCBI) of CDK5 was also genotyped through enzyme restriction with PvuII.

Results: We found that the TT genotype of the GSK3-beta -50T/C polymorphism was overrepresented in PSP patients compared to controls (48.7 % in PSP patients vs 30.2 % in controls; chi-square, $p = 0.0086$). The frequency of allele T was also significantly increased in patients compared to controls (70.4 % in PSP patients vs 57.3 % in controls; chi-square, $p = 0.009$). No significant differences between patients and controls were observed for the CDK5 polymorphism.

Conclusions: Variations in the promoter region of the GSK3beta gene could represent a genetic risk factor for PSP. Further association studies should be performed in other populations to confirm our results. Moreover, functional studies are necessary to elucidate whether the -50T/C polymorphism has any effect on gene expression.

P285

**Study of autosomal recessive spastic ataxia of Charlevoix-Saguenay in southern Italy.** C. Criscuolo, M. Orio, G. De Michele, V. Scarano, M. Carella, L. Santoro, P. Gasparini, S. Banfi, A. Filla, University "Federico II", Tigem (Naples, I)

Objective: To study occurrence of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) in a Southern Italian population.

Background: Early onset cerebellar ataxia with retained tendon reflexes (EOCA) is clinically and genetically heterogeneous. A form of early

onset autosomal recessive spastic ataxia has been described, with high prevalence, in Charlevoix-Saguenay area in Quebec. Features include ataxia, dysarthria, spasticity, nystagmus, retinal striation and absence of peripheral nerve sensory potentials. The gene responsible for ARSACS maps to chromosome 13q11. SACS gene encodes the protein sacsin and presents only one gigantic exon spanning 12,794 bp. This exon is the largest identified in any vertebrate organism. Two causal mutations have been described: g.6594 delT resulting in a frameshift with introduction of a stop codon, reported in 96% of the patients, and g.5254 C® T a nonsense mutation. Recently, one Tunisian and two Turkish families have been reported linked to 13q11.

Methods: Twenty-three patients with an ARSACS-like phenotype were recruited from a series of 85 EOCA patients. Linkage analysis using fluorescent microsatellites spanning SACS gene was performed in the nine patients born from consaguineous marriages. Screening for the known mutations in the SACS gene was performed in the remaining patients by denaturing high performance liquid chromatography (dHPLC).

Results: Cerebellar ataxia with dysarthria was present in all patients. Thirteen showed pyramidal signs (brisk reflexes, increased tone and/or extensor plantar response). Clinical and neurophysiological signs of peripheral neuropathy were present in all but four patients. Known SACS gene mutations were excluded in all patients. Two affected sisters from a consanguineous marriage showed homozigosity for all tested markers suggesting linkage to ARSACS locus. g.6594T and g.5254C mutations were not identified in these two patients.

Conclusion: Failure to detect g.6594 delT and g.5254 C® T mutations in our series suggests that these two mutations do not play a significant pathogenetic role in our EOCA sample. The linkage to 13q11 and the exclusion of the two known mutations in one family suggest the presence of at least one additional mutation in SACS gene.

P286

**Clinical profile and genotype-phenotype correlations in Spanish dominant ataxia families.** J. Infante, O. Combarros, V. Volpini, J. Corral, J. Llorca, J. Berciano, University Hospital Marqués de Valdecilla, IRO, University of Cantabria (Santander, Barcelona, E)

Background: Clinical and molecular profile of dominant ataxias (SCAs) is heterogeneous in different populations.

Objective: To analyze the clinical profile of SCAs in our environment and the influence of CAG repeat length on phenotypic variability.

Patients and Method: The study included 30 families (One SCA 1, nine SCA 2, six SCA 3, three SCA 7, one SCA 8, one DRPLA and nine in which no mutation could be identified). Seventy-three patients were examined and disability and severity scales were applied. Regression analysis was carried out to study the relationship between CAG repeat length and age at onset per SCA gene.

Results: Some constellations of clinical signs were highly suggestive of certain SCA forms: areflexia, slow saccades and hypopallesthesia in SCA 2; nystagmus, pyramidal signs or areflexia restricted to legs in SCA 3; and retinal degeneration, pyramidal signs and slow saccades in SCA 7. The degree of intergenerational instability of the CAG repeat and the anticipation phenomenon was greater in SCA 7 (mean increase 10.3 ± 17.6 CAG repeats and mean anticipation 29.7 ± 3.5 years). There was an inverse relationship between length of expanded allele and age at onset in all SCA subtypes (SCA 2: $r^2 = 0.70$; SCA 3: $r^2 = 0.22$; SCA 7: $r^2 = 0.79$). Larger expansions correlated with areflexia in SCA 2, with pyramidal signs in SCA 3 and with early visual impairment in SCA 7. Disease severity also correlated with expansion length.

Conclusions: Clinical guides may be useful in rationalizing the order of genetic loci to investigate. CAG repeat length determines not only age at onset but also certain clinical features in each form of SCA.

P287

**Change of secondary structure of the tRNA(Asn) anticodon loop due to a mitochondrial point mutation associated with CPEO.** J. Schmiedel, H. Reichmann, Technical University Dresden (Dresden, D)

Background and Purpose: Point mutations within mitochondrial tRNA genes are an important cause of mitochondrial diseases. We have previously described one such mutation, located at nucleotide position 5692 within the anticodon loop of tRNA(Asn) and associated with the clinical phenotype of chronic progressive external ophthalmoplegia (CPEO). Here we provide evidence that the mutation leads to changes in the secondary structure of the anticodon loop, due to formation of an additional base pair.

Methods and Results: A suitable model that would display characteristics of the original wildtype tRNA structure was established. Both anticodon domains were represented by two oligonucleotides only differing at position 5692, resulting in either a wildtype sequence or mutant sequence. Evaluation of secondary structure formation of both oligonucleotides was done by hybridization with a short molecule (wildtype-oligo) which was complementary to the wildtype-sequence and could form base pairs with the single stranded nucleotides of the anticodon loops. The wildtype anticodon loop, consisting of seven unmatched nucleotides would allow the formation of six base pairs with the wildtype-oligo. In contrast, a structural change, resulting in a small five-base loop would decrease the possibility of hydrogen-bond formation with the wildtype-oligo. We found significantly stronger hybridization signal intensities with different amounts of wildtype-loop than with mutant-loop.

Conclusions: Here we provide evidence that the tRNA(Asn) mutation at nucleotide pair 5692 leads to changes in the secondary structure of the anticodon loop, due to formation of an additional base pair associated with a change of secondary structure in the anticodon loop. Since this region represents one primary element for tRNA identity, alterations could cause disturbances of the tRNA-functionality.

P288

**Genetic analysis of Parkinson's disease in Crete, Greece.** C. Spanaki, A. Plaitakis, University of Crete (Heraklion, GR)

Although a growing number of genetic loci have been linked to familial forms of PD, the role of genetic factors in the primary or idiopathic type of the disorder, remains uncertain. A genetic analysis was performed on late-onset, L-dopa responsive PD that occurs on the island of Crete. Four multigenerational PD kindreds were selected for these analyses. Microsatellite markers for the chromosomal loci: PARK1 (4q23, á-synuclein gene), PARK2 (6q25.2, parkin gene), PARK3 (2p13), PARK4 (4p16), PARK5 (4p14-ubiquitin C terminal hydrolase L1 gene), PARK6 (1p35–36) and PARK7 (1p36) were used for genotypic analysis. Data were recoded for linkage analysis using FASTLINK program under an "affected only" analysis. Marker allele frequencies in the population were based on 50 Cretan healthy natives. Two-point linkage analysis was performed using the MLINK Program and multipoint linkage using the LINKMAP and the VITESSE programs.

The largest pedigree spanned 4 generations and encompassed 287 family members. Twenty eight family members were affected by PD. Of these, 7 were alive at the time of the study and were evaluated personally. The second pedigree spanned 4 generations and encompassed 120 family members. Twelve family members were affected by PD. Eight of them were alive at the time of the study and 6 were evaluated personally. The third pedigree spanned 3 generations and encompassed 78 family members, 5 of whom were affected from PD. Three were alive and were evaluated by the authors. The 4th pedigree spanned 3 generations and included 32 family members. Nine family members were affected by PD. Three out of four PD patients, who were alive, were personally evaluated. Analyses of the family study data revealed that the PD phenotype of these patients, including age at disease onset, was similar to that of the sporadic PD. The mode of genetic transmission appeared to be autosomal dominant with reduced penetrance based on segregation ratios. Genotypic data for PARK-1 (markers: D4S234, D4S423, D4S1647) gave two point Lod Scores varying from −2.57 to 0.65.; for PARK-2, (markers: D6S441, D6S1579, D6S305, D6S264), two point Lod Scores ranged from −2.48 to 0.16; for PARK-3, (markers: D2S441, D2S358, D2S1394, D2S211) two point Lod Scores ranged from −2.91 to 0.4; for PARK-4 (markers: D4S391, D4S3350, D4S230) two point Lod Scores ranged from −1.133 to 0.97; data for PARK-5 (markers D4S405) gave two point Lod Scores that ranged from 0.28 to 1.3; for PARK-6 (markers D1S199, D1S478) two point Lod Scores ranged from −0.8 to 1.02 and data for PARK-7 (markers D1S2663, D1S2845) gave Two Point Lod Scores that varied from 0.03 to 1.1.

Conclusion: The present data did not show linkage of the Cretan PD pedigrees with late-onset, L-dopa responsive PD to any of the 7 chromosomal loci studied. Further genetic analysis of these families may help to elucidate the pathogenesis of familial PD on the island of Crete and, hopefully, of the primary or idiopathic disorder.

P289

**Muscle strength correlates linearly with molecular genotype in myotonic muscular dystrophy (DM1).** G. Galassi, R. Suozzi, M. Leone, A. Percesepe, University Hospital (Modena, I)

Myotonic muscular dystrophy (DM1) is an autosomal dominant disorder with highly variable clinical manifestations and severity. Degree of weakness and wasting may vary among different muscle groups in the same

subject as well as in patients carrying the same length of abnormal CTG repeats. We examined 26 DM1 patients (14 males and 12 females) from 14 unrelated kindreds. Age ranged from 13 to 66 years (mean 42 ± 16). Genetic testing was performed using Southern Blot analysis after enzymatic digestion with ECOR1, BAMH1 and BGL1 of leukocyte DNA. Based on CTG repeat length the 26 patients were divided into three groups: 9 patients (34.6%) presented CTG expansion in the E1 range (50–250 repeats), 8 patients (30.8%) in the E2 range (251–800 repeats) and 9 patients (34.6%) in the E3 range (801–2000 repeats). Mean age of patients at the time of initial clinical diagnosis for the E1 group was 53 years (SD14), for the E2 group 42 years (SD12) and for the E3 group 29.4 years (SD 13.6). All three groups at the time of diagnosis presented involvement of cranial and limb muscles, myotonia, increased serum creatine kinase, typical discharges on electromyography in at least two muscles. The patients were examined along the duration of the study (3 years) by the same neurologists. Severity of muscular involvement in each subject was graded in 17 selected muscles using a 5 point scale (MRC) for a total amount of 442 muscles evaluated every four months. Strength was tested in five compartments: cranial (M1), proximal (M2) and distal (M3) muscles of right upper and proximal (M4) and distal (M5) of right lower limb. Muscle strength of the five compartments (M1, M2, M3, M4, M5) was compared in the three DM1 groups (E1,E2,E3) by means of a pentavariate MANOVA. Patient groups significantly differed in muscle strength ($E:F[10, 38]= 3.8537$ $P < 0.02$). Worsening from E1 to E3 followed a linear trend (Elinear: $F [5, 19]= 10.1917$ $P < 0.0008$; Equadratic: $F[5, 19]< 1$), and resulted to be non homogeneous in the five compartments (Elinear x M: $F[4, 20]= 4.4020$ $P < 0.01$). The E3 patients were significantly more affected in muscle strength than the E2 patients, who were more affected than the E1 patients. Multiple comparisons provided evidence that the muscles significantly sensitive to high number of repeats were the cranial, the proximal and distal muscles of upper and the distal of the lower limb as well as the multiple combinations of the four extremity compartments.

**P290**

**The ACE polymorphism in intron 16 is no vulnerability factor for complex regional pain syndrome type I.** S. Leis, K. Huehne, B. Rautenstrauss, M. Schmelz, B. Neundoerfer, F. Birklein, University Erlangen-Nuremberg, University Heidelberg, University Mainz (Erlangen, Mannheim, Mainz, D)

Background: Exaggerated neurogenic inflammation has been recognized as one reason for many symptoms of complex regional pain syndrome type I (CRPS I). Since angiotensin-converting enzyme (ACE) plays a key role in the termination of neurogenic inflammation, it has been selected as a candidate gene for CRPS predisposition. An insertion/deletion (I/D) polymorphism in intron 16 within the ACE gene was previously described and there is one report of an increased risk to develop CRPS I associated with the D allele.

Methods: Two groups were investigated: 48 non-related CRPS I patients (34 female, 14 male) in group one (sporadic CRPS), and 12 CRPS I patients (11 female, 1 male) from 6 families together with 21 of so far unaffected relatives (13 female, 8 male) in group two (familial CRPS). The ACE I/D polymorphism was determined by PCR amplification after DNA was extracted from peripheral lymphocytes.

Results: The distribution of genotypes in group one was DD: ID: II = 29.2%: 43.7%: 27.1% with a frequency of 0.51 for the D allele and 0.49 for the I allele, what is consistent with the Hardy-Weinberg equilibrium and does not differ from the allele frequency in the normal population. Furthermore, in none of the 6 CRPS I families a co-segregation of any genotype with the CRPS phenotype was found.

Conclusions: Our results render this particular ACE gene polymorphism unlikely to be a single predisposing factor for CRPS I.

**P291**

**Familial spinocerebellar degeneration with progressive myoclonus epilepsy: exclusion of known causes.** G. De Michele, G. Coppola, C. Criscuolo, A. E. Lehesjoki, F. M. Santorelli, F. Saccà, P. Striano, F. Barbieri, A. Filla, University "Federico II" (Naples, I; Helsinki, FIN; Rome, I)

Objective: To describe a Southern Italian family affected by epilepsy, myoclonus, mental deterioration and ataxia, clinically resembling Unverricht-Lundborg disease.

Background: Progressive myoclonus epilepsies are characterized by myoclonus, epilepsy and progressive neurologic deterioration, with cognitive impairment. This group of diseases is genetically heterogeneous. In recent years, responsible genes have been identified for Unverricht-Lundborg and Lafora diseases, some ceroid lipofuscinoses and sialidoses, DRPLA and MERRF.

Design/Methods: We examined a family originating from Southern Italy, with two couples of affected sibs (first cousins).

Complete laboratory testing was performed, including ceruloplasmin, lactate, hexosaminidases A and B, arylsulfatase and urine sialyloligosaccharides. All patients underwent EEG and MRI scan. Peripheral nerve conduction studies, myoclonus assessment and muscle biopsy were performed in two patients, skin biopsy in one.

Blood samples were obtained after informed consent from 4 patients and 9 unaffected family members. Linkage analysis was performed to exclude Unverricht-Lundborg (21q22.3) and Lafora (6q24) diseases. Qualitative and quantitative analysis of mtDNA was performed, and MELAS (A3243G, C3271T), NARP (T8993G, T8993C) and MERRF (A8344G, T8356C) mutations were ruled out.

Results: The clinical picture is characterized by onset between 8 and 12 years, with grand-mal seizures. Myoclonus was present in all patients, and was generalized, present at rest and stimulated by voluntary movements. Mental impairment was moderate, and present in all patients. Ataxia was evident in two patients, and seizures were frequent in one.

Routine laboratory examination, including ceruloplasmin, lactate, hexosaminidases A and B, arylsulfatase and urine sialyloligosaccharides was normal. Anti-glyadin and anti-endomysial antibodies were absent.

EEG showed slow background activity, generalized spike/wave paroxysms and absent photosensitivity. Back-averaging showed the cortical origin of myoclonus. MRI showed cerebellar atrophy in all patients, and peripheral nerve conduction studies were normal in two examined patients. Muscle biopsy showed normal morphology and biochemistry, with absent ragged red fibers. No Lafora bodies or 'fingerprint' inclusions were present at skin biopsy.

Genetic analysis excluded linkage to 21q and 6q. mtDNA analysis showed normal results, and absence of MELAS, NARP and MERRF mutations.

Conclusions: We report a new family with autosomal recessive spinocerebellar degeneration and a clinical picture resembling Unverricht-Lundborg disease. We excluded in this family known causes of progressive myoclonus epilepsy. This finding confirms the genetic heterogeneity in this group of diseases.

**P292**

**Congenital glaucoma and vanishing white matter disease: common aetiology or coincidence?** D. Pugin, A. Fogli, A. Bottani, C. Korff, J. Delavelle, O. Boespflug-Tanguy, Hôpital Cantonal Genève, Inserm U 384 (Geneva, CH; Clermont-Ferrand, F)

Congenital glaucoma (CG) is a rare disease (1/12,500 live births). It is most often sporadic, but familial cases are associated with newly isolated mutations of the genes GLC3A and GLC3B, respectively located in 2p21 and 1p36. Cerebellar ataxia with central hypomyelination (CACH), an autosomal recessive leukodystrophy also called vanishing white matter disease (VWM), is also a very rare disorder. Its age of onset and course are variable, ranging from rapidly fatal infantile forms to a slowly progressive adult-onset course. Fever and minor head trauma commonly lead to rapid neurological deterioration. Even in the presymptomatic stage, brain MRI is diagnostic, revealing diffuse abnormality of the cerebral white matter with progressive cavitations, without contrast enhancement or cortical atrophy. Recently, several mutations have been found in each of the 5 subunits of the translation initiation factor eIF2B, principally in the epsilon (3q27) and beta (14q24) subunits. Despite the ubiquitous expression of eIF2B, CACH/VWM apparently affects only the central nervous system.

We describe a 23-year-old woman with the peculiar association of CG and CACH/VWM. The congenital glaucoma was treated surgically twice before the age of 2 years. From the age of 3 years, the patient suffered from episodes of neurological deficits, often triggered by fever or trauma. Recovery was usually incomplete. Her neurological status slowly deteriorated over the years, leaving her with cerebellar ataxia, spasticity, mild mental decline, and dysarthria. She was admitted in a prolonged focal epileptic state with severe global aphasia and right-sided hemiplegia. Benzodiazepines worsened her seizures, while levetiracetam and valproate stopped them. She improved to her pre-seizure state over 3 weeks. Diagnosis of CACH/VWM was made on the basis of serial MRIs showing increasing zones of white matter cavitations over 10 years, and was confirmed by genetic analysis which identified a homozygous R113H mutation in the epsilon subunit of eIF2B.

The association of two rare diseases in the same patient suggests a possible etiological link between these conditions. Detailed ophthalmological examination of CACH/VWM patients will be necessary to determine the existence of a true involvement of the anterior segment of the eye in CACH/VWM or, alternatively, of a purely coincidental association such as seen in our patient.

## Multiple sclerosis

P293

**Long-term effects of intravenous high dose methylprednisolone pulses on bone mineral density in patients with multiple sclerosis.** M. Zorzon, R. Zivadinov, L. Locatelli, D. Giuntini, M. Toncic, A. Bosco, D. Nasuelli, A. Bratina, M. A. Tommasi, R. A. Rudick, G. Cazzato, Neurological Clinic (Trieste, I)

Background: There are some suggestions for a role of cyclical pulses of high-dose methylprednisolone (HDMP) as disease modifying therapy in both relapsing-remitting (RR) and secondary progressive (SP) multiple sclerosis (MS), but there are concerns about long-term adverse effects, in particular drug-induced osteoporosis.

Objectives: To determine the effects of HDMP pulses on bone mineral density (BMD) and bone turnover in patients with multiple sclerosis (MS).

Methods: We studied 25 MS patients who received regular pulses of HDMP as well as pulses of HDMP for relapses, 18 MS patients who received HDMP at the same dose schedule only for relapses, and 61 healthy controls. Median follow-up of patients was 8 years. We measured BMDs at lumbar spine, femoral neck and calcaneus. Additionally, we measured biochemical markers of bone metabolism and turnover. The average lifetime dosage of MP was 75.4 (SD 11.9) g in the pulsed HDMP group and 28.6 (SD 18.3) g in the HDMP for relapses group ($p < 0.0001$).

Results: Two MS patients (4.7%) and four controls (6.6%) had osteoporosis ($p = NS$), whereas 25 patients with MS (58.1%) and 21 controls (34.4%) had osteopenia ($p = 0.016$). BMDs measured at lumbar spine and femoral neck did not differ in MS patients and controls, whereas at calcaneus a marginally significant decrease in MS patients was found. Biochemical indices of bone turnover did not differ significantly in MS patients and controls. BMD and biochemical indices of bone turnover were not associated with lifetime methylprednisolone dosage. Controlling for age, gender, menopausal status, and disability in multivariate analysis did not change the results. BMD at femoral neck correlated significantly with EDSS score ($r = -0.31$, $p = 0.05$).

Conclusions: Treatment with repeated HDMP pulses was not associated with osteoporosis in patients with MS who participated in a trial of methylprednisolone. However, osteopenia was observed more frequently in MS patients than healthy controls. Although multiple HDMP pulses should not be avoided owing to concerns about osteoporosis, the risk of osteopenia is not negligible and recommends a strict surveillance of BMD. The data suggest that decreased mobility, as measured by EDSS, may contribute to bone loss more than corticosteroid use.

P294

**NAWM myoinositol at presentation as a potential predictor of CDMS; preliminary results.** K. T. M. Fernando, C. M. Dalton, D. T. Chard, S. M. Leary, K. M. Miszkiel, D. Altmann, M. A. McLean, D. G. MacManus, G. T. Plant, A. J. Thompson, D. H. Miller, Institute of Neurology for the NMR Research Unit (London, UK)

We have observed that myoinositol (Ins) is significantly raised in the normal appearing white matter (NAWM) of patients presenting with clinically isolated syndromes (CIS) suggestive of Multiple Sclerosis (MS). The aim of this study was to assess the predictive value of NAWM Ins in determining conversion to clinically definite MS (CDMS).

Methods: Forty eight patients presenting with CIS were followed up for a period of 3 years as part of a longitudinal study using multi parameter MRI techniques. All 48 patients had a baseline MRI study of the brain and cord within 12 weeks of presentation (mean 5.81 weeks). They then underwent a combined MRI/MRS study of the brain 12 weeks after the baseline scan (mean 13.2 weeks). 44 healthy controls were also studied. A single voxel spectrum was acquired from NAWM in the posterior parietal/centrum semi-ovale region. The MRS acquisition was a PRESS sequence with a TR 3000ms, TE 30ms and 192 averages. Automatic shimming and water suppression was adhered using the PROBE proprietary software (GE medical systems). The spectra were fitted with the full set of metabolites thought to be present at short TE. Concentrations of the following were estimated using the LC Model; N-acetyl aspartate (NAA), total NAA, Creatine, Choline and Ins. Differences between patients and controls were assessed by multiple regression of metabolite concentrations on a binary disease status variable and age and gender covariates to control for potential age/gender confounding.

Results: Thirty eight of the CIS patients presented with optic neuritis, 6 with brainstem syndromes and 4 with spinal cord syndromes. The median age was 33 years (range 18–50). The median EDSS was 1 (range 0–6). The median age for the controls was 38 years (range 22–62). 22/48 of the patients developed CDMS at 3 years as defined by the Poser criteria. The concentration of Ins was significantly raised in the NAWM of the CIS patients who developed CDMS at 3 years compared with the control group (mean 4.65 mM, standard deviation (SD) 1.40 vs 3.89 mM, SD 1.03, $p = 0.003$) and compared with the CIS patients who did not develop CDMS at 3 years (mean 3.64 mM, SD 1.12, $p = 0.002$).

Conclusions: This study suggests that NAWM Ins at presentation has potential to predict the likelihood of developing CDMS in patients presenting with CIS. Larger studies are needed however to confirm these preliminary results.

P295

**Clinical, radiological and cerebrospinal fluid findings of acute disseminated encephalomyelitis in children and adults.** M. Eraksoy, M. Kurtuncu, Z. Yapici, F. Bilgili, G. Akman-Demir, H. Ozcan, Istanbul University (Istanbul, TR)

Acute disseminated encephalomyelitis (ADEM) is a rare inflammatory demyelinating disease of the central nervous system affecting predominantly children and adolescents. The long-term risk of patients with ADEM for development of MS has been reported as 25%.

This study was designed to reveal the clinical, radiological and cerebrospinal fluid (CSF) findings of ADEM in children and adolescents and to establish the risk for development of MS.

Criteria for the diagnosis of ADEM in this study were history of preceding infections or vaccinations; association with constitutional symptoms; prominence of cortical signs as mental disturbances and seizures; CSF, EEG, EMG and MRI findings. Response to a standardized treatment during acute phase of disease was analysed by chart review and re-evaluated of the patients in December 2002. Twenty-six patients who were managed at our department between 1982 and 2002, fulfilled the diagnostic criteria of ADEM.

There were 17 women/girls and 9 men/boys with mean age at onset 21.5 years (range 1.5 to 50). In 10 patients, clinical features presented between 1.5 and 15 years of age, on the other hand, in the rest of the patients, initial manifestations were seen between 16 and 50 years. Upper respiratory tract infection occurred in 10 patients 1–2 months before the ADEM. The commonest clinical manifestations were mental status changes ($n = 8$), cranial nerve involvements with focal motor deficits ($n = 6$); sensory-motor hemiparesis/paraparesis ($n = 5$); right hemiparesis with dysphasia ($n = 3$); simultaneous brain stem and spinal cord involvement ($n = 2$); simultaneous bilateral optic neuritis ($n = 1$) and pure dysphasia ($n = 1$). Two children developed seizures at the initial phase and during the follow-up.

Mean duration of disease was 7.8 years and mean duration of follow-up was 6.7 years. Three patients died within 3 months of the disease and necropsy was performed in two. Two patients spontaneously improved within 6 months, the rest of the patients recovered completely ($n = 18$) or incompletely ($n = 3$) with therapy.

Brain MRI findings fulfilled the Poser and co-workers radiological criteria in 3 patients, the rest of the patients had white matter abnormalities. Of 26, 12 patients had mild pleocytosis and slightly increased protein content in CSF. In 20 out of 21 patients, oligoclonal bands were negative.

Final diagnoses were monophasic ADEM ($n = 18$); relapsing ADEM ($n = 7$); relapsing-remitting MS ($n = 1$).

In conclusion, although there are no reliable diagnostic criteria for ADEM, mental changes, seizures, CSF, MRI, EEG and EMG findings would be helpful for differential diagnosis of MS.

P296

**Prevalence of multiple sclerosis in Genoa province, Italy.** C. Solaro, C. Alemanni, L. Berardinelli, G. L. Mancardi, M. A. Battaglia, Ospedale P. A. Micone, University of Pavia, University of Genoa, University of Siena (Genoa, Pavia, Siena, I)

Objective: The objective was to assess the incidence and prevalence of multiple sclerosis (MS) in a large Italian area the Genova province (north-west Italy).

Background: Several epidemiological surveys carried out in Italy in the last ten years identified Sardinia as a very high risk area (prevalence rate 140/100.000) and north Italy as high risk area (70/100.000) but no studies are available in north-west areas.

Methods: The territory is mostly on the sea but also a hilly and mountainous area is present expanding to the inland. The city of Genova is one of the main Mediterranean ports and is an important industrial and commercial centre. The percentage of birth is one of the lower of Italy and indicates a remarkable aging of the population. The province in divided in 76 "communes". The health care system is equally distibuted in the studied area: 6 neurological departments are present. Moreover in Genova the headquarter of MS society is located. Ms cases were identified as follow-

ing: archives of the hospitals in which a neurological or a rehabilitation ward is present, neurologists serving the community, files of Italian MS society branch, all requests for oligoclonal bands analysis on CSF in the studied area. All the family doctors were contacted by a letter.

The prevalence day was December 31 1997; patients included in the study were MS cases diagnosed before December 31 1997 according to the Poser criteria resident in Genova province.

If the subject agreed to be interviewed, he/she was examined directly by a neurologist expert in MS and by a social worker in every case if it was possible. Patients evaluation consist in EDSS score and FIM.

Results: A total of 856 were alive and resident in Genova province on prevalence day, 31 December 1997. The crude prevalence rate was 94/100,000; 291 were male (33.9%) and 565 were female (66.1%); mean disease duration mean was 15 years (SD 10.2) for male and 15.9 years (SD 11.3) for female. 506 subjects were directly interviewed: 54% had relapsing-remitting course, 31% had secondary progressive and 15% primary progressive. The EDSS was less than 3.5 in 42% of subject and greater than 7 in 20%.

Conclusion: We confirmed that north Italy is a very high risk area for MS. We found a high rate of patients with a primary progressive course; also the figure of patients with elevated disability score is higher compared to previous studies.

P297

**High-dose high frequency interferon beta-1b treatment is effective in early stage relapsing-remitting multiple sclerosis.** R. Gold, University of Wurzburg – The IFNB Multiple Sclerosis Study Group and the UBC MS/MRI Analysis Group

Interferon beta-1b (Betaferon(R)/Betaseron(R)) is an effective treatment for relapsing-remitting (RR) and secondary progressive MS. However, the efficacy of high dose, high frequency interferon beta therapy in patients with low levels of disability or early stage RRMS has yet to be fully explored. To evaluate the efficacy of interferon beta-1b in early or mild RRMS we performed a post-hoc subgroup analysis of the data from the original pivotal trial of interferon beta-1b (50 and 250 mcg) versus placebo in RRMS.

Two subgroups were analysed, one with low levels of disability (EDSS $\leq 2$) the other with disease duration of $\leq 2$ years. In patients with low levels of disability (placebo 43, low dose 49, high dose 41), high dose interferon beta-1b produced significant reductions in mean relapse rate versus placebo (1.0 vs 1.5, $p = 0.013$) and a significant decrease in MRI disease activity as measured by the mean percentage change in lesion area at 2 years (20.1% vs 54.1%, $p = 0.0001$). There were also dose-dependent trends for the median time to first relapse (370 vs 146 days) and the proportion of patients remaining relapse free (31.7% vs 14%) although neither was significant.

In patients with a disease duration of $\leq 2$ years (placebo 48, low dose 48, high dose 38), high dose interferon beta-1b treatment significantly reduced MRI disease activity, compared with placebo, as assessed by mean percentage change in lesion area (9% vs 52.5%, $p = 0.0001$). There were also dose-dependent trends in the proportion of patients remaining relapse free, median time to first relapse and mean relapse rate, although none of these achieved significance.

The data indicate that 250 mcg interferon beta-1b administered every other day is effective in patients with either early stage RRMS or little physical impairment, reinforcing the rationale of early high dose, high frequency treatment intervention. Further controlled studies would be useful to confirm these findings, and the BENEFIT (Betaferon(R)/Betaseron(R) in Newly Emerging MS for Initial Treatment) study has recently been initiated to address this issue.

P298

**Brain atrophy in relapsing-remitting multiple sclerosis: the scope of treatments.** W. Cendrowski, Neurological Out-Patients Clinic (Warsaw, PL)

Background: Brain atrophy (BA) is important early feature of white matter pathology in relapsing-remitting multiple sclerosis (RR MS). Recent therapeutic trials provide opportunity to measure by MRI methods the changes of BA in RR MS patients.

Objective: To compare the effects of immunomodulating drugs on BA in clinically isolated syndromes (CIS), suspected MS (SMS) and RR MS.

Patients and method: Eight cohorts of CIS, SMS and RR MS patients investigated on T1-weighted MRI scans were included in this comparison. BA was measured by change in brain volume (BV, WBV, NBV, BCV) and brain parenchymal volume or fraction (BPV, BPF). Baseline MRI scans prior to the treatment were compared with endpoint scans after cessation of therapy. Patients were treated either with Rebif® (22 µg or 132 µg s. c. per week), Betaferon® (250 µg s. c. every other day), Avonex® (30 µg i. m. per week), Copaxone (20 mg s. c. daily), methylprednisolone (MP i. v. 67.6 g per whole course) or placebo. The trials lasted from 9 months to 5 years.

Results: WBV continued to decrease despite Rebif® treatment in 154 CIS patients ($p = ns$). Nevertheless, BA measured by NBV in subgroup of 131 SMS patients treated with Rebif® significantly improved ($p = 0.003$). Sixty-eight RR MS patients with BA benefited in one controlled study of Avonex® and 30 RR MS patients in another uncontrolled study of Betaferon® ($p = 0.03$ and $p = 0.008$). Regular high-dose pulses of MP i. v. reduced change in BPV (diminished BA progression) in 44 RR MS patients ($p = 0.003$). Whereas 9-month trial of Copaxone® failed to alter favourably BV in 113 RR MS patients, the 2-year study of this drug slowed BA course in 14 RR MS patients ($p = 0.003$).

Conclusion: Progression of BA was modestly slowed by IFN beta, methylprednisolone and glatiramer acetate in RR MS patients.

P299

**Real time RT-PCR determination of mRNA levels of cytokine, chemokine and chemokine receptors in PBLs from MS patients: clinical and MRI correlations.** R. Furlan, M. Rovaris, A. Bergami, F. Martinelli-Boneschi, M. Gironi, A. Kurne, M. Deleidi, F. Agosta, G. Comi, M. Filippi, G. Martino, San Raffaele Scientific Institute, Fondazione Don Gnocchi (Milan, I)

Quantification of mRNA levels of 25 immunological molecules (chemokines: IP-10, Rantes, MCP-1, MIP-1alpha, MDC, Eotaxin, MIP-1beta, MIP-3alpha, I-309, BCA-1; chemokine receptors: CCR5, CCR4, CXCR-5, CCR3, CCR6, CCR7; cytokines: IL-1 alpha, TNF-alpha, TGF-beta, IL-10, IL-4, TNF-beta, IL-1beta, IFN-gamma, IL-5) has been correlated with neurological examination and magnetic resonance imaging (MRI) analysis in 119 patients with definite multiple sclerosis (MS) [49 with relapsing remitting (RR)-MS: 17 in a clinically active phase and 32 in remission, 36 with secondary progressive (SP)-MS, 13 with benign (BB)-MS, and 21 with primary progressive (PP)-MS]. mRNA levels were quantified by real time RT-PCR (Taqman) in peripheral blood lymphocytes obtained at the time of neurological or MRI examination and expressed as arbitrary units (AU). Several significant correlations, of moderate strength, were found. In the whole MS group, we found a positive correlation between the disease progression index and the mRNA level of IL-1beta ($p = 0.044$, Spearman correlation). MS patients with gadolinium (Gd)-enhancing lesions (Gd+) had significantly higher MIP-3 beta mRNA levels compared to Gd–patients ($424.5 \pm 234.8$ AU vs $103.9 \pm 59.3$ AU). RR-MS patients experiencing a clinical relapse had increased levels of TNF-alpha mRNA compared to stable RR-MS patients ($13.4 \pm 5.5$ AU vs $3.0 \pm 0.8$ AU).

Our results suggest that the combined measurement of immunological, clinical and MRI parameters may ultimately lead to the definition of surrogate markers of disease activity and disease progression which might be useful to monitor the disease course in patients with MS.

P300

**Comparison of 3D SPGR and 3D FIESTA at C2 level in the assessment of multiple sclerosis.** M. Kirkegaard, A. D. Blankholm, D. Zeidler, L. Rohl, L. Sorensen, J. Jakobsen, University Hospital of Aarhus (Aarhus, DK)

Purpose: In the assessment of MS reduction of spinal cord area (SC area) has shown correlation with disability measured by EDSS (Kurtzke's Expanded Disability Status Scale) score. The aim was to compare clinical status with two different MRI sequences.

Method: Eight patients with known MS from a larger cross sectional study were scanned with both a 3D FIESTA and a 3D SPGR. The MR-system was a General Electric 1.5 Tesla, Signa Horizon. The cross sectional area of the SC at C2 level was measured by an experienced neuroradiologist (LS) blinded to the patients using a semi-automated software package (ALICE, Hayden Image Processing Solutions, Boulder, Colo., USA). The patients were clinically examined including EDSS within two weeks of the MRI scan by a trained neurologist (MK).

Statistics: Paired t-test was used to detect a possible difference in Signal Noise Ratio (SNR) and in Contrast to Noise Ratio (CNR). Spearman's correlation was used to correlate the SC areas measured on the two sequences and to correlate the SC area on the two sequences to the EDSS scores.

Results: The median age of the patients was 43.5 years (ranging 27–50 years). Three had relapsing remitting MS, 5 had secondary progressive MS. Five of them were women. EDSS ranged from 1.5 to 6 (median 4). Median time from diagnosis was 7.5 years (ranging 2–15 years). The SNR was similar for both sequences and without any significant differences $p = 0.41$. Comparing the CNR for the two sequences; the mean value for FIESTA

(35.83) was three times the value of the SPGR (13.58), however this was not significant p = 0.08 probably due to the limited number of patients. Neither was there any significant difference in the SC area measured with the two different sequences. Using FIESTA the median area was 75.72 (ranging 64.39–94.67) and with SPGR 83.11 (ranging 70.26–101.33).The correlation coefficient for the two sequences with respect to SC area showed strong correlation (r = 0.80 p = 0.01). The EDSS score correlated (r = –0.76 p = 0.03) with the FIESTA sequence, but not with the SPGR sequence.

Conclusion: With respect to estimation of SC area there was no significant difference between the two sequences. There is a significant correlation with EDSS using the FIESTA sequence indicating that SPGR might be replaced by FIESTA for estimation of the SC area. Furthermore, this saves scan time. However further investigation with a larger number of patients is recommended.

P301

**Mx protein and neutralizing interferon antibodies in relapsing remitting multiple sclerosis patients treated with interferon beta-1b: correlation with clinical and MRI efficacy.** A. Ricci, M. A. Cucci, E. Verdun, M. Clerico, A. Pipieri, P. Barbero, L. Durelli, University of Turin (Turin, I)

Background: Recent studies have shown that the efficacy of interferon (IFN) beta in relapsing-remitting multiple sclerosis (RRMS) patients is dose dependent. The bioavailability of IFN beta can be estimated by measuring markers in the blood. Of these, Mx protein has the highest specificity for IFN-beta, and is induced selectively by type I IFNs in a dose-dependent manner. A multicenter randomized study (OPTIMS) is underway in Italy, which aims to compare the efficacy of two different doses of IFN beta-1b (250 mcg vs 375 mcg).

Objective: To study Mx protein and neutralizing antibody (NAb) levels in RRMS patients receiving treatment with IFN beta-1b.

Methods: After 6 month's treatment with 250 mcg subcutaneous every-other-day (EOD) IFN beta-1b, partially responding RRMS patients (those with persisting clinical/MRI signs of disease activity) are randomized to either continue on the same IFN beta-1b dose or receive an increased dose (375 mcg EOD). Laboratory assessments are performed at baseline and repeated every 3 months. Mx protein production is determined by a two-site chemiluminescent sandwich in whole blood samples, whose Mx level reflects those of circulating blood cells. NAb titration is performed by an assay based on the induction of Mx protein in a lung carcinoma cell line.

Results: We present the preliminary immunological data from the first 23 patients completing the first 6 months (pre-randomization phase) of the study. Eleven of the 23 patients responded well to IFN-beta-1b 250 mcg EOD, while 12 displayed a partial response. In all patients we measured an increased level of Mx protein after the first 3 months of treatment ($p < 0.01$). The increase in Mx protein was 4-fold greater than the baseline level in the 12 partially responding patients ($p = 0.01$), and 6-fold greater than baseline in the 11 who responded well ($p < 0.01$). The difference between the two groups was statistically significant ($p < 0.05$). The increase in Mx levels peaked after 3 months of treatment, decreasing thereafter. At month 6, the difference between responders and partial responders was not significant. Five of the 23 patients developed NAb (titer < 60), all at month 6 of treatment; one of these patients had responded well to therapy, while the others had a partial response.

Conclusions: Levels of Mx protein correlated with the response to treatment. Further follow-up will clarify the effects of an increase in the IFN beta dose to 375 mcg on both Mx protein and NAb.

P302

**The influence of a functional variant of the glutamate kainate receptor 3 on susceptibility to and severity of multiple sclerosis.** J. A. Woolmore, A. A. Fryer, M. D. Boggild, R. C. Strange, C. P. Hawkins, North Staffordshire Hospital, The Walton Centre for Neurology and Neurosurgery for the Keele Multiple Sclerosis Research Group

Introduction: Excitotoxicity mediated via the AMPA/kainate type of glutamate receptor is known to damage oligodendrocytes. Altered glutamate homeostasis in multiple sclerosis (MS) lesions has been correlated with oligodendrocyte and axonal damage in post mortem studies. Antagonists of kainate receptors ameliorate the neurological score of animals with experimental autoimmune encephalomyelitis and increase oligodendrocyte survival. The first described functional polymorphism of kainate receptors is of glutamate kainate receptor 3 (GRIK 3), causing an amino acid change (ser310ala) in the extra cellular N-terminus of the protein. An association study was therefore performed to investigate whether this variant was associated with susceptibility to or severity of MS.

Methods: 181 Caucasian patients (27.7 % female, 72.3 % male) and 100 controls of Northern European origin were recruited. Mean onset age of MS was 31.1 ± 8.6 years and mean disease duration was 13.3 ± 9.3 years. DNA was extracted from leucocytes and a polymerase chain reaction RFLP-based assay using digestion with SmaI was used to identify the ser310ala variant. Outcome was assessed by the expanded disability status scale (EDSS) and cases stratified into mild/moderate disability (EDSS 0–5.5) and severe disability (EDSS 6–10) after disease duration of 10 years. Results were analysed using chi square testing and logistic regression to correct for independent covariants as below. Significance levels were set at $p < 0.05$.

Results: Genotype frequencies in controls (62 % ser/ser, 34 % ser/ala, 4 % ala,ala) and MS patients (54 % ser/ser, 40 % ser/ala, 6 % ala/ala) were not significantly different ($p = 0.432$). Compared with homozygosity for the ser310 allele, hetero and homozygosity for ala310 was not significantly associated with development of severe disability (EDSS 6–10) in MS patients with a disease duration of 10 years after correcting for age of onset, gender and disease duration ($p = 0.617$, OR = 1.3, 95 % CI 0.5–3.1).

Conclusions: The ser310ala functional polymorphism of GRIK3 does not appear to be associated with susceptibility to, or prognosis of MS. Examination of further polymorphic variants of receptor types involved in excitotoxic cell death may be of value in illustrating the role and importance of excitotoxic damage in the MS lesion. Such work may support consideration of future treatment strategies designed to ameliorate such damage, and prevent progression of disability.

P303

**The role of polymorphisms in the PVR- and nectin-genes for the development of multiple sclerosis.** B. Rosche, S. Cepok, S. Stei, V. Grummel, B. Hemmer, Neuroimmunology Group (Marburg, D)

Multiple Sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). Although the immune system seems to play a crucial role in the pathogenesis of disease, pathogenetic pathways and target antigens are still uncertain. However, both genetic and environmental factors are important for the development of disease. In this study, we focused on the Poliovirus Receptor (PVR) and Poliovirus-related Receptor (PRR2) receptor genes, which are located on chromosome 19q13, a region previously linked to MS. Both receptors are not only important for the entry of neurotrophic viruses (such as Herpes simplex or polio viruses) but also expressed in the brain and involved in inter-cellular adhesion. The exons of both genes were sequenced in a group of MS patients to identify genetic alterations. In the PVR genes, we identified 6 new polymorphisms. Two of them are located in the promoter region of the gene and 4 in exonic regions. All exonic polymorphisms altered the amino acid sequence of the receptor (exon 2, 120 G à A, exon 5 41 G à A and exon 7, 29 A à G). We did not detect any polymorphism in the PRR2 genes. In subsequent studies, we determined the frequency of these polymorphisms by RFLP in 187 MS patients and 176 healthy unrelated controls. The prevalence of the polymorphisms was: exon 2: MS 5.88 %, controls 8.74 %; exon 5: MS 0.54 %, controls 0 % and exon 7: MS 2.14 %, controls 4.40 % ($p > 0.05$ for all three polymorphisms). Further studies will focus on the polymorphisms in the promotor region and additional genes involved in virus entry to the CNS. In conclusion, we found several new polymorphisms, which alter the structure of the PVR receptor. None of them seem to be associated with MS.

P304

**Multiple elements of the allergic arm of the immune response modulate autoimmune demyelination.** R. Pedotti, J. DeVoss, S. Youssef, D. Mitchell, J. Wedemeyer, R. Madanat, H. Garren, P. Fontoura, M. Tsai, S. Galli, R. Sobel, L. Steinman, Neurological Institute C. Besta, Stanford University (Milan, I; Stanford, USA)

Analysis of mRNA from multiple sclerosis (MS) lesions revealed increased amounts of transcripts for several genes encoding molecules traditionally associated with allergic responses, including prostaglandin D synthase (PGDS), histamine receptor 1 (H1R), platelet activating factor receptor (PAFR), immunoglobulins (Ig) Fc epsilon receptor 1 (FceRI), and tryptase.

We now demonstrate that in the animal model of MS, experimental autoimmune encephalomyelitis (EAE), mediated by Th1 T cells, histamine receptor 1 and 2 (H2R) are present on inflammatory cells in brain lesions. Th1 cells reactive to proteolipid protein (PLP) expressed more H1R and less H2R than Th2 cells. Pyrilamine, an H1R antagonist, blocked EAE and the PAFR antagonist CV6209 reduced the severity of EAE. EAE severity was also decreased in mice with disruption of the genes encoding Ig Fc gamma RIII and both Fc gamma RIII and Fc epsilon RI. PGDS and tryptase transcripts were elevated in EAE brain. Taken together, these data reveal ex-

tensive involvement of elements of the immune response associated with allergy in autoimmune demyelination.

P305
**Utilization of complementary and alternative medicine by multiple sclerosis patients with special focus on coping and reasons for use of alternative medicine.** A. Apel, B. Greim, U. K. Zettl, University of Rostock (Rostock, D)

Objective: To evaluate the use of complementary and alternative medicine (CAM) in patients with multiple sclerosis (MS) and influence of coping as well as reasons for the use of CAM.

Background: Recent research could show that two thirds of patients with MS are using alternative medicine at one time point during their course of illness. Research in oncology indicates that utilization of CAM is part of the coping process. There are also evidences that reasons for the use of CAM are connected to satisfaction with the physician-patient relationship. But there is no detailed investigation about the relationship between CAM and coping with MS or reasons for use of CAM.

Design/methods: In 2002 105 patients with a clinically definite MS were investigated in a standardized interview. Patients were asked sociodemographic variables, aspects of their illness and variables of the use of CAM. The interview was completed by a 12 item self-developed questionnaire to explore motivation and reasons for the use of CAM and the TSK (Trier scales of coping with illness) to measure aspects of coping with illness.

Results: 73.3 % out of the 105 interviewed patients were women which approximates the general MS population. The mean age of patients at the time of study was 43.3 years (SD = 12.7) and the mean duration of illness 6.7 years (SD = 12.7) with a mean EDSS score of 3.4 (SD = 2.2). 83 patients (79 %) with MS reported the use of CAM during their illness. The average number of CAM therapies used was 3.6 (SD = 2.6). There was a positive correlation found between the number of used CAM and both the EDSS score ($r = 0.44$, $p < 0.01$) and the age of the patients ($r = 0.23$, $p < 0.05$). Compared with patients receiving only conventional treatment, users of CAM reported more defense of threat than non-users ($p < 0.05$) and they were searching more often for information and exchange of experience ($p < 0.01$). The number of used CAM was correlated to discontent with treatment ($r = 0.22$, $p < 0.05$) as well as with the desire for active participation on treatment of the MS ($r = 0.30$, $p < 0.01$).

Conclusion: Patients with MS are using CAM more often at one time point during their disease than former research has suggested. As reasons for this development MS patients report dissatisfaction with their medical care and the wish for an active involvement in the therapy process. Users of CAM are more active in their coping when searching for information and tend to a more fighting and optimistic attitude against the disease.

P306
**Patient comfort during interferon beta treatment – a comparison of interferon beta-1b and interferon beta-1a.** D. S. Goodin, University of California (San Francisco, USA)

Interferon (IFN) beta is an effective treatment for relapsing forms of MS. Recent clinical studies have demonstrated the superior efficacy of higher, more frequently administered subcutaneous doses of both IFN beta-1b (Betaseron(R)/Betaferon(R)) and IFN beta-1a (Rebif(R)) over once-weekly intramuscular IFN beta-1a (Avonex(TM)). In the absence of comparative efficacy data between these two high-dose, high frequency preparations, tolerability may be an important consideration when choosing therapy. In the pivotal clinical studies of these two agents, the respective side-effect profiles looked remarkably similar. However, there has been some suggestion, mostly based on anecdotal evidence, that injection site pain may be more a frequent problem in patients receiving three times weekly IFN beta-1a than in patients receiving IFN beta-1b, possibly due to differences in the pH of the two formulations (IFN beta-1a has a pH of 3.8, whereas IFN beta-1b has a pH of 7.2).

A patient satisfaction survey (addressing the tolerability of the different high-dose IFN beta preparations) was carried out in Canada and Germany. A group of 409 MS patients participated in this survey (189 treated with IFN beta-1b 250 mcg qod, 88 treated with IFN beta-1a 22 mcg tiw, and 132 treated with IFN beta-1a 44 mcg tiw). In addition, a prospective study has been conducted in a group of 64 healthy volunteers to evaluate the effects of different high-dose IFN beta formulations. During the study, subjects were assigned randomly to inject either IFN beta-1b (250 mcg) qod or IFN beta-1a (44 mcg) tiw for a period of 4 weeks. Each subject had blood samples drawn daily for the duration of the study period. As part of this study, the short-term tolerability of the two preparations was assessed by an investigator blind to treatment assignment.

Of the 409 patients surveyed, 49 % of the IFNB-treated patients were either very or extremely satisfied with their therapy, and 39 % were satisfied, with no differences in satisfaction levels between the two treatments. Nevertheless, IFN beta-1a users did experience more injection site pain compared to IFN beta-1b users.

The full analyses from both these studies are in progress, and the final results from both will be presented.

P307
**Intravenous cyclophosphamide therapy in multiple sclerosis: a prospective study in a clinical cohort.** E. Portaccio, V. Zipoli, G. Siracusa, S. Piacentini, S. Sorbi, G. Ponziani, M. P. Amato, University of Florence (Florence, I)

Objectives: To assess the safety and tolerability of cyclophosphamide (CPM) pulse therapy in a cohort of Multiple Sclerosis (MS) patients who have been treated and prospectively followed-up in our Department since 1997.

Materials and Methods: Patients had a definite diagnosis of MS, a Primary Progressive (PP), Secondary Progressive (SP) or Relapsing-Remitting (RR) course with high relapse rate, and were non respondent to previous treatments. CPM (700 mg/m$^2$) was administered intravenously (i. v.) monthly for 12 months, then bimonthly for another 12 months. CPM was associated with i. v. methylprednisolone 1 g, ondansetron 8 mg and mesna 800 mg. Monitoring tests included: complete blood count and differential, platelet count, hepatic enzymes, bilirubin, protein fractions and urine cultures performed twice a month. Overall tolerability of the therapy was assessed by the patients on a visual analogue scale.

Results: We treated 108 patients (62 women; 6 RR, 102 SP or PP cases; mean age 47 ± 9.4 years; mean EDSS score 5.9 ± 0.9; mean disease duration 13.8 ± 6.8 years). The mean number of pulses was 10 ± 5, and the mean duration of the follow-up period 2.2 ± 1.4 years. Side effects were cause of suspension of the therapy in 20.4 % cases: infections of the urinary and respiratory tracts were observed respectively in 58 (54 %) and 7 (6.5 %) subjects. Moreover, 35 (32.4 %) patients suffered from nausea and vomiting. Microscopic or macroscopic hematuria and hemorrhagic cystitis occurred respectively in 4 (3.6 %), 5 (4.6 %) and 3 (2.8 %) patients. Reversible menstrual abnormalities were reported by 2 women while definitive amenorrhea occurred in 4 patients, all over 35 years. Finally, transient hair loss was observed in 2 cases. Lymphopenia occurred in 17 (15.7 %), increase of hepatic enzymes in 12 (11 %) and hypogammaglobulinemia in 6 (5.6 %) patients. A mammary and a basal cell carcinoma were diagnosed in 2 women, both previously treated with azathioprine. Finally, 67 % of the patients judged the treatment regimen as acceptable and well tolerated.

Discussion: Pulse therapy with CPM has been preliminarily reported to be effective in patients with progressive or very active MS, and our findings point to a reasonable safety and tolerability of this therapy.

Conclusions: Further trials are needed to define the efficacy of CPM as an alternative therapeutic option for the most active forms of MS.

P308
**Devic's neuromyelitis optica in Turkey: report of 11 cases.** D. Uluduz, S. Saip, A. Altintas, F. Mutluay, A. Siva, Istanbul University Cerrahpasa Medical Faculty (Istanbul, TR)

Introduction: Devic's Neuromyelitis optica (NMO) is an idiopathic inflammatory demyelinating disease of CNS characterized by attacks of optic neuritis and myelitis. The incidence and prevalence of the disease that affects young adults much like multiple sclerosis (MS) are unknown. As it is more common in Japan and India first it was named as Asian type MS. Though definite ratio is unknown it is more uncommon than MS in Europe. Objective: We think NMO is a rare disease in Turkey. Therefore we retrospectively evaluated clinical, imaging and serologic features of 11 NMO cases at our department within the criteria proposed by Wingerchuk et al. in 1999. Furthermore therapeutic approaches and prognostic clues were also reviewed under the light of literature.

Method: We evaluated 11 NMO cases (9 female). The onset ages were between 18 and 52 (mean 27.6). Symptoms at onset was optic neuritis in 6 patients and transverse myelitis in 5. NMO was clinically relapsing in all cases. In 3 cases NMO was associated with autoimmune disorders and only 2 had oligoclonal bands in CSF. All cases had normal brain MRI. Cervical MRI showed T2 hyperintense lesions extending over 3 or more vertebral segments in 8 cases. 2 cases died because of respiratory failure, 3 were restricted to bed and others were fully ambulatory. All cases received intravenous corticosteroid treatment in acute attacks. Plasmapheresis was used in 4 cases with myelitis attacks that were not responsive to methylprednisolone and the results were hopeful. We concluded that prophylactic

therapeutic approaches (e. g., immunesuppresive, immunemodulator) had no effect in the course of the disease.

Result: NMO is a rare disease in our country. Clinical, serologic, imaging and therapeutic results supported the literature that pathogenetic mechanisms different from MS play a role in NMO.

**P309**

**Predictors of efficacy of rehabilitation in patients with multiple sclerosis.** F. Martinelli Boneschi, M. Rovaris, F. Agosta, L. Mugnaga, R. Gatti, P. Rossi, M. Comola, A. Raschi, G. Comi, M. Filippi, Department of Neurology, HSR, Neuroimaging Research Unit, Neurorehabilitation Unit, HSR (Milan, I)

A total of 60 MS patients underwent a standard rehabilitation treatment, of whom 12 (20 %) had relapsing-remitting (R-R), 26 (43.3 %) with the secondary progressive (S-P), 18 with the primary progressive (P-P) (30.1 %) and 2 each (6.6 %) with the progressive-relapsing and transitional forms of the disease. The median time of the rehabilitative treatment was 29 days, ranging from 0 to 51 days. The aim of the study was to assess: 1) whether rehabilitation has a short-term impact on clinical disability in MS patients; 2) whether there are predictors of rehabilitation effectiveness in order to select patients best fitted for rehabilitation. Rehabilitation was considered to be effective if associated with an improvement of the EDSS score of at least 0.5 (if baseline EDSS was greater than 5.5) or of 1 point (if baseline EDSS was equal or lower than 5.5) and/or an improvement as measured by the Functional Independence measure scale (FIM) of at least 5 points.

According to our pre-defined EDSS outcome measure, 38 (63.3 %) of the patients benefited from rehabilitation, while, according to the FIM outcome measure, this was 40.8 %. If we consider as effective an improvement on at least one of the two of the scales, 55.1 % of the patients improved. When patients were stratified according to their disease courses, we noticed that, according to the either FIM or EDSS improvement, 63.6 % of patients with the R-R form, 65 % of patients of those with the S-P form and only 40.2 % of those with the P-P form improved. Baseline EDSS did not predict rehabilitation effectiveness when patients were stratified into baseline EDSS lower or equal than 5.5 versus greater than 5.5, while the number of days of hospitalization were associated with different effectiveness when stratified into 2 groups, less or more than 30 days (41.9 % versus 75 % of rehabilitation effectiveness respectively; $p = 0.02$).

We conclude that rehabilitation treatment has a moderate clinical impact on patients with MS. It is still unclear whether the efficacy is subtype-dependent, namely if patients affected with the R-R and S-P form benefit more than patients affected with the P-P form. Moreover, it is still unclear whether the benefit of rehabilitation is short-term or lasts over a significant period of time.

**P310**

**Acute disseminated encephalomyelitis complicating campylobacter gastroenteritis.** B. Sharrack, D. Orr, M. McKendrick, Royal Hallamshire Hospital (Sheffield, UK)

The association of Campylobacter infection and Guillain-Barré syndrome is well recognised. We report a case of acute disseminated encephalomyelitis (ADEM) complicating Campylobacter gastroenteritis in a previously fit man.

A 24 year old man presented to his general practitioner with a four day history of non bloody diarrhoea associated with fevers and sweats. Campylobacter species was later isolated from stool samples. Sixteen days into the illness he complained of slurring of speech, intermittent diplopia and difficulty in walking. Examination revealed dysarthria, mild left pyramidal limb weakness and decreased sensation in the left leg. His gait was ataxic. Cranial MRI showed abnormalities consistent with ADEM. His CSF was normal with negative oligoclonal banding.

He was treated with intravenous Methylprednisolone, 1 g daily for three days. One day later he noticed an improvement and after 7 days of starting treatment he had no ataxia and was discharged home.

Campylobacter gastroenteritis, is the most common cause of acute gastroenteritis in the United Kingdom, accounting for over 56,000 cases in 2000. It is estimated that approximately one in every 1000 reported campylobacteriosis cases leads to Guillain-Barré syndrome and around 33 % of Guillain-Barré syndrome cases in the western world may be triggered by campylobacteriosis. There has been one case report of combined ADEM and acute motor axonal neuropathy following Campylobacter jejuni infection and hepatitis A immunisation. However, there have been no reported cases of ADEM following Campylobacter infection alone. This is perhaps surprising given the strong association between Campylobacter jejuni infection and Guillain-Barré syndrome and the pathogenesis of the latter. In these cases Campylobacter jejuni induces humeral and cellular immune responses due to molecular mimicry between specific lipopolysaccharides epitopes on the infecting agent and target epitopes on the surface components of the peripheral nerves resulting is myelin destruction and axonal degeneration.

We describe the first identifiable case of ADEM complicating Campylobacter gastroenteritis alone. Our patient made an excellent recovery associated with therapy with high dose methylprednisolone.

**P311**

**Connective tissue diseases mimicking multiple sclerosis (MS).** S. Pelidou, S. Tzavidi, N. Tsifetaki, A. Kostula, G. Kitsios, D. Stefanou, E. Arkoumani, A. Drosos, A. Kyritsis, Medical School of Ioannina (Ioannina, GR)

Objective: To analyze the clinical, laboratory, and imaging findings of MS-like manifestations in a cohort of 105 patients presented at our University Hospital.

Background: Clinical syndromes mimicking MS, mainly in its relapsing-remitting pattern, are reported to occur in association with several connective tissue diseases, especially systemic lupus erythematosus (SLE), Sjögren syndrome (SS) and antiphospholipid syndrome (APS).

Methods: Proposed clinical and laboratory diagnostic criteria for MS, SLE, SS and APS were systematically assessed in 105 consecutive patients at our MS-clinic. The authors questioned all patients about xerophthalmia and xerostomia, biopsied minor salivary glands, performed a Rose-Bengal and Schirmer test, and measured antinuclear (ANA), anti-Ro (Ssa), anti-La (SSb) and anticardiolipin (aCL) antibody by standardised methods. All patients underwent complete screening for MS, including brain and spinal cord magnetic resonance imaging (MRI), visual evoked potentials (VEP), and CSF analysis.

Results: Thirteen patients (12,3 %) met criteria for collagen vascular diseases (SLE, SS, APS) while additional fourteen patients (13,4 %) in whom a diagnosis of MS was established had positive ANA tests.

Conclusions: This prospective study confirmed that in the absence of pathognomonic clinical findings or a definitive laboratory test, the diagnosis of MS is a diagnosis of exclusion. SLE, SS and APS should be screened for systematically in all patients with MS.

**P312**

**Hemicraniectomy as a successful treatment of mass effect in acute disseminated encephalomyelitis.** S. v. Stuckrad-Barre, C. Foerch, E. Klippel, J. Lang, R. du Mesnil de Rochemont, M. Sitzer, Klinik für Neurologie, Klinik für Neurochirurgie, Institut für Neuroradiologie (Frankfurt am Main, D)

In general, the prognosis of acute disseminated encephalomyelitis (ADEM) has been reported as favorable. However, fulminant forms with space-occupying lesions are associated with a substantial risk of fatal outcome due to herniation or secondary brain damage. Few reports about treatment of elevated intracranial pressure (ICP) in fulminant ADEM are available.

Here, we report on a 34-year-old woman presenting with a 6-day history of fever and headache. At initial presentation in a general hospital neurologic examination was unremarkable except for nuchal rigidity. Lumbar puncture revealed 67 lymphocytes per µl (no RBC), 52 mg/dl glucose, and 2 mg/dl protein. After developing complete left hemiplegia, and bilateral extensor plantar responses she was referred to our institution. MRI revealed an isolated high intensity lesion involving the frontal, temporal, and parietal white matter of the right hemisphere as well as the corpus callosum. Brain biopsy revealed diffuse and extensive demyelination, macrophages containing myelin lipid-degradation products, and no hemorrhages confirming the diagnosis of fulminant ADEM. Treatment with IV-methylprednisolone (1 g/d for 5 days) was started. Despite antiedematous treatment with IV-mannitol and hyperventilation, she developed clinical and neuroradiological signs of uncal herniation. Consequently, emergency right-sided hemicraniectomy was performed. The patient continued to improve, and neurological examination at 3-month follow-up after symptom onset revealed only a mild left-sided hemiparesis.

In ADEM, high-dose steroids are widely used as first-line treatment, and immunoglobulins, plamapheresis, or cytostatic drugs are alternative treatment options in patients with poor or no response to steroids. Our patient continued to deteriorate and developed uncal herniation, as shown by serial brain imaging, despite the use of high-dose steroids. To achieve substantial ICP-reduction, hemicraniectomy was performed with good clinical outcome.

Whether unfavorable long-term outcome in such cases reflects primary brain injury due to ADEM itself or secondary effects due to raised ICP could not be determined from the available literature.

This case illustrates that aggressive treatment options such as hemi-

craniectomy may provide a lifesaving therapeutic option in a subset of patients with fulminant ADEM who deteriorate with conventional immunosuppressive and antiedematous therapy.

P313
**Pilot study: ingested IFN-alpha alters MxA/TNF-alpha mRNA in MS.** S. Brod, M. Nguyen, University of Texas (Houston, USA)

14 active MS patients not on immunomodulators for six months or steroids for one month were assayed for TNF-a reduction/Mx induction after ingesting IFN-a at 100, 300, 1,000, 3,000, and 10,000 IU. 10,000 IU dose was used as the highest dose since it is at the upper limits of effect in reducing new MRI lesions in MS. The relative numbers of transcripts for human b-actin, human TNF-a and MxA were measured. The mean measured MxA transcript levels were normalized to the b-actin control (normalized mean = MxA mean/b-actin x 100) and expressed as % b-actin molecules.

The number of patients at which maximum MxA message induction occurs: 100 units: 2; 300 units: 3; 1,000 units: 5; 3,000 units: 2; 10,000 units: 2. Goodness-of-fit test is commonly used to examine if the frequency is different across the groups. If the frequency is different, we will see where the maximum increase occurs. Exact goodness-of-fit test shows $p = 0.762$. This suggests that there is no significant difference in the dose levels at which maximum increase occurs after examining this limited number of samples. However, there is a clear trend for doses < 10,000 to be the optimal dose. The mean dose = 2,400 IU. If we examine MxA inductions > 100 % baseline (5/14), we see the following: 100 units: 2; 300 units: 1; 1,000 units: 2; 3,000 units: 0; 10,000 units: 0. MxA inductions > 100 % baseline occur at doses less than or equal to 1,000 IU.

The frequency of the dose level at maximum decrease occurs in TNF-a: 100 units: 6; 300 units: 0; 1,000 units: 4; 3,000 units: 3; 10,000 units: 1. Exact goodness-of-fit test yields p-value = 0.093. This suggests a clear trend without as yet a significant difference in the dose levels at which maximum decrease occurs. There is also a clear trend for doses < 10,000 to be the optimal dose. The mean dose = 1,700 IU. If we examine TNF-a reductions > 50 % baseline (8/14), we see the following: 100 units: 3; 300 units: 1; 1,000 units: 2; 3,000 units: 2; 10,000 units: 0. TNF-a reductions > 50 % baseline occur at doses < 10,000 IU. In 10/14 instances, the maximal induction of MxA mRNA and maximal repression of TNF-a mRNA occur at the same or contiguous doses. These data suggest a connection between Mx expression and TNF-a repression. Additional sampling will provide more definitive results.

P314
**Using alternative therapy in multiple sclerosis patients in Poland.** D. Mirowska, W. Fryze, M. Wiszniewska, A. Czlonkowski, A. Czlonkowska, Medical University of Warsaw (Warsaw, Gdañsk, Pila, PL)

Despite the great progress that has been made in medicine, treatment of many chronic diseases especially of not fully known aetiology, including multiple sclerosis (MS) remains very difficult and hardly effective. The use of complementary and alternative medicine is booming in many countries. MS patients, their families and even some professionalists prompt to use alternative medicine. To obtain more data about the scale of this phenomenon in Poland we have distributed questionnaire concerning alternative therapy (AT) among MS patients treated in 3 hospitals of different regions of our country. Data from 176 patients (117 women and 59 men) were obtained. The mean age of patients was 44 ± 11 years, mean disease duration was 8,76 ± 8 years.

120 patients (68 % of 176 questioned MS patients) declared using now or in the past AT. The majority of them declared more than one alternative method, some of them tried up to 10 different treatments. Among patients who have ever tried AT, the most popular (56 %) was Oenothera seeds oil, the second place was taken by vitamins, especially of B group (43 %), the third most common was the massage (34 %). The next places were taken by herbal medicine (28 % responders), diet restriction (21 %), acupuncture (19 %), bioenergotherapy (17 %), homeopathy (14 %), hypotherapy (13 %) and magic practitioner (8 %). There were no significant demographic differences (in sex, age, education, place of living) between users and non-users. Neither disease duration nor disability progression had any impact on the decision of using AT. It should be said that the patients in our study regard alternative treatment more as complementary than purely alternative. The majority of them used standard treatment prescribed by physicians during receiving AT. 51 % of patients have heard about AT form their physicians, 94 % of AT users regulary visit their neurologists.

The phenomenon of using AT by MS patients is not fully understood but it seems that long-term efficacy limitation of so-called immunomodulators as e. g. interferon-beta or copaxone and symptomatic treatment determines seeking other treatment possibilities. Patients confirm that the main advantage of AT is improvement in general self-being (26 %) and in movement ability (26 %). Without doubt a very important aspect of AT is general belief that such therapy is safe and even if it does not help it will not harm.

P315
**Case-based learning in neurology.** H.-P. Hartung, P. Posel, N. Riachi, C. Wernsdorfer (Dusseldorf, D; Paris, F; Beirut, LBN; Ismaning, D)

It is well accepted that individuals learn best when their education mirrors personal experience. In a study by Frankford et al. (2000), it was found that medical professionals were most motivated to learn when the learning seemed useful, particularly in the short term. Hence, education should be based on situations derived from medical practice and the problems that medical professionals face in daily work.

Teaching the approach to a clinical problem in neurology demands methods for the training of practical skills, clinical problem-solving, and decision-making. In multiple sclerosis (MS), for example, the skills required to examine, establish the diagnosis, exclude differential diagnoses, and assess disability can only be acquired through systematic training. Establishing the diagnosis early during the disease by careful history taking, clinical examination, and thoughtful use of ancillary techniques (in particular MRI) following an intricate algorithm has become mandatory given the availability of effective immunomodulatory therapy in this stage of disease.

BrainLINC™ is a web-based, case-based educational tool that was developed to train neurologists on basic and advanced skills. Cases for BrainLINC™ were selected and evaluated by an editorial board and a steering committee, were published quarterly, and an electronically interactive program was developed. The tool emphasizes the distinction between doing and planning to do in the decision-making process.

We discuss the benefits of case-based learning in terms of how it [1] contributes to the appropriate organization of information for later recall in clinical reasoning situations; [2] generates experience that physicians would not have otherwise gained in daily practice; [3] increases the visibility of the clinical reasoning processes; and [4] strengthens the confidence of the neurologist. Our evaluation also explores three examples of case-based education and learning: use of written cases, use of standardized patient cases, and use of web-based cases for learning assessment and diagnostic skills. Finally, we compare and contrast each of these methods in terms of their relative effectiveness in achieving each of the defined benefits.

Presentation and discussion of a clinical case and the case-method were considered to reflect more faithfully "real-life settings," to better introduce clinical problems and testing clinical skills than traditional methods (eg, lectures). Case-based learning is a valuable tool in neurology.

P316
**An open study on modafinil as fatigue treatment in multiple sclerosis.** G. Iuliano, R. Napoletano, M. Tenuta, A. Esposito, Ospedali Riuniti di Salerno (Salerno, I)

Background: Modafinil is a recent drug, approved for sleepiness in narcolepsy. Some reports showed improving of fatigue in multiple sclerosis (MS). Our aim was to assess its effectiveness and tolerability on fatigue in a group of patients with MS.

Patients and methods: 13 patients (7 females, 6 males, age onset 16–45 yrs, m 32.38) with definite MS are studied, with fatigue in daily activities. They received modafinil (200 mg) raised to 400 mg daily if necessary. They were evaluated at the beginning and monthly by clinical examination, EDSS, and Fatigue Impact Scale (FIS). Baseline data were: EDSS m 2.8 (0–6.5); FIS 74.92 [37–130]. Seven had a diagnosis of depression. Statistical evaluation was performed by non parametric tests (Kruskal-Wallis) and multivariate analysis (linear regression).

Results: EDSS score did not change significantly. Fatigue Impact Scale (FIS) was significantly improved after 1 month, (74.923 (SD 26.484) vs 60.077 (SD 22.581), $P = 0.001$). Stratification for sex showed significance only in females (82.857 (SD 27.120) vs 64.429(SD 27.269), $P = 0.004$). Improving was significant either in presence (78.286 (SD 29.646) vs 64.571 (SD 19.303), $P = 0.038$) or in absence of depression (71.000 (SD24.380) vs54.833 (SD 26.746), $P = 0.014$). Also stratification for EDSS did not modify the significance of improving (EDSS < 3.5 = 73.125 (SD 32.577) vs 54.875 (SD 26.595), $P = 0.006$; EDSS > 3.5 = 77.800 (SD 15.172) vs 68.400 (SD 12.300), $P = 0.046$).

In spite of the good results, after the first month only 3 patients continued therapy. The other 10 discontinued it, three for nervousness, three

for prescription problems (Italian Health Service gives this drug only for narcolepsy); four without reason. Two of these raised the dosage to 200 mg/day, and soon after they dropped. Besides nervousness and insomnia (which is for the main indication a therapeutic effect), other side effects, as nausea, dry mouth, headache, and diarrhea, were not observed.

Discussion: Fatigue is common in MS, but it does not indicate a neurological localization. So one cannot expect changes in EDSS, at least in a short time, as it is in this series. The effectiveness seems well documented, and the reduction of fatigue is not associated to other variables as impairment differences or depression. Nevertheless, the patients discard the therapy, either for side effects, or for the cost of the drug. So, the best schedule for fatigue in MS seems to be 100 mg daily, in short courses (3 weeks-1 month).

P317

**Quality of life in multiple sclerosis patients: interrelations with clinical and MRI T2 lesion load.** C. Tortorella, A. Ceccarelli, D. Carrara, M. Lopez, M. F. De Caro, A. Bellacosa, I. Pavone, F. Girolamo, G. Tedeschi, L. Lavrogna, D. Dinacci, P. Livrea, I. L. Simone, Department of Neurological and Psychiatric Science, Department of Neurology (Bari, Naples, I)

Background: Quality of life (QOL) is dramatically affected in MS patients. Over the past decade the assessment of QOL has become an important issue for the evaluation of therapy and care strategies in MS. Objective: The aim of the study was to investigate the interrelations between quality of life and clinical disability, fatigue, depression and magnetic resonance imaging (MRI) T2 lesion load in MS patients.

Patients and methods: We studied a subgroup of 67 MS patients participating in a "multicenter study of mobile MRI evaluation in MS patients", characterized by a definite relapsing remitting clinical course. Age was 39.3 ± 11 (range 17–58 years) and disease duration was 10.15 ± 6.4 (range 0.93–27.25 years). No patient was under disease modifying treatments, nor corticosteroid therapy was administered during three months preceding the study. Quality of Life was assessed using MSQOL-54 inventory. All patients underwent a complete clinical assessment including the evaluation of clinical disability (Expanded Disability Status Scale-EDSS), cognitive function (Mini Mental State Examination), depression (Beck Depression Inventory) and fatigue (Fatigue Severity Scale). T1 and T2 weighted images were acquired by 1.0 Tesla GE scanner. T2 lesion load was evaluated by using a semi-automated segmentation technique based on local thresholding.

Results: A significant relation was found between fatigue and depression ($p < 0.0001$) and EDSS score ($p < 0.0001$), whereas both fatigue and depression were not related to total T2 lesion load. Multivariate analysis showed that the clinical disability was significantly associated with fatigue severity ($p = 0.004$) and T2 lesion load ($p = 0.003$). MSQOL-54 physical composite score was inversely related with fatigue severity ($p < 0.0001$), EDSS score ($p = 0.036$) and depression score ($p = 0.026$). The main determinants of MSQOL-54 mental health composite score was the depression score ($p < 0.0001$) and the disease duration ($p = 0.006$). No relation was found between quality of life and T2 lesion load.

Conclusions: This study demonstrated that the impairment of quality of life in MS is significantly associated with increasing clinical disability, depression and fatigue score and disease duration. Quality of life assessments are not related to lesion load.

Grant: Study supported by Serono Foundation

P318

**Long-term clinical experience with intramuscular interferon beta-1a in relapsing-remitting multiple sclerosis patients.** V. Brescia Morra, C. Florio, G. Coppola, R. Lanzillo, P. Vivo, G. Vacca, E. Salvatore, S. Ascione, V. Schiavone, G. Orefice, V. Bonavita, Università Federico II, Ospedale Cardarelli (Naples, I)

Background: Interferon (IFN) beta-1a 30 mcg IM once weekly has been shown to reduce disability progression and relapses in the treatment of relapsing-remitting multiple sclerosis (RR-MS) patients. Few data are available about the long-term follow up and outcome in large cohorts of patients.

Objectives: To monitor the long-term efficacy of IM IFN beta-1a as used in clinical practice in Naples, Italy; to identify possible predictors of the therapeutic response.

Methods: An open-label postmarketing study was conducted to evaluate the efficacy and tolerability of IFN beta-1a for up to 66 months of treatment. A total of 225 patients with RR-MS received treatment with IFN beta-1a, 30 mcg IM once weekly. We considered baseline characteristics (including EDSS basal score and prior annual relapse rate) and clinical outcome (EDSS, relapse rate and relapse-free proportion) in the group reaching a 2-year follow-up. On whole series, we evaluated time to EDSS 1-point progression and time to first relapse during the treatment, by means of survival analysis. We also studied the influence of baseline parameters (baseline EDSS and prior relapse rate) on the outcome measures.

Results: Mean therapy duration was 27.6 ± 17 months (range: 2–66), and a follow-up longer than 24 months was available in 156 patients (69%). Median ± SD EDSS at baseline was 2.5 ± 1.2 [1–5]. Mean prior relapse rate was 1.0 ± 0.9 [0–6]. The mean interval between onset and starting of the therapy was 7.5 ± 6.2 years (0.6–31). During the first 2-year follow up, 37 patients (24%) dropped-out, for disease activity (relapses and disability progression, 68%), voluntary decision (17%) and side effects (17%). Among 119 patients completing the first 2 years of therapy, 66% did not worsen at the EDSS, and 50% remained relapse-free. Mean relapse rate in this period was 0.4 ± 0.5 ($p < 0.01$).

Survival analysis on 225 patients showed a 75% estimated proportion of EDSS stable patients and a 50% of relapse-free, after a 2-year therapy. Patients with baseline EDSS ≤ 2 (55%) showed significantly longer estimated time to both EDSS progression and first relapse.

Conclusions: In this series we observed, after 2 years of therapy, 50% of relapse-free and 66% of disability progression-free patients. Twenty-four per cent of the patients dropped-out, mainly for disease activity. Survival analysis showed that about 75% of the patients are likely to not have a 1-point worsening on EDSS score, and to not experience a relapse after a 2-year therapy. Lower baseline EDSS appeared to be predictor of good response to therapy.

P319

**Does interferon-beta trigger proliferative retinopathy in patients with multiple sclerosis and diabetes mellitus? Report of two cases.** D. Maimone, S. Crimi, Garibaldi Hospital (Catania, I)

Background: Mild retinal changes may occur during interferon (IFN)-alpha in patients with chronic hepatitis C or neoplasms. Occasionally, IFN-alpha-associated retinopathy may be responsible for permanent loss of visual acuity. Although IFN-beta shares several activities with IFN-alpha, its use has been associated with retinopathy only in one MS patient [1].

Methods: We describe two patients with relapsing-remitting multiple sclerosis (RRMS) and diabetes mellitus (DM) type I developing a severe proliferative retinopathy after starting IFN-beta treatment.

Results: Patient 1: A 34-year-old woman affected by DM type I and RRMS had been receiving IFN-beta1b (250 μg subcutaneously e. o. d.) for 18 months when she was diagnosed a bilateral proliferative retinopathy. Ophthalmoscopic findings consisted of preretinal neovascular proliferation with intravitreal hemorrhages. Previous funduscopic examinations performed yearly had revealed only minor signs of diabetic non proliferative retinopathy. IFN-beta1b was stopped and the patient was given glatiramer acetate (20 mg subcutaneously q. d.) and laser photocoagulation with stabilization of retinopathy and neurological conditions for up to 4 years.

Patient 2: A 35-year-old woman affected by DM type I and RRMS had been on IFN-beta1a (30 μg intramuscularly o. w.) for 27 months when she suddenly complained of marked reduction of visual acuity in the right eye. A severe vitreal hemorrhage was found in the right eye with extensive retinal neovascularization in both eyes. Prior retinal examinations obtained yearly had shown only mild signs of diabetic non-proliferative retinopathy. IFN-beta1a was suspended and the patient received glatiramer acetate (20 mg subcutaneously q. d.) without worsening of neurological status for the following 8 months. Proliferative retinopathy also stabilized and a partial recovery of visual acuity in the right eye was achieved after laser photocoagulation.

Conclusions: Severe retinopathy is extremely rare in RRMS patients receiving IFN-beta treatment. However, concomitant DM type I may act as predisposing factor, increasing the incidence and severity of this ocular complication. Careful monitoring of retinal conditions should be undertaken during IFN-beta treatment in patients with RRMS and DM type I. Alternative disease-modifying drugs may be safer than IFN-beta when RRMS and DM type I coexist in the same subject.

## Neuro-ophthalmology

P320

**Treatment of downbeat nystagmus with 3,4-diaminopyridine – a placebo-controlled, double-blind study.** O. Schüler, M. Strupp, S. Krafczyk, K. Jahn, F. Schautzer, U. Büttner, T. Brandt, Ludwig-Maximilians University, LKH Villach Carinthia (Munich, D; Villach, A)

Background: Several drugs that primarily act on GABA or muscarinic receptors have been used to treat downbeat nystagmus (DBN) syndrome despite their having only moderate success and causing several side effects that limit their effectiveness. These drugs were tested under the assumption that DBN was caused by a disinhibition of a physiological inhibitory cerebellar input on vestibular nuclei.

Objective: We evaluated the effects of a single dose of the potassium-channel blocker 3,4-diaminopyridine (3,4-DAP), which is known to increase the excitability of Purkinje cells, on DBN in a prospective, placebo-controlled, double-blind study with a crossover design.

Methods: Seventeen patients with DBN due to cerebellar atrophy [5], infarction [3], Arnold-Chiari malformation [1], or of unknown etiology [8] were included in the study (one out of 18 patients had to be excluded). Mean peak slow-phase velocity (PSPV) was measured before and 30 min after randomized ingestion of 20 mg 3,4-DAP or placebo orally; at least 1 week later the treatments were switched.

Results: 3,4-DAP reduced mean PSPV of DBN from 7.2 ± 4.2 deg/s (mean ± SD) before treatment to 3.1 ± 2.5 deg/s 30 min after ingestion of the 3,4-DAP ($p < 0.001$, two-way ANOVA). Placebo had no measurable effect. In 10 of 17 subjects the mean PSPV decreased by more than 50 %, in 12 of 17 by more than 40 %. In parallel the subjects had less oscillopsia and felt more stable while standing and walking. Nine of the subjects continued to take the drug with success. Except for transient, minor perioral or digital paresthesia reported by three subjects and nausea and headache reported by one, no other side effects were observed. Conclusions: In this study we demonstrated that a single dose of 3,4-DAP significantly improved DBN. In view of animal studies reporting that micromolar concentrations of 4-aminopyridine increased the excitability of Purkinje cells, we suggest that its efficacy may be due to an increase of the physiological inhibitory influence of the vestibulocerebellum on the vestibular nuclei.

P321

**Linearity in visual gap perception.** S. C. Manson, J. C. Gore, Yale University (New Haven, USA)

It has been proven that the brain does not respond linearly to a compounded series of visual impulse stimuli [1]. Instead, there is a saturation limit after which the brain will react no further to additional stimulation. This principle was applied to a reverse situation where there was a continuous 15 second continuous stimulus interspersed with gaps ranging between 500ms and 4500ms. It was shown that there is a time threshold at 1500ms below which the brain no longer recognizes the presence of the gap and that the brain also responds non-linearly to a lack of stimuli.

Method: 7 right handed adults were used as subjects. A circular black and white checkerboard and its converse white and black checkerboard were used as visual stimuli, and were alternated at a rate of 10Hz creating the illusion of motion. Each subject was simply asked to focus on the flashing screen and keep eyes open at all times. The baseline was 15 seconds of checkerboard followed by 15 seconds of white screen, repeated 5 times. The actual task involved alternation between checkerboard and impulse gap with the same 15 second checkerboard sequence followed by white screen gaps of 500ms, 1000ms, 1500ms, 3000ms and 4500ms with seven blocks in each run. The imaging was done in a 1.5 Tesla magnet using an epibold sequence at TR = 1500ms, TE = 60ms, flip angle = 60°. Because of the soporific nature of the task, subjects could only be kept alert in the scanner for half an hour, so repeating each gap length and baseline three times, it was only possible to give each subject two different gap lengths.

All image analysis was done in matlab.

Results: My data indicated that the BOLD response to the increasing gaps was consistently the same, no matter the size of the stimulus. A prolonged gap didn't result in a prolonged response. Instead the response was a uniform shape that only varied in magnitude. Regardless of length of gap, the maximum deactivation was observed between 7500ms and 9000ms after the onset of the gap.

The longest gap (6000ms) elicited the largest mean magnitude response with a 1.4 % ± 0.42 % deactivation. The 4500ms gap showed a deactivation of 0.63 % ± 0.14 %. The 3000ms gap deactivation was 0.27 % ± 0.1 %, the 1500ms gap deactivation was 0.22 % ± 0.03 % and the 1000ms gap deactivation was 0.145 % ± 0.078 %. The error on the deactivation from the 1000ms gap was half the size of the actual deactivation. In addition, the time course and the activation maps don't indicate any type of robust change. Therefore the cut-off gap length for BOLD detection of deactivation was deemed to be between 1000ms and 1500ms.

A regression analysis was run on the values above to determine the relationship on gap size and deactivation magnitude. Linear regression resulted in an R value of 0.8539. However, by doing exponential analysis, R was improved to 0.966, therefore confirming that deactivation, similar to activation, is a non-linear process.

P322

**Age-dependent increase and decrease of torsional eye movement responses to galvanic vestibular stimulation.** K. Jahn, A. Naessl, E. Schneider, M. Strupp, T. Brandt, M. Dieterich, Ludwig-Maximilians University, Johannes Gutenberg University (Munich, Mainz, D)

To investigate age dependent changes we analyzed torsional eye movement responses to binaural and monaural galvanic vestibular stimulation (GVS) in 57 healthy subjects (20–69 years old). GVS (1–3 mA) induced torsional eye movements consisting of static torsion toward the anode (amplitude 1–6 deg) and superimposed torsional nystagmus (slow phase velocity 0.5–3 deg/s, quick phase amplitude 0.5–2 deg, nystagmus frequency 0.75–1.5 s-1). Static ocular torsion and torsional nystagmus increased from the third to the sixth decade and decreased in older subjects, e. g., slow phase velocity increased from 1.5 deg/s (20–29 years) to 2.9 deg/s (50–59 years) and decreased to 2.5 deg/s for the seventh decade (60–69 years). Thus, an inverse U-shaped curve was found for the dependence of torsional eye movement responses on age.

All structures relevant for vestibular function degenerate with age, but at varying times. Since hair cell loss precedes those seen in the vestibular nerve and Scarpa's ganglion, the decrease in hair cell counts could be compensated for by increased sensitivity of afferent nerve fibers or central mechanisms. Increased sensitivity could thus maintain normal function despite reduced peripheral input. As GVS acts at the vestibular nerve – thereby bypassing the hair cells – electrical stimulation should be more efficient in subjects with reduced input from hair cells, as seen in our data up to the sixth decade. The degeneration of nerve fibers, ganglion cells, and central neurons becomes evident at older ages. Thus, the compensatory increase in sensitivity breaks down, and GVS-induced eye movements decline, a finding that is reflected by the inverse U-shaped curve for age dependency presented in this study.

P323

**The congenital cranial dysinnervation disorders.** N. J. Gutowski, T. M. Bosley, E. C. Engle, Royal Devon and Exeter Hospital, King Khaled Eye Specialist Hospital, Children's Hospital Boston on behalf of the European Neuromuscular Centre International Workshop

A multi-disciplinary group of 13 clinicians and researchers from six countries convened for the first time, hosted and facilitated by the European Neuromuscular Centre (ENMC), to form an international consortium to study a group of congenital neuromuscular diseases characterized by abnormal eye, eyelid, and/or facial movement. This group of diseases includes Duane syndrome, congenital fibrosis of the extraocular muscles (CFEOM), Möbius syndrome, horizontal gaze palsy, congenital ptosis and congenital facial palsy. Although these disorders were previously referred to in the literature under various terms, including "congenital fibrosis syndromes", we have now chosen to refer to them as the "congenital cranial dysinnervation disorders" or CCDDs. This name reflects our belief that these disorders result from developmental errors in innervation of the ocular and facial muscles. Thus far, members of the consortium have identified ten CCDD genetic loci and two CCDD disease genes (PHOX2A mutated in CFEOM2 and SALL4 mutated in Duane syndrome with radial ray anomalies). A CCDD classification scheme and an International CCDD Consortium have been established. The goals of the Consortium are to foster continuing research into the genetic basis of these disorders by identifying new families and affected individuals and by the sharing of genetic resources. Future studies of the CCDD genes should enhance our understanding of the pathophysiology and treatment of these disorders.

P324
**Activations at the basal ganglia-thalamus level during optokinetic stimulation and voluntary saccades (fMRI study).** A. Nolte, T. Stephan, A. Mascolo, T. Yousry, H. Brückmann, T. Brandt, M. Dieterich, Department of Neurology, Department of Neuroradiology (Mainz, Munich, D; Pavia, I; London, UK)

Neurophysiological studies in animals have shown that different neuronal assemblies in the brainstem are responsible for the generation of saccades and optokinetic nystagmus. How the visual information during both paradigms reaches the frontal eye fields and from there the brainstem areas is still under discussion. The aim of this fMRI study was to compare the activation patterns of the basal ganglia-midbrain-thalamus region during the stimulation of the ocular motor system by horizontal OKN and horizontal voluntary saccades.

We examined 12 healthy right-handed volunteers during (A) voluntary horizontal saccades (± 12°, 1 Hz), (B) OKN by fixation of horizontal black and white stripes (5° thickness, 8°/s), and (C) the rest condition with fixation of a white dot straight ahead. The computer-generated visual stimulations of both paradigms were presented on MRI-compatible video glasses with a visual field of 30° in horizontal and 20° in vertical directions.

The functional images were acquired on a 1.5 T standard clinical scanner with echo planar imaging (EPI) with a T2*-weighted gradient-echo multislice sequence (TE = 60 ms, voxel size 3.75x3.75x4 $mm^3$, matrix 64x64, interscan interval 2.5 s).

Activations were found bilaterally at the midbrain level (red nucleus, substantia nigra, superior colliculus), the basal ganglia level (putamen, globus pallidus, caudate nucleus, parahippocampal gyrus, anterior internal capsule) and at the thalamus-insular level (dorsomedial and posteromedial ventral nucleus of the thalamus, genicular body, superior temporal gyrus, anterior insula). These activation loci were identical during both paradigms (conjunction analysis). Differential areas were seen during OKN only in the right superior colliculus, left pallidum, putamen, internal capsule, anterior insula, and caudatus (OKN vs saccades), whereas in the opposite condition (saccades vs OKN) no differences were found.

In conclusion, both ocular motor paradigms share the same anatomical areas within a network at the basal ganglia-thalamus level, parts of which belong to the subcortical efferent pathways of the basal ganglia-thalamo-cortical motor loop. Activations seemed to be stronger during OKN than during saccadic stimulation.

P325
**Alcohol does not decompensate a compensated unilateral peripheral vestibular deficit.** V. C. Zingler, M. Strupp, S. Krafczyk, T. Brandt, Klinikum Grosshadern, LMU (Munich, D)

Introduction: Incidental reports of reduced tolerance to low dose alcohol intake by patients with centrally compensated vestibular neuritis led to the hypothesis that alcohol may decompensate a compensated peripheral vestibular deficit. This is supported by animal studies on compensated hemilabyrinthectomized cats, which have shown that spontaneous nystagmus to the intact side recurred after alcohol injection. We, therefore, investigated the influence of alcohol on recurrence of nystagmus, displacement of subjective visual vertical (SVV), and increased body sway in patients with compensated vestibular neuritis but a persisting peripheral deficit.

Methods: We examined 11 male patients (mean age 60 ± 12.7 years, range 35–74 years) with persisting unilateral peripheral vestibular deficit after vestibular neuritis. Vestibular neuritis had occurred on the average 29.2 months prior to the study (range 3–69 months). A blood alcohol level of approximately 0.1 % was achieved by ingestion of 0.8 g alcohol/kg body weight (2.5 ml vodka/kg body weight). The alcohol was dissolved in orange juice [1:4] and presented in four portions, one of which was drunk every 10 minutes. The following three parameters were determined before the first and 30 minutes after the last ingestion of alcohol: video-oculography (to detect spontaneous nystagmus), SVV (adjustment measured in degrees for perception), and posturography (total sway path values (SP) for postural control, measured in meter/minutes).

Results: None of the patients showed spontaneous nystagmus after alcohol ingestion. Alcohol also did not cause a significant displacement of the SVV in any of the patients (mean ± SD before ingestion: 1.8° ± 2.0°, 35 minutes after ingestion 1.4° ± 1.3°). Patient stance showed no significant deterioration of postural stability after alcohol ingestion (condition: standing on a foam-padded platform, eyes closed: total SP 1.96 ± 1.48 m/min before ingestion, 40 minutes after 1.36 ± 0.83 m/min).

Conclusions: Our results showed no significant deterioration of the three specific variables for the vestibulo-ocular, vestibulo-spinal, and visual systems. Thus, alcohol in a dose up to 0.1 % does not cause a decompensation of the central compensatory achievement following unilateral peripheral vestibular deficit.

P326
**Functional monocular blindness due to visual neglect.** M. Vokaer, J.-C. Bier, P. Fery, J. Garbusinski, S. Blecic, E. J. Bartholomé, Erasme Hospital (Brussels, B)

Background: Oculomotor nerve disease is a common cause of diplopia. When strabismus is present, absence of diplopia has to induce the research of either uncovering of visual fields or monocular suppression, amblyopia or blindness. We describe the case of a patient presenting with right oculomotor III paresis and left object-centered visual neglect. She never suffered from diplopia despite binocular vision and progressive recovery of strabismus excluding uncovering of visual fields.

Case report: A 41-year-old woman presented with a right fronto-parietal haemorrhage expanding to the right peri-mesencephalic cisterna caused by the rupture of a right middle cerebral artery aneurysm. Neurological examination disclosed a right oculomotor III paresis with eyelid ptosis and reflectic mydriasis. Complete neuropsychological examination was performed. Description of complex visual scenes was impaired for the left elements. A drawing copy task showed a left object-centered visual neglect. Visual acuity was normal for both eyes (10/10). Oculomotor palsy was confirmed by Lancaster test. Bagolini striated lense test revealed a normal binocular vision without monocular suppression. Even when recovering, the patient never suffered from diplopia. However, she often closed the right eye while being unable to explain why.

Discussion: In this case, usual causes of absence of diplopia were excluded. Considering the normal visual acuity, there were clearly no monocular blindness or amblyopia. The Bagolini striated lense test excluded monocular suppression as the cause of the absence of diplopia. Uncovering of visual fields was not the explanation of our case's presentation, as diplopia never appeared despite the progressive recovery of the oculomotor III paresis. Moreover, the fact that the patient frequently closed the right eye argues for an unconscious perception of two images and so for a covering of her visual fields. Hemispatial visual neglect is defined as a defect of perception, attention, representation, and/or performing actions in the area contralateral to a cerebral lesion. Since all other causes were excluded in this case, we hypothesize that the absence of diplopia was due to the object-centered visual neglect.

Conclusion: Our case is the first description of functional monocular blindness due to visual neglect.

P327
**Delayed bilateral optic neuritis caused by low-dose fludarabine: a case report.** B. Kis, M. L. Mono, P. Berlit, Alfried Krupp Hospital (Essen, D)

Objective: To report a case of bilateral optic neuritis with transient oligoclonal banding caused by low-dose fludarabine treatment.

Background: The purine analogue fludarabine phosphate is indicated for the treatment of patients with B-cell chronic lymphatic leukemia (B-CLL). CNS toxicity is known to appear with a high-dose regimen (> 96 $mg/m^2$), but is reported infrequently in patients treated with currently recommended doses. The onset of symptoms varies and is occasionally delayed up to several weeks. The occurrence of optic neuritis and brain demyelination was reported in the Phase II Study in rare cases treated with high-dose fludarabine (J Clin Oncol 1986;4;74–79).

Design/Methods: Report of a patient with bilateral optic neuritis after low-dose treatment with fludarabine who was admitted to our hospital in 2002.

Results: A 72-year-old woman was diagnosed of B-CLL (RAI stage II) when aged 68 years. Two years later, she was treated with chlorambucil when splenomegalie, leukocytosis and lymph node enlargement appeared. Due to disease progression, a treatment with fludarabine with a dose of 25 $mg/m^2$ was initiated, which was administered for 5 days in XII/2000 and I/2001 and for 3 days in II/2001. Several follow-up examinations showed the patient in excellent clinical state with good partial remission. In XI/2001 the patient developed a subacute visual loss over three days. The neurological examination revealed complete blindness of the left eye and an extensive vision loss on the right. Two MRI scans done in XI/2001 and III/2002 presented symmetrical hyperintensive T2 lesions of both optical tracts without gadolinium enhancement or evidence for focal cerebral demyelinisation or leukencephalopathy. Cerebrospinal fluid (CSF) evaluation revealed oligoclonal banding (IgM & IgG) at the same time. The patient was treated with oral corticoids and intravenous immunglobulins in I/2002 without clinical benefit. At her last visit in VIII/2002, the neurological examination and the MRI remained unchanged. Visual-evoked poten-

tials were absent. The ophthalmoscopic examination revealed a bilateral optic atrophy. At this time, CSF was entirely normal, and there was no oligoclonal banding. The results of an extensive workup for known causes of optic neuritis were all negative.

Conclusion: This is the first case of bilateral optic neuritis with transient inflammatory activity leading to irreversible optic atrophy associated with regular-dose fludarabine and a delay of 11 months. We recommend caution in the administration of fludarabine, with prompt investigation even at conventional doses and a delayed onset of neurological symptoms. The underlying mechanism of the CNS toxicity might be a demyelinating degeneration caused by an autoimmune process.

## Pain and headache

P328

**Serotonin transporter deficient mice have reduced thermal hyperalgesia.** F. Palm, M. Zelenka, R. Moessner, M. Gerlach, K.-P. Lesch, C. Sommer, University of Wurzburg (Wurzburg, D)

Background: Serotonin is involved in pain transmission at different levels of the nervous system. We have previously shown that mice deficient of the serotonin transporter (5-HTT -/- mice) have reduced thermal hyperalgesia after a nerve lesion. Injection of complete Freund's adjuvant (CFA) induces ectopic activity and sensitization in afferent nerve fibers without overt nerve damage. Here we investigated the role of the serotonin transporter in pain after injection of CFA.

Methods: 5-HTT -/- mice of C57Bl/6 background and wild type (WT) controls were used. CFA was injected subcutaneously into one hind paw and the development of paw swelling and pain related behavior was monitored. Thermal hyperalgesia was tested according to the Hargreaves method, mechanical allodynia was assessed using von Frey hairs.

Results: The CFA-injected hindpaw was swollen from 4 hours to 4 days after injection without differences between genotypes. Thermal hyperalgesia was present in WT mice from 4 hours to 5 days after CFA, in 5-HTT -/- mice it was only transiently present until day 1. Mechanical thresholds as assessed with von Frey hairs were not different between genotypes throughout the experiment.

Discussion: The 5-HTT -/- phenotype protects from CFA-induced thermal hyperalgesia, but not from the other sequels of inflammation like swelling and reduction of mechanical thresholds. Given that serotonin levels are reduced in peripheral tissues of 5-HTT -/- mice, we propose a prominent and selective role for peripheral serotonin in the development of ectopic activity in a subset of afferent nerve fibers.

P329

**Efficacy and safety of Topiramate in migraine prevention: a double-blind, placebo-controlled dose-response trial.** M. J. A. Láinez, W. Neto, J. Schmitt, Hospital Clinico de Valencia, Spain, J&J PRD, USA and TOPMAT-MIGR-001 Investigators

Objective: To assess the efficacy and safety of topiramate in the prevention of migraine headaches.

Background: Topiramate (TPM) is an antiepileptic drug which inhibits glutamate-mediated neurotransmission at the AMPA/kainate receptor subtype, enhances GABAA receptor-mediated chloride flux, and inhibits high voltage activated Ca++ channels. These actions could be beneficial to migraine prophylaxis, as suggested by open pilot trials.

Method: This multi-centre, randomised, double-blind, placebo-controlled trial was conducted over 26 weeks. Patients with migraine (± aura) received placebo or TPM, after 4 weeks baseline. TPM was escalated at 25 mg/day weekly increments in 8 weeks from 25 mg to target doses of 50, 100, and 200 mg/day (divided doses). Mean monthly migraine frequency defined as periods with migraine pain for 24 hrs or less; migraine pain persisting or recurring for at least 24 hrs = a new event), responder rate (at least 50% reduction in monthly migraine frequency), mean monthly migraine attack rate (IHS criteria), monthly migraine days, number of days per month requiring rescue medication, and safety data were analysed.

Results: Of the 487 patients, 469 entered the intent-to-treat analysis (89% female, aged 41 [13–70] years, n = 117–128 per group). When compared to placebo, TPM 100 and 200 mg (but not 50 mg) daily significantly reduced mean monthly migraine frequency (–2.1 and –2.2 vs. –0.8 with placebo; $P < 0.001$, as early as the first month of treatment), mean monthly migraine days (–2.7 and –2.6 vs. –1, $P < 0.001$) and monthly need for acute medications ($P = 0.005$ and $= 0.002$). Mean monthly migraine attack rate (IHS) at trial end was significantly reduced by –1.3, –1.9 and –2.1 with TPM 50, 100 and 200 mg/day versus –0.7 with placebo ($P = 0.069$, $< 0.001$ and 0.001 respectively). The percentage of responders was significantly higher for each of the TPM dosages when compared with placebo (36–54–52% versus 23%, $P = 0.039$, $< 0.001$ and $< 0.001$). Drop outs due to adverse events increased dose-dependently with TPM to 17–20–33% respectively (most common: paresthesia, anxiety, difficulty sleeping and hypoesthesia), versus 10% with placebo. TPM 50, 100 and 200 mg/day was associated with a decrease of 2.4, 3.8 and 3.8% in body weight, vs. placebo (0.3%).

Conclusions: A TPM dose as low as 100 mg/day consistently improved migraine parameters. The apparent dose-dependency of TPM's efficacy and safety suggests a role for titration to optimal treatment response in migraine.

P330

**Prevalence and clinical features of headache among medical students.** M. R. Gheini, N. Shafizade, Sina Hospital (Tehran, IR)

Headache is a highly prevalent condition with considerable impact on social activities and work. In order to show prevalence, clinical features and social impact of headache, we conducted this study on medical students of Tehran University of medical science in 2000. The data were gathered by a self-administered questionnaire method. The study population consisted of 582 persons, 307 male and 275 female, 17–35 years old. At first the persons who reported at least one attack of headache in every month, or had consulted a doctor for headache, or had impairment of activities because of headache, were separated. These persons responded to another questionnaire, which was about their headache characteristics. After information was obtained, each type of headache was classified according to International Headache Society criteria. 10% responders reported that they had never suffered from headache. 47.1% had moderate or severe impairment of their daily activities because of headache. The overall prevalence of tension-type headache, migraine without aura, migraine with aura and unclassified headache were 30%, 15%, 4%, and 10% respectively. Prevalence of migraine was 19% in women and 11% in men, the corresponding prevalence of tension-type headache was 32% and 29%. 11.2% of responders had consulted a doctor for their headache and 25% had used different medications for their headache. We conclude that the headache disorders are very common but they are neglected even in medical students.

P331

**Cranial magnetic resonance imaging findings in cases with migraine.** E. Gozke, O. Ore, N. Dortcan, Z. Unal, M. Cetinkaya, PTT Teaching Hospital, MEB Validebag Hospital (Istanbul, TR)

Objective: To investigate the frequency of cranial magnetic resonance imaging (MRI) abnormalities in patients with migraine and to find their relationships with type of migraine, duration of disease and frequency of attacks.

Methods: Forty-five patients (43 female, 2 male) with migraine whose ages ranged between 19–53 (mean 40.91 ± 7.69) were evaluated. Of 45 patients 20 had migraine with aura and 25 had migraine without aura. The diagnosis of migraine was made according to International Headache Society criteria.

Results: In 13 (28.8%) out of 45 cases white matter foci (WMF) were present. Migraine with (n:8; 61.5%) or without aura (n:5; 38.4%) was detected in these 13 cases. The number of patients with WMF was significantly higher in patients with aura than in patients without aura (8/20; 40% versus 5/25; 20%). It was found that as the frequency of attacks per month increased, the number of patients with WMF also increased. Although mean duration of disease was higher in patients with WMF than without WMF, there was no significant difference (149.5 ± 87.9 versus 134.1 ± 88.3 months, $p > 0.05$).

Conclusion: Although there is no specific MRI finding peculiar to migraine detection of WMF should be taken into consideration in patient with migraine (especially migraine with aura) and frequency of attacks are important for existence of WMF.

P332

**Prevalence and clinical features of headache in Tehran.** M. R. Gheini, A. Soltanzade, H. Sikaroodi, Sina Hospital, Doctor Shariati Hospital (Tehran, IR)

Headache is a highly prevalent condition that can be disabling. Widely differing prevalence rates are reported in various populations. Overall and sex specific prevalence of headache including migraine, for Iranians, are

unknown. In 1995–1996 a population-based study of prevalence of headache in a sample of 808 persons, aged 15 and above, was carried out in Tehran. They were interviewed using questionnaires. At first we separated the persons who had reported at least one attack of headache in every month, or had consulted a doctor for headache, or had impairment of activities because of headache. These persons were asked to respond to detailed questions about symptoms, frequency, and severity of headaches. Headaches were classified using the International Headache Society criteria (1988). 10.6% of men and 8% of women reported that they had never suffered from headaches. Disability, defined as being absent from work, was relatively common (30.7% in total population). The overall prevalence of tension-type headache, migraine without aura and migraine with aura were 20.4%, 10.8% and 1.2% respectively. Prevalence of migraine without aura was 13.7% in women and 7.3% in men. The corresponding prevalence of tension-type headache was 21.6% and 19%. Only 51.7% of migraine and 40% of tension-type headache sufferers had ever consulted a physician for their headache problem. We conclude that the headache disorders are extremely prevalent and represent a major health problem.

P333

**Prevalence and clinical characteristics of migraine headache in Edirne, Turkey.** Y. Celik, G. Ekuklu, B. Tokuc, U. Utku, University of Trakya (Edirne, TR)

Objective: Describe the prevalence and clinical features of migraine headache in Edirne, Turkey.

Design/Methods: This descriptive cross-sectional study was conducted in Edirne city center, Turkey, a city of 110,580 resident according to the 2001 census, between February 2002 and May 2002.

In this study, 386 subjects aged 14 and older were personally interviewed by a practitioner. Migraine diagnosis was made according to the classification criteria of migraine proposed by the International Headache Society, 1988. Subjects were asked to respond to questions about severity, frequency, location, duration, associated symptoms of their headache.

Results: A total of 77 lifetime migraineurs (61 females and 16 males) were identified. The mean age of cases with migraine was detected as 34.3 ± 10.8. The lifetime prevalence of migraine was 29.7% in women, 8.7% in men, and 19.9 in both sexes. Among 77 migraineurs, 61 (79.2%) were women and 16 (20.8%) were men; 63.7% had migraine without aura, 36.3% migraine with aura. The lifetime prevalence of migraine, migraine without aura, migraine with aura was 19.9%, 12.7%, and 7.2%, respectively.

Conclusions: The prevalence of migraine in this Turkish population showed quite similar to those reported in Europe and the United States.

P334

**Precipitating factors of migraine attacks in patients with migraine without aura.** O. Deniz, R. Aygül, N. Koçak, A. Orhan, M. Kaya, Atatürk University (Erzurum, TR)

Objective: Studies specifically devoted to precipitating factors in migraine are rare. The aim of the study was to determine the distributions of triggers of migraine in a population attending neurology clinic.

Methods: One hundred eighty five patients who fulfilled the diagnostic criteria for migraine as proposed by the International Headache Society were evaluated by means of a personal interview.

Results: The mean age at consultation was 32.37 ± 10.16 years, and the mean duration of migraine history was 9.30 ± 7.58 years; 74.6% (138 patients) met criteria of migraine without aura and 25.4% (47 patients) of migraine with aura. Stress and mental tension (70,8%) were found to be most common precipitants of both migraine without aura and migraine with aura. Other common precipitants were lack of sleep (48.4%), noise (42.2%), not eating on time (41,6%), foods/drinks (32.9%), light (31.9%), and menstruation (31%). Afterwards, in descending order of frequency, were cited smell (25.9%), fatigue (21.6%), heat/cold weather (16.8%), smoking (15.7%), travel (9.2%), and sexual activity (1,1%). The distributions of triggers in patients with migraine with aura and migraine without aura did not differ significantly from each other, except for fatigue, travel, and smoking. The same was true for the results of the female and male patients.

Conclusion: It is concluded that endogenous and exogenous factors seem to play an important role in the triggering of migraine.

P335

**Fitness for work in late whiplash syndrome: long-term efficacy of an inpatient multimodal treatment evaluated on 197 patients.** B. Grunder, A. Brüderlin, V. Stange, H. E. Kaeser, U. Kischka, T. Ettlin, Rehaklinik Rheinfelden (Rheinfelden, CH)

Aim: To assess the long term effect of an inpatient multimodal treatment after whiplash injury on the development of fitness for work. There are only limited data available in the literature.

Method: 197 patients between 17 and 64 years of age (124 female) who had participated in an inpatient therapy programme at Rehaklinik Rheinfelden Rehabilitation Centre the first time, were sent a questionnaire assessing the current fitness for work. It was filled in and returned by 138 patients.

Results: At 6 months after the end of the rehabilitation programme, 52 patients (38%) had a higher fitness for work than at admission to the rehabilitation centre. The average increase in fitness for work was 54%. Of these 52 patients, 16 patients were 100% fit for work, 36 patients were partially fit for work. 79 patients (57%) had the same fitness for work at 6 months as on admission. Out of those 79 patients, 45 had 0% fitness for work, 29 had partial fitness for work, and 5 had 100% fitness for work. Four patients (3%) had lower fitness for work at 6 months than on admission. Three patients (2%) were undergoing vocational retraining organized by the Invalidenversicherung (Incapacity Insurance). 71 patients (51.5%) had started the inpatient rehabilitation less than one year after the accident. Patients whose fitness for work improved had a significantly shorter posttraumatic interval before starting the inpatient treatment than those whose fitness for work failed to improve.

Conclusion: Inpatient rehabilitation has a positive effect on the long term development of fitness for work. The effectiveness of inpatient rehabilitation on fitness for work after whiplash injury is greater if it starts early after the accident.

# Poster session 2

## Cerebrovascular disorders

P336

**Visual hallucinations and haemorrhagic stroke.** F. Flocard, H. Taillia, P. Kouna, T. De Greslan, J.-L. Renard, D. Bequet, Hôpital du Val-de-Grâce (Paris, F)

Hallucinations that is perceptions without object (Lhermitte) may concern all modes of somesthesia. Visual hallucinations have been the subject of many works, especially following the 1914–18 conflict when focused cranio-cerebral wounds were frequent. We report on the observation of a 81 year old patient, hypertensive, who is subject to visual hallucinations of a very rich variety. The clinical manifestations start with temporary balance disorders (for about 12 hours) associated with rotational dizziness. Thereafter the patient describes a modification of his visual perception involving an impression of distortion of his face when seen in a mirror. Then, in succession, projected on his local environment, he sees various complex visual hallucinations (i. e. an officer of the mounted Canadian-police with horses and cattle; fishes swimming from left to right; colored roadmap; down-rolling pearls of different colors; black and white horses on a TV screen background; flies clotted in the corner of a room; boats and anglers. The hallucinatory process concerns the entire visual field of this patient who suffers from a left lateral homonymous hemianopsia. The descriptions made by the patient are especially detailed. Although some scenes are static (for instance a wallpaper), most are moving and colorful. The troubles come and go and disappear over 48 hours. The patient remains conscious and always critical of the perceived visual scenes. The MRI detects a right haemorrhagic occipital stroke (V2 and V3 area). We investigate the distinct rôles of the right occipital lobe, the right temporal lobe, the associative pathways and the left contro-lateral hemisphere of the lesion. Further, we discuss the associated physio-pathologic mechanisms (focal epileptic process or deafferentation).

P337
**Abrupt warfarin withdrawal in patients with stroke is associated with hypercoagulable state.** I. Tezer, G. Gurer, H. Ay, O. Saribas, Hacettepe University (Ankara, TR)

Abrupt termination of warfarin in patients with previous history of myocardial infarction or deep venous thrombosis has previously been linked to thrombotic events including stroke. A rebound hypercoagulable state caused by over-release of coagulation factors from the suppression by warfarin has been suggested to be the most likely mechanism for these thrombotic events. Accordingly, previous studies of patients with myocardial infarction revealed that abrupt cessation of warfarin was associated with higher F1 + 2 levels than tapering the dose gradually. In the present study, we aim to investigate the question of whether rebound hypercoagulabity occurs in patients with stroke following abrupt or gradual cessation of warfarin.

A total of 16 patients with stroke who were put on short-term warfarin treatment (3 to 6 months) at the discretion of their treating physician were included. Patients were randomly stratified into two groups. Warfarin was stopped abruptly in the first group and gradually decreased by one third of the weekly dose in every three days and stopped in six days in the second group. Blood samples for INR, Factor VIIa, II, TAT, F1 + 2, and d-dimer were collected at baseline and on days 3,6,9,12,15 and 21 after the cessation of warfarin. The time course of changes in the levels of hemostatic factors was determined in each group.

In group 1, Factor II, d-dimer, and Factor VIIa levels increased during the first 6 days ($p = 0.001$, $p = 0.021$, $p = 0.039$), but only Factor II levels continued to rise thereafter ($p = 0.001$). Except for Factor II, none of the hemostatic factors changed during the study period in group 2. Factor II levels showed a significant increase from baseline by day 9 ($p = 0.001$). TAT and F1 + 2 levels exhibited no change with respect to baseline in either group. No thrombotic event occurred during the course of study period.

This pilot study shows that abrupt cessation of warfarin in patients with stroke is associated with a hypercoagulable state characterized by elevated levels of factor II.

P338
**HAMLET hemicraniectomy after MCA infarction with life-threatening oedema trial.** J. Hofmeijer, H. B. van der Worp, A. Algra, G. J. Amelink, J. van Gijn, L. J. Kappelle, UMCU (Utrecht, NL)

Background: Patients with massive space-occupying hemispheric infarction have a poor prognosis. Non-randomized studies suggest that decompressive surgery reduces mortality and improves functional outcome of survivors. HAMLET is a randomized controlled trial to study the efficacy of decompressive surgery to reduce mortality and to improve functional outcome in patients with supratentorial infarction and space-occupying edema.

Methods: The study design is that of a multi-center, open, randomized clinical trial, which will include 112 patients aged up to 60 years with a space-occupying infarct in the territory of the middle cerebral artery in either hemisphere leading to a decrease in consciousness.

Patients will be randomized to either decompressive surgery, consisting of a large hemicraniectomy and a duraplasty, followed by intensive care treatment, or conservative treatment, consisting of either intensive care treatment or 'standard' therapy on a stroke unit. Randomization will be stratified according to the intended mode of conservative treatment.

The primary outcome measure is functional outcome according to the modified Rankin Scale at one year. Other outcome measures include the Barthel Index, the NIH Stroke Scale, the Montgomery and Asberg Depression Rating Scale, and quality of life as determined by the SF36 as well as a visual analogue scale.

Trial status HAMLET started in September 2002.

P339
**Localization of integrins in the normal rat brain and after middle cerebral artery occlusion followed by reperfusion.** A. Muellner, C. U. A. Kloss, G. Raivich, G. F. Hamann, Klinikum Großhadern, Max-Planck-Institute for Neurobiology (Munich, D)

Integrins are a large family of noncovalent bound, heterodimeric cell surface glycoproteins composed of alpha and beta subunits, that play a crucial role in different cell-cell and cell-matrix adhesion processes. In their function as endothelial receptors for basal lamina components like collagen and laminin, they contribute to the microvascular integrity. During cerebral ischemia and reperfusion the basal lamina antigens disappear, the vascular permeability is impaired, and edema and petechial hemorrhages develop. In the current study, we investigated the localization of different integrin subunits in the normal rat brain and after middle cerebral artery occlusion and reperfusion (MCAO/R).

Ultrastructurally findings in the normal brain showed, that integrins were located on the luminal and on the abluminal side of the endothelia and on perivascular cells. A clear immunoreactivity could be detected for alpha4 on astrocytes and alpha1, alpha6 and beta1 integrins on endothelia. Alpha6 immunoreactivity was stronger on larger vessels, while alpha1beta1 stained all vessels similarly.

Following 3 hours of cerebral ischemia and reperfusion intervals of 0, 9 and 24 hours (I3R0, I3R9 and I3R24, $n = 15$), the number and staining intensity of immunoreactive vessels in the ischemic area were compared to the contralateral side and classified according to their diameters.

Whereas beta1 integrin positive capillaries (6 to 9 µm) showed a significant reduction (by −12 % at I3R0 and −15 % at I3R9 that greatly decreased to −43 % at I3R24 (all $p < 0.05$)), all other vessel sizes remained unaffected. Also the beta1-staining intensity decreased homogeneously over all vessel sizes (by −4 % to −6 % at I3R0, −8 % to −12 % at I3R9 and −16 % to −23 % at I3R24 ($p < 0.05$)). In addition we measured a less pronounced decrease of alpha1 staining intensity. Interestingly, the alpha6-positive capillaries also were reduced by −21 % at I3R24 ($p < 0.05$), but the decrease of the alpha6-staining intensity was confined to vessels larger than 15 µm (−15 % at I3R24, $p < 0.05$), so that we had a diameter-selective loss of vascular integrin presentation. This points to a size-specific interaction between the endothelium and the basal lamina. The prominent capillar vulnerability may significantly contribute to the impairment of the microvascular integrity during ischemia and reperfusion.

P340
**Predictive role of 24-hour blood pressure values on brain oedema formation in patients with acute stroke.** G. Tsivgoulis, K. Spengos, E. Manios, V. Kotsis, A. Synetos, P. Konstantopoulou, K. Vemmos, University of Athens (Athens, GR)

Background: Elevated systolic and diastolic blood pressure (SBP and DBP respectively) values during acute stroke are widely reported. Aim of this study was to examine a possible association between brain edema formation and blood pressure (BP) values during the acute stroke stage by means of 24 hour (24-h) BP monitoring.

Methods: 24-h-BP monitoring was performed in 250 first-ever acute (< 24 hours) stroke patients. CT-brain-scan was performed within 24 hours of ictus and 5 days later to determine the presence of brain edema. Known stroke risk factors were documented. Neurological deterioration on admission was assessed using the Glasgow Coma Scale (GCS). The relationship of 24-h-BP variables, stroke risk factors (age, atrial fibrillation, heart failure, history of hypertension) and patients' clinical characteristics (stroke subtype, headache, vomiting, GCS) with edema formation were studied by univariate and multivariate logistic regression analyses.

Results: From 250 patients (mean age 69.5 ± 8.3) edema formation was present in 76 (30.4 %). Patients with brain edema had significantly higher 24-h SBP ($p = 0.000$), 24-h DBP ($p = 0.000$), 24-h mean BP ($p = 0.000$), 24-h pulse pressure ($p = 0.002$) and 24-h heart rate ($p = 0.000$) values than patients without edema. In the multivariate analysis the odds ratio for edema formation associated with each 10-mmHg increase in 24-hour SBP on admission was 1.25 (95 % CI: 1.04–1.51, $p = 0.019$). Vomiting, atrial fibrillation, intracerebral hemorrhage and GCS were also associated with edema formation.

Conclusions: High 24-h-SBP values in the acute (< 24 hours) phase of stroke are possible prognostic indicators for brain edema formation.

P341
**Diabetes mellitus: a predictor of stroke recurrence among acute first-ever stroke patients.** G. Tsivgoulis, P. Konstantopoulou, K. Spengos, E. Manios, A. Dimitriou, M. Panas, K. Vemmos, University of Athens (Athens, GR)

Background: Diabetes Mellitus (DM) is considered as an independent stroke risk factor. Aim of this study is to assess stroke recurrence among first-ever acute stroke patients with history of DM.

Methods: We studied a consecutive series of first-ever stroke patients admitted to our hospital within 24 hours after symptoms onset from June 1992 to June 2002. Patients were classified into groups according to history of DM and the etiopathogenic mechanism of stroke. They were followed up to 10 years. Recurrent stroke definition was based on WHO diagnostic criteria. Statistical analysis was performed by using Kaplan-Meier estimates.

Resuts: From 1511 study patients (mean age 70.02 ± 11.93), 284 (18.8 %) had a history of DM and in total 217 (14.2 %) suffered a first recurrent stroke. DM was present in 69 (31.8 %) patients with recurrent stroke. Mean

time of stroke recurrence after the first event was 89 months and 97 months for the diabetic and non-diabetic group respectively ($p < 0.04$). The cumulative risk of survival free of stroke recurrence after a 10 year follow-up period was 63.4 % for diabetic and 67.7 % for non-diabetic patients ($p < 0.002$). Stroke recurrence was significantly higher among patients with intracerebral hemorrhage ($p < 0.04$).

Conclusion: Patients with acute stroke and history of DM are more prone to suffer a recurrent stroke after the index event and this happens sooner than in the group of non-diabetic acute stroke patients. DM increases the prevalence of stroke recurrence among patients with intracerebral hemorrhage.

P342

**Pulsatile tinnitus in the ear as a symptom of intracranial arterial stenosis. The role of screening transcranial Doppler (TCD) in diagnosis of this symptom.** J. Wojczal, A. Szczepanska-Szerej, T. Jargiello, Z. Stelmasiak, M. Szczerbo-Trojanowska, University School of Medicine (Lublin, PL)

Tinnitus in the ear is a common symptom in the arteriovenous malformation, the cerebellopontine angle tumor or hypoacusis. We report cases of an intracranial artery stenosis in which the pulsatile tinnitus in one ear, ipsilateral to the stenosed artery was the main symptom and the initial diagnosis was based on screening TCD.

From 660 stroke free patients with tinnitus subjected to screening Transcranial Doppler examination (TCD) from 1999 to January 2003 we observed 20 patients (14 women and 6 men, mean age 57 ± 12,31 years) in whom > 70 % stenosis of the intracranial (terminal) portion of internal carotid artery (tICA) (n = 13) or > 50 % stenosis of the middle cerebral artery (MCA)(n = 7) were diagnosed. TCD diagnosis of tICA and MCA was based on ultrasound criteria of Christou (2001) and of Baumgartner (1999), respectively. Diagnosis was confirmed by digital subtraction angiography in 15 cases, and in 4 cases by magnetic resonance or computed tomography angiography. In 12 patients pulsatile tinnitus was an isolated symptom and in 8 patients it was associated with TIA from the stenosed artery. In all cases the tinnitus increased markedly with the increase in arterial blood pressure.

In differential diagnosis of pulsatile tinnitus in the ear the possibility of the stenosis of an intracranial artery should be taken into account. TCD is a very suitable screening, noninvasive test in such cases.

P343

**Recurrent exertion-induced spinal cord ischaemia due to infra-renal aortic occlusion.** V. C. Zingler, M. Strupp, R. Brüning, L. Lauterjung, T. Waggershausen, T. Brandt, Klinikum Grosshadern, LMU (Munich, D)

Introduction: Recurrent transient paraparesis has been reported in various spinal diseases, in particular in arteriovenous malformation (AVM). We describe a case of recurrent exertion-induced spastic paraparesis in a patient who had the typical features of AVM, but suffered from complete focal occlusion of the infrarenal aorta.

Case report: A 40-year-old man, a heavy smoker, was admitted to our hospital with a 3-months history of painful weakness of the lower extremities and a loss of bladder control, both of which were induced by walking. Neurological examination after a 200-m-long walk revealed a symmetrical spastic paraparesis combined with numbness below L1 and pain in the buttocks and legs. The knee and ankle reflexes were exaggerated, and Babinski's sign was absent. Within 10 minutes of rest normal strength and sensation, as well as normal knee and ankle reflexes returned. Peripheral pulses were absent during and after exertion. Magnetic resonance imaging (MRI) of the spine showed several dilated dural vessels at the thoracic and lumbar levels of the spine but no signs of ischemic myelopathy. Abdominal CT-angiography and digital subtraction angiography revealed a complete occlusion of the infrarenal aorta with reconstitution at both common iliac vessels via a complex network of collaterals. A thrombendarterectomy with an alloplastic Dacron patch was performed. At discharge the patient was symptom-free. Four weeks later he had a relapse with more symptoms than before surgery. A second angiography showed a recurrence and progression of the patient's disease. A second surgery failed due to massive adhesions between the aorta and vena cava. Finally, the patient was treated with warfarine.

Discussion: We assume that our patient had over time developed a chronic thrombotic occlusion of the infrarenal aorta, since a net of collaterals had formed to ensure sufficient residual flow to both common iliac arteries at rest. However, walking probably caused ischemia of the spinal cord, which manifested as recurrent spastic paraparesis. Transient spinal cord ischemia most probably resulted from a steal phenomenon. This is supported by the MRI, which showed dilated collateral spinal vessels.

Conclusions: This is an exceptional case, in which the patient's symptoms and MRI findings suggested an arteriovenous malformation. The absence of peripheral pulses indicated an aortic disease, which was confirmed by angiography.

P344

**Knowledge of stroke risk factors in people with modifiable risk factors.** A. Koçer, N. Ince, Ü. Börü Türk, E. Koçer, Dr. Lutfi Kirdar Education Hospital, Istanbul University, Duzce Medical Faculty (Istanbul, Duzce, TR)

With this study evaluation of modifiable risk factors of stroke, recognition of these factors by patients at risk and effecting factors in a teaching hospital. Structured questionnaires consisting of multiple questions (interrogating age, gender, education, risk factors, awareness of modifiable risk factors, sources of information and treatment status) were completed using face to face interview techniques. Data were evaluated by using frequency, percentage ratio, multiple logistic regression, and chi- square tests. The incidence of awareness of risk factors of stroke was 58.3 percent. Awareness of the risk was not influenced by gender, age and level of education. It was statistically significant for the participants carrying risk factors such as heart disease ($p < 0.0001$ OR: 2–2.4 95 % CI: 1.53–3.28) and hyperlipidemia (p: 0.007 OR: 2.01 95 % CI:1.20–3.36) to know that these factors are also the risk factors for stroke. Compliance of the participants to the treatment was mainly effected by the awareness of the patients that their diseases could be a risk factor for stroke. The presence of multiple risk factors, a nonsmoker status also influenced compliance to the treatment positively. The distribution of informative sources also differed according to the level of education. For uneducated patients and cases with primary education, the impact of informative sources such as environment, doctors, media and books were reported to be 75.1 %; 48.2 %; 18.1 % and 3.1 % respectively, while the corresponding percentages for patients having middle or upper levels of education were 66.5 %; 42.7 %; 43.3 % and 22.0 percent. The increase in the rate of obtaining information from media and books was noteworthy.

P345

**Reduced P-selectin expression on platelets and C-reactive protein after statin therapy in chronic atherosclerotic ischaemic stroke.** J.-K. Cha, K.-M. Yoo, Dong-A University, Kosin University (Busan, KOR)

Background and Purpose: It has been well known that HMG-CoA reductase inhibitor (statin) could reduce the recurrence of atherothrombotic vascular events beyond the lipid lowering mechanism. In this study, we investigated the effect to modulate the surface expression of P-selectin and C-reactive protein (CRP) in chronic atherosclerotic infarction.

Methods: Simvastatin 20 mg daily was administered to 38 patients with chronic atherosclerotic infarction. Normal subjects recruited as controls. The influence of simvastatin on the surface expression of P-selectin on platelets and plasma concentration of CRP was studied.

Results: After simvastatin treatment for 12 weeks, the surface expression of P-selectin ($p < 0.01$) and plasma concentration of CRP ($p < 0.05$) was significantly decreased before the statin therapy. These effects had no relation with cholesterol level. Among the 38 patients, two patients had experienced fatigue.

Conclusions: Short period stain therapy may reduce the prothrombotic and inflammatory mechanism in the processes of atherothrombosis without harmful complications. These modulation effects of statin will make a promise the beneficial effect to prevent the recurrence of atherothrombotic ischemic stroke.

P346

**Systemic thrombolyis with Abciximab beyond the three-hour time window.** I. Dzialowski, G. Gahn, U. Becker, A. Kunz, V. Pütz, H. Reichmann, University of Technology (Dresden, D)

Background: In acute ischemic stroke, intravenous fibrinolysis with tissue plasminogen activator (t-PA) is limited to the first three hours after stroke onset and is therefore a treatment option only in a minority of patients. Thrombolysis with Abciximab, a platelet glycoprotein IIb/IIIa receptor inhibitor, is currently being studied within a 3 to 6 hours time window.

Methods: Since January 2001 we treated 15 selected patients with acute ischemic stroke beyond the 3 hrs time window with Abciximab (0,125 mg/kg bolus, followed by 0,125 microgram/kg/min x 12h). Nine patients additionally received t-PA (0,45 mg/kg). On admission we examined all patients for intracranial arterial occlusion with computed tomography angiography or transcranial color coded duplexsonography. National In-

stitute of Health Stroke Scale (NIHSS) was assessed on admission and at discharge. We treated five patients with acute basilar artery occlusion with Abciximab and t-PA. Six patients with an anterior circulation stroke and middle cerebral artery (MCA) occlusion received either Abciximab alone (n = 3) or Abciximab and t-PA (n = 3). Four other patients with progressive stroke without MCA-occlusion received Abciximab alone.

Results: No symptomatic hemorrhage occurred. Two patients (12.5 %) had asymptomatic cerebral hemorrhagic transformation. In 4/5 (80 %) patients with acute BA occlusion we achieved recanalization. Two out of these died from severe brainstem ischemia. In the remaining 3 patients mean NIHSS changed from 12.7 to 11.7. In the anterior circulation, 6/6 initially occluded MCAs recanalized within 24 h after baseline examination. In this subgroup mean NIHSS improved from 10.4 to 5.2. The remaining four patients without MCA occlusion had an improvement in mean NIHSS from 6.0 to 0.6.

Conclusion: In our heterogenous patient group, intravenous thrombolysis with Abciximab within a 3 to 6 hour time window was a safe and effective treatment option. Outcome remained poor in acute BA occlusion in spite of successful recanalisation. Patient outcome markedly improved in the anterior circulation.

**P347**

**Association of insulin sensitivity and plasma leptin level with ischaemic stroke.** Y.-D. Zhang, Nanjing Brain Hospital (Nanjing, CHN)

Objective: To investigate the association of insulin-sensitivity and plasma leptin level with cerebral infarction.

Methods: The plasma leptin, by means of ELISA, insulin, by means of radioimmunoassay, glucose, lipids levels and body-mass index (BMI) of 31 patients with atherothrombotic cerebral infarction (ACI), 30 patients with lacunar infarction (LI) and 21 healthy controls were determined in this study.

Results: Compared with those in the controls ($3.49 \pm 1.85$μIU/ml and $-2.75 \pm 0.54$), there were an elevation of plasma insulin level ($6.17 \pm 4.33$μIU/ml) ($\ddot{o}2 = 6.060$, $P = 0.014$) and a reduction of insulin-sensitivity index (ISI) ($-3.22 \pm 0.79$) ($t = 3.124$, $P = 0.03$) in the ACI patients. The plasma leptin level was correlated negatively to ISI ($r = -0.633$ in male and $-0.503$ in female, both $P < 0.01$) in the stroke patients.

Conclusions: Insulin resistance is a risk factor for ACI, and increased plasma leptin level plays an important role of pathogenesis of insulin resistance.

**P348**

**The use of thermal diffusion regional cortical blood flow sensors (SABER system) in the management of spontaneous hypertensive hematoma undergoing evacuation and receiving intensive care therapy.** J. Abdullah, Z. Idris, A. Ghani, S. Awang, S. Sayuthi, J. George, A. Tahir, University Sains Malaysia (Kubang Kerian, Kelantan, MY)

The intensive care management of spontaneous hypertensive hematomas secondary to hypertension abruptly may cause cerebral and myocardial ischaemia. A study was done to assess the effect of management of reduction of hypertensive encephalopathy associated with intracerebral hematoma in relation to invasive cerebral blood flow of the pathological site and the 30 day outcome, using the SABER 2000 system.

Materials and methods: 15 chronic hypertensive patients of whom 8 were females and 7 males, had a Glasgow Coma Scale of less than 8 and hematoma size > 40 ml were studied. Exclusion criteria were those with myocardial ischaemia on ECG, bilateral fixed and dilated pupils and no consent for operation. All patients had a ventricular external damage and jugular bulb catheter in the right internal jugular vein. A transcranial Doppler middle cerebral and internal carotid artery measurement was done every 8 hours using a DWL 2 Mega Hz probe for 30 minutes. Autoregulation test was done in all patients for 5 days. All patients were managed with intravenous labetolol.

Results: Those who had on clinic follow up prior to bleeding with a systolic pressure of between 140 and 160 mmHg tolerated MAP of 60 and lower with CBF > 60 ml/100 gm/min with transcranial Doppler values of 50–100 cm/sec. Those who had higher systolic blood pressure of 160–180 mmHg did not tolerate MAP of 60 or less and required a MAP of 80 to 100 mmHg to maintain a CBF of > 80 ml/100 gm/min and a TCD flow of 50–100 cm/sec. There was no correction with diastolic blood pressure for both groups with CBF values. External ventricular fluid drainage to 10 mmHg improved cerebral blood flow even though MAP was low. Head down position improved CBF (0–15 degree compared to 20–35 degree). Good outcome was determined by size of hematoma, age of patient and presence of autoregulation prior to operation ($P < 0.01$).

**P349**

**Endovascular stent-assisted angioplasty for symptomatic intracranial internal carotid artery stenosis.** T. H. Lee, D. H. Kim, B. H. Lee, H. J. Kim, D. S. Jung, C. H. Choi, Pusan National University Hospital, Chosun University Hospital, Metrohospital Anyang (Pusan, Kwangju, Anyang, KOR)

Purpose: To assess the feasibility, safety, and effectiveness of stenting for symptomatic intracranial internal carotid artery stenosis.

Subjects and Methods: Between January 1999 and December 2002, 12 patients with 15 symptomatic intracranial internal carotid artery stenosis (> 50 %) were treated with stent-assisted angioplasty. We retrospectively analyzed the technical success rate of stenting, the procedure-related complications, clinical and angiographic outcome for 2 to 26 (mean 6.4) months of follow-up.

Results: Stent-assisted angioplasty was technically successful in 14 of 15 (93.3 %) lesions. The stent catheter did not reach to the stenotic portion due to the tortuosity of carotid artery, and so it failed in one (6.7 %) patient with a supraclinoid stenosis. In 14 lesion of 11 patients with successful stenting, the postprocedural angiography showed restoration of normal luminal diameter with smooth inner margin. The procedure-related complication was acute thrombosis of stent in only one (n = 1) patient, which was completely dissolved with intraarterial administration of abciximab. All 11 patients were neurologically stable at the clinical follow-up. Angiographic follow-up (n = 1) after 8 months revealed no restenosis of stent.

Conclusion: Endovascular stent-assisted angioplasty for symptomatic intracranial internal carotid artery stenosis was feasible, safe, and effective for alleviating symptoms and improving cerebral blood flow. However, long-term follow-up study would be necessary to evaluate its prolonged effectiveness on the prevention of stroke and durability.

**P350**

**Vertebral artery dissection: observations on radiologic findings, clinical presentation and outcome.** A. Tavernarakis, N.-M. Alexandri, S. Vassilopoulou, P. Papageorgiou, C. Potagas, N. Matikas, Evangelismos (Athens, GR)

Spontaneous dissection of vertebral arteries is a well-recognized cause of ischemic events. However, it can be asymptomatic and it is often accused for subarachnoid hemorrhage (SAH). We present a series of 17 patients admitted from 1990 to 2002 (5 women, 12 men, 20 to 54 years old, mean age 41.05 years). In 8 patients, dissections were multiple, and in 6 there was a bilateral vertebral dissection. Dissections were intracranial in 7. Dissected arteries were occluded in 14 patients (82.35 %).

Our results were compared with literature data. We notice a clear male predominance unlike other studies. Clinical presentation varied from asymptomatic to severe tetraplegia or coma, although clinical outcome was generally favorable as only two patients were left with severe neurologic deficit and no one died. No patient suffered SAH.

**P351**

**Acute pseudobulbar mutism.** M. Bös, C. Grothe, H. Urbach, T. Klockgether, A. Hartmann, University of Bonn (Bonn, D)

A 43-year-old man presented with acute speech arrest and problems swallowing for 3 weeks. Six years earlier he had suffered from an ischemic stroke resulting in severe rightsided hemiparesis. He did not have any speech problems at that time. CT scan on admission showed the old left-sided anterior choroidea artery capsule infarct but neither a new infarct nor a cerebral bleeding. However, diffusion weighted MRI disclosed a new infarct contralateral to the older lesion in "mirror position". Cardiac investigations including TEE, ultrasound of the extra- and transcranial arteries and coagulation testings did not reveal any reason.

Acute pseudobulbar mutism is a rare, but well characterized syndrome caused by bilateral infarction of the anterior choroidal artery territory interrupting corticobulbar tracts within the internal capsule. In contrast, unilateral lesions can be well compensated because of bihemispheric innervation of the caudal motor nuclei.

## Clinical neurophysiology

P352
**Effects of interstimulus interval on early components of the somatosensory evoked magnetic fields in patients with migraine.** E. Lang, M. Kaltenhäuser, S. Seidler, B. Neundörfer, Department of Neurology (Erlangen, D)

Introduction: Amplitudes of the early components (N20m, P35m) of the somatosensory evoked magnetic fields (SEFs) after median nerve stimulation depend on interstimulus interval (ISI). In healthy subjects, the first cortical response (N20m) was stable between the ISIs 0.3 and 5 s whereas the second cortical response (P35m) decreased steadily throughout this range (Wikström et al. 1996). Based on known effects of the ISI on intracellular evoked potentials the authors hypothesized that N20m represents early excitatory postsynaptic potentials (EPSPs) and P35m early inhibitory postsynaptic potentials (IPSPs) in pyramidal neurons of the somatosensory (SI) cortex. Based on this hypothesis we examined the excitatory and inhibitory activity that determine the excitability of the pyramidal neurons of the primary somatosensory cortex in patients with migraine. Hyperexcitability of the visual, auditory and motor cortex have been described interictually in migraineurs.

Methods: We examined 12 patients with migraine (9 women, 3 men, 5 with and 7 without aura) and 7 control subjects without migraine or regular headache. Right median nerve was stimulated electrically at the wrist with stimulus intensity just above motor threshold and at ISIs of 0.3, 0.5, 1.0, 1.5, 2.0, 3.0 and 6.0 ms in random order. SEF were recorded with a 37 channel neuromagnetometer (Magnes II, BTI). The sensor was placed with the center above C3 according to the 10–20 system and 200 stimulus responses were averaged. Amplitudes of N20m and P35m were determined as the root mean square of all channel deflections at the corresponding latencies.

Results: N20m and P35m could be recorded in all subjects. In both groups, amplitudes of N20m were significantly reduced at ISI of 0.3 s as compared to all other ISIs. Furthermore, amplitudes of P35m decreased significantly in both groups with ISIs of 3 s and shorter. However, data of patients with migraine with aura, migraine without aura and control subjects did not differ significantly.

Conclusions: ISI dependent excitation and inhibition of pyramidal tract neurons in the somatosensory (SI) cortex of patients with migraine are normal. Data do not support an interictual hyperexcitability of the somatosensory (SI) cortex in migraine.

P353
**The contribution of electrophysiological examination in the diagnostics of lumbar spinal stenosis.** B. Adamova, S. Vohanka, University Hospital (Brno, CZ)

Background Data: Monoradicular or polyradicular lumbosacral involvement is typical of lumbar spinal stenosis (LSS). The needle electrode examination is considered the most useful procedure to evaluate patients with suspected radiculopathy. The clinical relevance of motor and somatosensory evoked potentials (MEPs, SEPs) is uncertain.

Objectives: The aim of the study was to evaluate the diagnostic contribution of electrophysiological tests in patients with LSS.

Methods: One hundred and two patients (44 males, 58 females, aged 62 ± 13 years) with clinically symptomatic LSS, documented by CT scan, volunteered to participate in the study. Patients suffering from diabetes mellitus or other disease causing polyneuropathy were excluded. All patients underwent electrophysiological examination, which included needle electromyography (EMG) and nerve conduction studies (NCS) of the lower extremities, somatosensory evoked potentials of tibial, sural and superficial sensory peroneal nerves and motor evoked potentials to the abductor hallucis muscle and tibialis anterior muscle.

Results: On the basis of nerve conduction studies and needle EMG, the presence of radiculopathy was established in 70% of patients with LSS; polyradicular involvement (46% of patients) was more common than monoradicular involvement (24% of patients). The involvement of L4 root had 37% of patients, L5 root was involved in 52% and S1 root in 51% of LSS patients. Abnormal MEPs were found in 31% of patients and abnormal SEPs in 59% of the patients with LSS. Normal needle EMG and NCS were recorded in 19% of LSS patients. When abnormalities of any evoked potentials (MEPs, SEPs) were considered, the number of patients with normal electrophysiological findings was reduced to 13%.

Conclusions: Nerve conduction studies and needle EMG are the most useful electrophysiological examinations for the evaluation of suspected radiculopathies in patients with LSS. The involvement of L5 and S1 roots is the most common. The diagnostic contribution of evoked potentials (SEPs, MEPs) is limited in patients with LSS.

Supported by the Internal Grant Agency of the Ministry of Health of Czech Republic; Grant No NF/5938–3 and NK 7129–3.

P354
**Electrophysiologic evaluation of phrenic nerve injury during open heart surgery – prospective, controlled, clinical study.** S. Canbaz, N. Turgut, U. Halici, K. Balci, U. Utku, E. Duran, Trakya University (Edirne, TR)

Objective: It is reported that the left hemidiaphragmatic paralysis due to phrenic nerve injury may occur following open heart surgery. The purpose of this study: document the effects of whole body hypothermia and use of ice slush around the heart on phrenic nerve injury.

Materials and Methods: Electrophysiology of bilateral phrenic nerves was studied in total 78 subjects undergoing cardiac or peripheral vascular surgery before and three weeks after operation. Coronary artery bypass surgery (CABG) with hypothermic cardiopulmonary bypass (CPB) in 38 patients, heart valve replacement with CPB in 11 patients, CABG with beating heart in 12 patients and several peripheral vascular surgery under general anesthesia in 17 patients were performed.

Results: In all patients, measurements of bilateral phrenic nerves function were normal limits before surgery. Total five patients in the CPB and hypothermia group – 3 in CABG (8%) and 2 in heart valve replacement (18%) – had abnormal left phrenic nerve function three weeks after surgery. No phrenic nerve dysfunction in the CABG with beating heart (no CPB) group and the peripheral vascular group was observed before and after surgery. Except five patients who have left phrenic nerve paralysis; mean phrenic nerve conduction latency time (ms) and amplitude (mV) were not statistically different in the four groups of the patients before and after surgery ($p > 0,05$).

Discussion: Our results were indicated that the cardiopulmonary bypass with hypothermia and local ice slush application around the heart play a role in the phrenic nerve injury following open heart surgery. Furthermore, incidence of phrenic nerve injury during open heart surgery was obtained 10,2% in our series.

P355
**Neonatal polissonography: a new methodology to measure sleep movements.** R. Scola, C. Silvado, L. Paola, L. Wernwck, R. Fernandes, UFPR, USP – Ribeirão Preto (Curitiba, BR)

Objectives: To determine the normal patterns of polissonographic findings in normal full-term newborns, in the first two days of life, showcasing the features of a complete sleep cycle, predominant state and quantifying the amount of movement in each different phase.

Background: In the visual analysis of neonatal EEG one must classify the different phase of the sleep-wake cycle based on polygraphic features and their changes according to each phase. In addition, quantifying the amount of each sleep phase may be of help in determining whether the polissonographic findings are those of a normal newborn or of an abnormal one.

Method: We analyzed the amount of movement, according to a proposed Motor Index (MI) of 32 polissonographic studies of normal full-term newborn babies born at the Hospital de Clínicas da UFPR. MI was dubbed positive (+) when there were findings suggestive of movement in more than 50% of an EEG epoch, negative IM (–) when no movements could be observed and Average MI (±) when findings of movement where observed in less than 50% of an EEG epoch. Movement features were obtained through annotations on the exam such as movement of feet, hands, head, mouth, suction movements and generalized movements, among others. An additional electromyographic channel aided in registering an increase in amplitude or irregularity of registry, when the EEG was obscured by muscular and/or movement artifacts. All of these variables were quantified in each different sleep-wake phase.

Results: The mean duration of registry was of 57.45 minutes and the total amount was of 1838.4 minutes. The mean duration of MI (+) was of 18.33 minutes, MI (±) of 16.36 and of MI (–) of 21.5 minutes. During quiet sleep the mean duration of MI (+) was of 22.3 minutes and during active sleep 323 minutes. Mean duration of MI (–) in quiet sleep was of 446.33 and in active sleep was of 171.6. In quiet sleep the mean duration of MI (±) was of 134.33, 240 during active sleep, 84.6 minutes in transitional sleep, and 67.6 minutes in the wake state. The sum of MI (+) and MI (±) was of 563 minutes (30.6% of the total time). Considering the incidence of each sleep phase, quiet sleep occurred at a rate of 3.1/exam (range 1–8/exam), active sleep 3.3/exam (raging 1–9/exam), transitional sleep 3.9/exam (range 0–9/exam) and awake 3.9/exam (range 0–10/min). When the number of phases is compared with the movements, in the instable sleep there are more movements. The statistical test used was regression multiple, with co-variance time of the electroencephalogram.

Conclusion: In all our exams the newborns remained the majority of time in sleep. Movements are observed in a greater amount during active sleep, predominantly in those patients who had a greater variation of sleep phases. There are more movements in instable sleep neonates.

**P356**

**Unusual cause of abnormal abdominal movements.** S. Grégoire, J. M. Raymackers, M. Ossemann, P. Laloux, Cliniques Universitaires de Mont-Godinne (Yvoir, B)

We report the case of a 75-year-old woman who developed involuntary jerks of the abdominal musculature, with no involvement of the limbs, trunk or face. They occurred spontaneously, while sometimes triggered by attempts to arise from the supine position. They were rhythmical and synchronous; they disappeared during sleep and could not be controlled by will. Auditive, painful or sensitive stimuli failed to provoke the symptomatology.

Clinical examination was normal, excepted for motor and sensitive signs due to lumbar stenosis. Extensive blood evaluation was normal. Electrophysiological study was conducted (electroencephalogram (EEG), 24h video-EEG, electromyography, somatory-evoked potentials and motor-evoked potentials), which excluded cortical epileptic activity as a cause of the abnormal movements and suggested a spinal origin. MRI of the spine revealed a syringomyelic cavity descending from T4 to T10. No associated Chiari malformation was associated. No previous traumatic event was reported.

The topological correlation of muscle activity and radiological findings were consistent with the diagnosis of spinal myoclonus. Propriospinal propagation did not seem to be associated. Fractionated doses of clonazepam rapidly permitted the control of the myoclonus, which systematically reappeared after drug cessation.

Video of the abnormal movement is presented. Electrophysiological studies are detailed. Similarities and differences between segmental spinal and propriospinal myoclonus are drawn. Pathophysiology of spinal myoclonus is discussed. Differential diagnosis of spinal myoclonus is shown. Syringomyelia has been previously reported as a rare (< 10 cases) cause of segmental spinal myoclonus.

**P357**

**Lumbosacral radiculopathy: correlation of clinical and electrophysiologic findings in 100 cases.** S. Nafissi, S. Niknam, A. Soltanzadeh, Tehran University of Medical Sciences (Tehran, IR)

Low back pain and lumbosacral radiculopathy is one of the most common disorders in general population. This study was conducted to evaluate the relation between clinical and electrophysiologic findings in patients presenting with lumbosacral radiculopathy.

Methods: In this cross-sectional study, 101 patients with the clinical diagnosis of lumbosacral radiculopathy were sequentially selected and clinical and electrophysiologic evaluation was performed.

Results: Mean age was 49 ± 13.1 years. 51 % were female. In 32 % symptoms were bilateral and in 68 % unilateral. Frequency of signs and symptoms: radicular pain 78 %, positive SLR test 48 %, hyposthesia 34 %, paresthesia 37 %, weakness 34 %, atrophy 9 %, absent Achilles reflex 27 %. 82 % had abnormal electrophysiologic study. Frequency of root involvement: S1 41 %, L5 43 %, L5-S1 9 %, L4 4 %, L2–3 2 %, S2–3 1 %. 77 % had abnormal paravertebral muscle electromyography and 73 % had denervation and/or reinnervation in lower extremity muscles. The only common NCS abnormalities were low amplitude peroneal CMAP in 24 % and abnormal H-reflex in 38 %. There was no significant difference in clinical findings between patients with normal and abnormal electrophysiologic findings. There was no correlation between sensory symptoms and signs and electrophysiologic findings. There was no relation between age, sex, pain type and electrophysiologic findings.

Conclusion: electrophysiologic study is a useful tool in evaluation of patients with lumbosacral radiculopathy and is a great companion to clinical and imaging studies.

**P358**

**R-R interval variation in migraine patients.** R. Aygül, O. Deniz, A. Orhan, N. Koçak, M. Kaya, Atatürk University (Erzurum, TR)

Objectives: The aim of this study was to evaluate possible cardiovascular autonomic dysfunction in migraine patients with the R-R interval variation (RRIV) measurement which is easy and reliable method for evaluation of parasympathetic function.

Material and methods: We studied 71 migraine patients in headache-free intervals (mean age: 31.56 ± 8.96 years, range 13–57 years, 52 females and 19 males), all without any known heart disease, and 51 age-matched healthy subjects (mean age: 28.81 ± 9.11 years, range 13–52 years, 25 females and 26 males). R-R interval variation at rest (R%) and during deep breathing (D%) were studied in all the subjects. The difference between D% and R% (D-R) and the ratio of D% to R% (D/R) were determined.

Results: The mean values of RRIV in migraine patients at rest [mean RRIV in patients, 15.98 ± 6.30 % vs controls, 18.92 ± 5.82 % ($P < 0.05$)] and during deep breathing [mean RRIV in patients, 28.72 ± 9.95 % vs controls, 34.57 ± 11.50 % ($P < 0.05$)] and D-R [mean in patients, 12.74 ± 7.90 % vs controls, 15.64 ± 8.20 % ($P < 0.05$)] were significantly lower compared with the controls, but D/R [mean in patients, 1.90 ± 0.58 % vs controls, 1.86 ± 0.42 % ($P > 0.05$)] was not significant.

Conclusion: Patients with migraine have hypofunction in the parasympathetic nervous system during normal daily activity in the headache-free period.

**P359**

**The effect of carpal tunnel syndrome in sympathetic skin responses recorded from the palm.** H. Moutopoulou, A. Argyriou, G. Katsoulas, S. Papapetropoulos, E. Chroni, Medical School of Patras-Neurology Department (Patras, GR)

Background: Sympathetic skin responses (SSR) are used to evaluate the sympathetic sudomotor function in response to different stimuli. Autonomic disturbances, i. e. dry palms or blanching of the hand, are common in carpal tunnel syndrome (CTS).

Purpose: to study the SSR in patients with idiopathic CTS and to assess whether this method could supplement the diagnostic sensitivity of conventional techniques.

Material and Method: Twenty three patients with no diabetes or other conditions associated to neuropathies were examined and CTS was documented in a total of 38 hands. These were classified, according to electrophysiological criteria, into two groups; a group of 20 hands with severe CTS (distal motor latency > 4.5ms, sensory conduction velocity < 35m/s) and a group of 18 hands with mild/moderate CTS and were compared with a group of 20 hands of age matched healthy controls. SSR were recorded simultaneously from the median and ulnar part of the palm following electrical stimulation at the wrist, in a mid-point between median and ulnar nerve. The amplitude, latency and habituation (ratio of the 5th to 1st response) were estimated.

Results: Five hands in the severe CTS group (25 %) showed no response from either the median or ulnar territory. Furthermore, the amplitude of median and ulnar SSR was lower than normal in the severe CTS group; mean amplitude values of median SSR were 2.2 mV in controls vs 1.5 mV in CTS group, $p = 0.07$ and of ulnar SSR were 2.5 mV, vs 1.5 mV respectively, $p = 0.03$. The SSR latency of both nerves tended to be longer than normal in the severe CTS group; mean latency values of median SSR were 1.3 s in the controls vs 1.5 s in CTS group $p = 0.04$ and of ulnar SSR were 1.3 s, vs 1.5 s respectively, $p = 0.06$. No differences were found in mild/moderate CTS group as compared to controls. The median-to-ulnar ratios and habituation of SSR did not differ between groups.

Conclusions: SSR is not a sensitive method for the early diagnosis of CTS, possibly because the large myelinated somatic nerve fibers are preferentially involved. Severe CTS might effect the SSR that are recorded from both the median and ulnar part of the palm. Involvement of the ulnar nerve in cases of severe CTS, which has also been previously suggested in respect to sensory potentials and, more likely, wider expand of sympathetic sudomotor fibers beyond the typical territory of median nerve could explain the similarity of median and ulnar SSR abnormalities.

**P360**

**Quantitative surface electromyography in cervical dystonia.** G. Filiz, M. Kiziltan, C. Caksibae, Haydarpasa Numune Research Hospital, Istanbul University Cerrahpasa (Istanbul, TR)

In this study we used quantitative surface electromyography (SEMG) for the evaluation of dystonic muscles in cervical dystonia to evaluate whether quantitative SEMG can be used as a diagnostic tool for cervical dystonia or not. Root mean square (RMS), mean rectified value (MRV), mean frequency (MF), turns per second (T/S), mean amplitude (MA) and ratio of turn number to mean amplitude were the parameters used for the quantitative evaluation of SEMG of the sternocleidomastoid (SCM) muscle and posterior neck muscles especially splenius capitis (SC) muscle in 22 patients not treated with botulinum toxin. 11 healthy subjects were examined for comparison.

RMS, MRV, MF, T/S, MA and T/A values of normal SCM muscles during maximal voluntary contraction (MVC) were statistically higher than the values of the dystonic SCM muscles at the resting position. At the comparison of normal and dystonic SCM muscles during MVC; RMS, MRV, T/S values of normal SCM muscles were statistically higher than dystonic SCM muscles. There was no statistical difference at MF, MA and T/A values of the same muscle groups during MVC. RMS, MRV, T/S, MA and T/A values were statistically higher during MVC of dystonic SCM muscles compaired to the resting position. There was no statistical difference at the MF value of the same muscle group at the same conditions. All the values for SC muscle were considered as unreliable because of the anatomical localization of that muscle.

It is concluded that MF might be most reliable parameter in the evaluation of cervical dystonia. We suggest that one should choose superficial muscles like SCM muscle for investigation to obtain reliable values of quantitative SEMG

P361

**Neuropsychological tests and P300 potential in the long-term assessment of memory decline in patients with unilateral cerebral lesion.** K. Slotwinski, A. Pokryszko-Dragan, R. Podemski, Medical Academy (Wroclaw, PL)

Auditory long-latency P300 potential is one of most frequently analysed endogenous evoked responses, which correspond with efficiency of cognitive processes engaging mainly cerebral cortex. Disturbance of these processes results in complete lack of endogenous response or in abnormal parameters of this response (prolonged latency and/or lowered amplitude).

The aim of this study was to evaluate latency and amplitude of auditory P300 potential in patients with memory dysfunction in the course of unilateral brain lesion, with electrophysiological parameters referred to degree and profile of memory deficits.

Material and methods: The study comprised 4 patients (2 men, 2 women, aged 26–47, mean age 38.6) with memory dysfunction as a consequence of haemorrhagic or infectious aetiology, localised in the dominant cerebral hemisphere. In all the patients routine neurological examination and CT head scan were performed. Memory dysfunction was evaluated by means of neuropsychological tests battery, including Auditory Verbal Learning Test – AVLT, Digit Span -DS, Rey Figure Test -RFT. Auditory P300 potential was evoked using basic "oddball paradigm". Superficial recording electrodes were placed in Fz, Pz, Cz (according to 10–20 system), with the reference electrode at the mastoid. Responses were recorded and averaged by means of ElmikoEEG Digitrack, with the filter bandpass 0.30/s – 70 Hz, sweep speed 1000 ms and prestimulus baseline of 250 ms. At least 30 target responses were averaged. Neuropsychological and electrophysiological examination was repeated after 12 and 24 months. Results of neuropsychological tests were referred to P300 parameters.

Results:

1. In all the patients marked regression in memory decline was found after 12 and 24 months in all neuropsychological tests performed (AVLT, DS, RFT).
2. In all the patients significant decrease in P300 latency and increase in P300 amplitude were found after 12 and 24 months.
3. In all the patients positive correlation was found between improvement in neuropsychological tests evaluating memory dysfunction and changes in P300 parameters in subsequent electrophysiological examinations.

Conclusion: Assessment of endogenous P300 potential, together with neuropsychological tests, is a useful method of long-term assessment of memory dysfunction caused by focal cerebral lesion of various aetiology.

## Dementia/Higher function disorders

P362

**Endothelial nitric oxide synthase gene polymorphism Glu298Asp and apolipoprotein E genotype in Alzheimer's disease.** I. Guidi, D. Galimberti, C. Fenoglio, R. Del Bo, A. Gatti, L. Perego, F. Cogiamanian, M. Tiriticco, P. Baron, G. Conti, G. P. Comi, N. Bresolin, E. Scarpini, IRCCS Ospedale Maggiore (Milan, I)

Recent evidences suggest that endothelial Nitric Oxide Synthase (eNOS) gene may be a potential risk factor for the development of Alzheimer's disease (AD). The presence of the homozygous genotype Glu/Glu of the common polymorphism Glu298Asp in eNOS gene was shown to confer an increased risk for late onset AD in British population. However, no association has been demonstrated in other populations. Besides these evidences, an involvement of ApolipoproteinE (ApoE) genotype and gender in the regulation of nitric oxide (NO) production has been reported. Significantly more nitric oxide was produced by peritoneal macrophages and microglia from male transgenic mice (tg) that only express the ApoE4 isoform than those from male tg mice that only express the E3 isoform, while this effect was not observed in females. Similarly, macrophages from humans carrying the e4 allele produce significantly greater amount of NO than individuals with e3. The distribution of the Glu298Asp polymorphism in the eNOS gene was studied in a population of 140 Northern Italian patients with probable AD, diagnosed according to NINCDS-ADRDA criteria, as well as in 90 healthy subjects. Genomic DNA was isolated from whole blood, Glu298Asp polymorphism was determined by PCR-RFLP assay. Allelic and genotypic frequencies were obtained by direct counting and Hardy Weinberg equilibrium was tested. $NO_2$-serum levels were determined using the Griess method. $NO_2$-levels were compared using the non parametric Mann-Whitney test. The frequency of the Glu298Asp polymorphism in both our AD population and controls was similar to the one reported for the Caucasian population. $NO_2$-levels in serum of patients carrying at least one mutated allele were slightly but not significantly increased compared with non carriers. Stratifying by the presence of e4 allele, a trend towards an increase of $NO_2$- levels in e4 carriers was observed. The effect of e4 allele appears to be dose dependent (e4 +/+: 37,33 ± 8,6; e4 +/−:30,44 ± 2,7; e4 −/−: 29,17 ± 2,58). Conversely, no differences in nitrite levels were observed stratifying for gender.

In conclusion, our data suggest that the eNOS Glu298Asp polymorphism is not an important genetic risk factor for the development of AD in the Italian population. However, the greater NO production in e4 carriers implies a high level of oxidative stress. This, leading to neuronal damage, could provide a mechanism that potentially explains the genetic association between e4 and AD.

P363

**Genotype-phenotype analysis in early-onset Alzheimer's disease due to Presenilin-1 mutations at codon 139.** F. Hanisch, H. Koelmel, Martin-Luther University Halle-Wittenberg, Klinikum Erfurt (Halle/Saale, Erfurt, D)

Mutations in the presenilin-1 (PS-1) gene are the main cause of autosomal-dominant early onset Alzheimer's disease (EOAD) and show a high penetrance of symptoms. There are more than 60 mutations in the PS-1 gene. Among them are four different missense mutations at position 139 on exon 5. Lack of genotyping in other family members may lead to the suggestion of sporadic cases.

We present 46-year old German female with EOAD, onset with cognitive decline at the age of 32, occurrence of myoclonic and tonic-clonic jerks in the later course. Disease symptoms were present in three generations of her family. Genetical analysis revealed the M139V mutation on exon 5 of the PS-1 gene.

We compared the clinical data of her family with seven previously reported families and two sporadic cases with mutations at the codon 139. The age of onset ranges between 32 and 50 years (mean: 41.6 years). The duration of the illness ranged 4–11 years (mean: 5.9 years). However, a substantially longer course is possible. Myoclonic jerks and generalized tonic-clonic seizures are prominent symptoms in the later course. The genotype-phenotype analysis showed marked intrafamilial homogeneity, but interfamilial heterogeneity concerning the onset, duration, and progression of illness. Onset and duration were not correlated to the amino acid exchanged. Another modifying genetic or environmental factor is possible.

P364

**Acetylcholine-esterase inhibitor might modify the "cytokine cycle" in Alzheimer's disease.** C. Iarlori, M. Reale, F. Gambi, G. De Luca, A. Salone, D. Gambi, University of Chieti (Chieti, I)

Background: The etiology of Alzheimer's disease (AD) is still unknown. The neuropathology of the disease hallmarks are neurofibrillary tangles and senile plaques containing amyloid-beta (A-beta) peptide. The increase of some cytokines and acute phase reactants in the blood of AD patients has suggested the possible role of a systemic chronic inflammation associated with the disease. A cytokine cycle may be the pathogenic mechanism for neuro-degeneration in AD. Acetylcholine-esterase inhibitors (AchEI) are now considered the first choice treatment for AD. It is known that AchEIs enhance neuronal transmission by increasing the availability of acetylcholine in muscarinic and nicotinic receptors.

Objective: to assess the immunological effects of AchEI treatment on MCP-1, RANTES, IL-1 and IL-4 production and expression in AD patients.

Methods: twentyone patients with clinical diagnosis of AD according to DMS IV-R and NINCDS-ADRDA were studied. All patients underwent blood withdrawal before and after 1 month of AchEI treatment. Peripheral blood mononuclear cells (PBMC) were purified and cultured without or with PHA (20mcg/ml). MCP-1, RANTES, IL-1 and IL-4 levels in 24-h supernatants were measured with commercially available ELISA kits. Reverse-transcriptase-polymerase chain reaction (RT-PCR) was used to determine mRNA expression in unstimulated and PHA-stimulated PBMCs. Statistical analysis was performed using Wilcoxon test. Results: AchEI treatment significantly reduced IL-1 and RANTES levels and increased MCP-1 and IL-4 production both in unstimulated or PHA stimulated PBMC cultures ($p < 0.001$). RT-PCR results confirmed the ELISA data.

Discussion: these findings confirm that inflammation may contribute to the pathogenesis of AD. Interaction between cytokines and chemokines may modify the "cytokine cycle" which is a proposed pathogenic mechanism for neuro-degeneration in AD. AchEI may contribute to the delay of the disease progression affecting the cytokine-chemokine network by promoting Th2 responses, inducing MCP-1 and IL-4, and reducing the proinflammatory cytokines, RANTES and IL-1. Further studies are needed to establish the diagnostic and therapeutic effects of acetylcholine-esterase inhibitors (AchEI) and to clarify the involvement in the amyloid precursor protein metabolism and in the inflammatory cascade.

P365

**Matrix-metalloproteinase (MMP)-9 and its tissue inhibitor (TIMP)-1 plasma levels in patients with Alzheimer's disease.** M. Inspector, J. Peretz-Aharon, L. Glass-Marmor, A. Miller, Carmel Medical Center, Rambam Medical Center (Haifa, IL)

Background & Objectives: Research of recent years provided accumulating evidence that Alzheimer's disease (AD) involves a CNS inflammatory process, which was found to be accompanied also by modulation of immunological indicators in the peripheral blood. Additionally, recent studies indicate the involvement of Matrix-Metalloproteinases (MMPs) and their endogenous inhibitors (TIMPs) in processes of primary inflammatory brain diseases. The present study was designed to assess plasma levels of MMP-9 and TIMP-1 in AD patients and their possible correlation to the degree of the dementia.

Methods: The study population included 19 patients with clinical AD and 10 controls. Patients with AD were divided into 2 groups according to their clinical dementia rating (CDR) as having mild dementia (CDR = 0.5 and 1) (N = 10) or as having moderate-severe dementia (CDR = 2 and 3) (N = 9). Plasma levels of total MMP-9 and TIMP-1 were determined by ELIZA (R&D systems).

Results: In patients with moderate-severe AD mean MMP-9 levels (24.09 ± 7.4 ng/ml) were lower then levels in patients with mild AD (33 ± 15.3 ng/ml and also compared to controls (30.8502 ± 13.46 ng/ml). Although a 27.9% decrease in the mean level of MMP-9 in moderate-severe AD patients compared to mild AD patients, this change was not found to be statistically significant (P = 0.277). Mean plasma TIMP-1 levels were higher in moderate-severe AD patients (189.38 ± 70.53 ng/ml), compared to levels in patients with mild AD (141.8983 ± 40.96 ng/ml) and also compared to controls (167.1172 ± 63.04 ng/ml), but this was not statistically significant (P = 0.234). When patients with AD were divided according to whether they are being treated or not by Acetylcholin-Esterase inhibitor (Ach-E-I) (N = 13 treated, N = 6 non-treated) a significant decrease in the levels of TIMP-1 (P = 0.008) was observed in the treated patients (140.96 ± 30.72 ng/ml in the treated patients versus 215.14 ± 79.56 ng/ml in the non-treated group). No significant affect of Ach-E-I was found referring to MMP-9 levels.

Conclusions: No significant difference was found in plasma levels of MMP-9 and TIMP-1 in AD patients compared to healthy controls, or between patients with mild versus moderate-severe dementia. Ach-E-I treatment was found to be associated with decreased TIMP-1 levels in AD patients, a finding that might suggest an immuno-modulatory affect of Ach-E-I, and which requires further investigation.

P366

**A Spanish version of "Memory Impairment Screening": a prospective blind validation for detection of cognitive impairment and dementia.** D. A. Perez-Martinez, J. J. Baztan, M. Gonzalez, A. Socorro, Hospital Central Cruz Roja (Madrid, E)

Introduction: "Memory Impairment Screening" (MIS) is an easy and brief (3–4 minutes) memory screening test for cognitive impairment. We develop a Spanish version of MIS and validate this version among a neurological and geriatric clinic population aged 60 years or older.

Methods: We selected 66 subjects from a neurological and geriatric clinic who came with suggestive symptoms of cognitive impairment. Patients were evaluated with a Spanish version of Mini-Mental State Examination, Global Deterioration Scale and a Spanish verbal memory test. We used a prospective blind method to assess our Spanish version of MIS. We used DSM-IV criteria for gold standard diagnosis of dementia, NINCDS-ADRDA criteria for Alzheimer's disease and Petersen criteria for mild cognitive impairment.

Results: 66 subjects (71.2% females) were tested. Mean age was 76.6 years old. The diagnostic efficiency for dementia was high (area under the curve ROC 0.923 [CI 95%: 0.878–0.969]) and efficiency for cognitive impairment was high, too (area under the curve ROC 0.937 [CI 95%: 0.896–0.977]). Sensitivity and specificity for dementia were 94.1% and 79.5%, respectively (cut-off of 2 or less). The positive likelihood ratio was 4.6 and the negative likelihood ratio was 0.07. The inter-rater reliability and test-retest reliability were 0.883 ($p < 0.001$), and 0.854 ($p < 0.001$), respectively. The convergent validity with Spanish verbal memory test was 0.726 ($p < 0.001$).

Conclusions: Our Spanish version of MIS has a good reliability and validity. For clinical use, a cut-off of 2 appears to be most useful for screening of dementia. A cut-off of 3 is most useful for screening of cognitive impairment.

P367

**Persistent right spatial neglect after left hemisphere damage.** C. Salathé, R. Ptak, B. Leemann, A. Schnider, University Hospital Geneva (Geneva, CH)

Spatial neglect, the lack of awareness of stimuli on the contralesional side of space, is a frequent consequence of right temporal-parietal damage. Nevertheless, occasional cases of right neglect after left hemisphere injury have been reported. Right neglect is mostly associated with aphasia and often shows a rapid recovery. The rareness of persistent right spatial neglect suggests a strong dominance of the right hemisphere for visual-spatial attention. We report a 66 year-old right-handed woman with persistent right neglect after occlusion of the left carotid artery siphon of probably thromboembolic origin. The patient presented a severe right motor and sensory right-sided deficit, as well as severe unawareness of visual stimulation coming from the right, simultaneous with a right hemianopia. A CT-scan showed a left hemisphere infarct in the entire territory of the left middle cerebral artery, involving putative Broca's and Wernicke's area. Neglect manifested itself clinically: the patient had a strong ipsilesional deviation of gaze, made few right-sided turns in her wheelchair, did not notice people talking to her from the right, and omitted food placed on the right side of the plate. A detailed neuropsychological examination two months after her stroke did not reveal any language, reasoning, or verbal memory deficits. In contrast the patient demonstrated severe right spatial neglect in cancellation tasks (up to 100% right targets omitted), as well as object-centered neglect on line bisection (up to 40% ipsilesional deviation), reading or copying of drawings. In a follow-up examination two years after brain injury, she still demonstrated marked neglect on cancellation tasks (75% right targets omitted), line bisection (mean, 23% ipsilesional deviation) and reading. Even at that time the patient was not aware of her neglect although she had noted some consequences of it such as bumping into obstacles. The absence of verbal deficits paired with symptoms typically presented by patients with right hemisphere injury indicates that our patient has a right-hemisphere dominance for language.

Therefore, this case suggests that persistent right spatial neglect is associated with partial or complete reversal of hemispheric dominance, supporting the notion of a right hemispheric dominance for visual-spatial attention.

## Epilepsy

P368

**Changes of gene expression induced by ketogenic diet.** N.-C. Choi, O.-Y. Kwon, K.-J. Park, B. Lim, Gyeongsang National University College of Medicine (Jinju, KOR)

Background and Purpose: The ketogenic diet (KD) is a high-fat, low-carbohydrate and -protein diet that has been used to treat refractory epilepsy in children. However, little is known about how the KD inhibits seizures or its effects on epileptogenesis. There are few studies about its effects on

gene expression. The influence of KD on gene expression was investigated at both Sprague-Dawley (SD) rat and ICR mouse.

Methods: The SD rats (postnatal day 21) were randomly separated into 2 groups (each n = 6); fed with either the KD or normal diet (ND) for 6 weeks. The ICR mice (postnatal day 21) were randomly separated into 4 groups (each n = 6); fed with the KD for 1 week, for 2 weeks, for 6 weeks, and with ND for 6 weeks. Kainic acid (KA) was injected via intraperitoneal route in 2 groups of ICR mice (each n = 6); fed with either the KD or ND for 6 weeks. The KA-treated mice were evaluated the onset and grade of seizure. The influence of KD on gene expression in rat hippocampus was determined using cDNA expression array. The difference of gene expression according to the period of KD in mouse hippocampus and the effect of KD on gene expression in hippocampus of KA-treated mouse were determined using reverse northern blot analysis on a 36 subset of genes.

Results: The onset of seizure was significantly delayed in KD-fed mice but the grade of seizure was similar between KD- and ND-fed mice. There were expressed 179 genes on cDNA expression array, among them 42 genes were strongly up- or down-regulated by the KD. The largest change is the metabolic pathway related gene in the category of up-regulated gene expression, such as mitochondrial ATP synthase D subunit, mitochondrial ATP synthase β, and mitochondrial ATP synthase B subunit precursor. The largest change is the intracellular transducer related gene in the category of down-regulated gene expression, such as protein kinase C. Reverse northern blot analysis according to the period of KD shows similar to the result of cDNA expression array with some exceptions. KA-treated mice with KD up-regulated heat shock protein 90 and synaptotagmin XI, also down-regulated potassium channel subunit protein.

Conclusions: This study supports hypothesis that KD alters the nature and degree of energy metabolism in the brain. And KD induces a complex cascade of cellular changes in the hippocampus of rat or mouse.

P369

**Lamotrigine and myoclonus exacerbation in idiopathic generalized epilepsy. Report of four cases.** C. Delpirou-Nouh, P. Gelisse, A. Crespel, M. Baldy-Moulinier, Centre Gui de Chauliac (Montpellier, F)

Objective: To describe four cases with Idiopathic Generalized Epilepsy (IGE) who presented an apparition or an exacerbation of myoclonus by treatment with lamotrigine.

Background: The efficacy of Lamotrigine is well documented in IGE, and in particulary in co-therapy with Valproate, or in monotherapy when Valproate is poorly tolerated. The cutaneous adverse effects are well known. Myoclonus apparition or exacerbation are rare.

Material and methods: Four female patients (ages ranged from 20 years to 63 years) fulfilled the clinical and electric criteria of IGE. Two patients had a Juvenil Myoclonic Epilepsia (JME). All of them were treated with Lamotrigine in a single drug regimen or as add-on therapy because of tolerance problems or lack of full efficacy with Valproate or Phenobarbital. The patients had been taking Lamotrigine for an average of ten months, with moderate doses of 125 mg to 150 mg per day.

Results and discussion: They developped an apparition or an exacerbation of myoclonus, leading to stop (2 cases) or decrease (2 cases) the Lamotrigine therapy. In this second group, the myoclonus disappeared at the dose of 100 mg per day, and a good control of seizure was obtained with or without additional therapy (Topiramate, Valproate, Phenobarbital). In the case of JME, Lamotrigine must be stopped completely, replaced by Topiramate or Phenobarbital.

This adverse effect is exceptionally describe. The underlying mechanism of action is unknown, but might be linked to inhibition of voltage gated Sodium Channels.

Conclusions: Although the efficiency of Lamotrigine is well documented in IGE, a risk of therapy-induced myoclonus must be seriously considered in IGE, and particulary in JME.

P370

**Descriptive analyses of epileptic seizures in the emergency department. Contribution of the neurologist to inpatient management.** E. Vidry, P. Decavel, E. Revenco, F. Vuillier, O. Retel, T. Moulin, L. Rumbach, Hôpital Jean-Minjoz (Besançon, F)

Goals: The objective of this study was to assess the role of the neurologist in the emergency room for patients with epileptic seizure (ES).

Background: The frequency and impact of inpatient assessed by a neurologist in the emergency room (ER) remain largely underestimated. The objective of our study was to analyse the impact of the neurologist in patient management in a primary care university hospital especially for patients with ES.

Methods: Over a period of 3 months, we prospectively recorded the demographics of patients requiring examination in the ER, the ER team's tentative neurological diagnosis (classified by signs, symptoms or syndromes), the final diagnosis of the neurological team and the patient's outcomes. For each patient, the time between admission, the call and the neurological examination were recorded. We analysed specific patients with epileptic seizures.

Results: Among 952 assessed by a neurologist, 107 (11 %) had an epileptic seizure. Neurological examinations were performed in the ER for eighty two percent. Of these 107 patients, 68 % were male and the mean age was 50 ± 21 years. The time between admission and the call was 110 mn ± 11, the time between the call and the examination was 36 mn ± 4. Seventy six percent had a correct tentative diagnosis and 24 % a false negative diagnosis (stroke: 7 %, syncope 12 %, confusion 5 %). A false positive diagnosis occurred in 26 % of patients. A new onset seizure was diagnosed in 51 % of the patients (62 % symptomatic) and the epilepsy was previously known in 49 % (symptomatic in 50 %). Forty three percent of the symptomatic seizures were poststroke seizures and 19 % were alcohol-related seizures. A status epilepticus (SE) was found in 4.5 %. Neurological examination modified treatment in 93 % of the patients.

A cerebral CT scan was performed in 87 % of patients with inaugural seizures and an EEG in only six patients (2/6 for SE). Of all patients with seizure, 75 % were admitted in medical ward (neurology ward in 50 %). Patients with known epilepsy were more frequently discharged 38 % versus 14 % ($p < 0.01$).

Conclusion: Emergency neurological examination improves neurological diagnosis and has a positive impact both on treatment and, more globally, in patient management.

P371

**Alcohol-related seizures: a prospective study in a cohort of hospitalized adult patients.** P. Polychronopoulos, A. Economou, I. Nilas, A. Argyriou, G. Katsoulas, S. Papapetropoulos, T. Papapetropoulos, Medical School of Patras-Neurology Department (Patras, GR)

Purpose: Seizures and alcohol are complexly interrelated. From a therapeutic view it is important to differentiate between situations related seizures, which occur only in connection with toxic events such as alcohol abuse, and seizures precipitated by alcohol in patients with epilepsy. In this study we tried to appreciate the clinical features of alcohol-related seizures and to clarify the role of alcoholism in the etiology of epilepsy.

Patients and methods: We prospectively evaluated adult patients (> 16 years old) admitted with seizures to our department from January 1999–December 2000. Demographic and clinical characteristics were obtained by neurologists using a detailed questionnaire. EEG and neuroimaging studies were performed to all patients.

Results: During the study period, 174 consecutive patients were hospitalized for seizures or epilepsy. Twenty nine of them (25 men and 4 women aged 18–74 years) were alcohol abusers, representing 16.6 % of the total number of hospitalized patients. Sixteen of twenty nine patients (55.17 %) manifested seizures for the first time in their life. The clinical type of seizures was generalized tonic-clonic in 22 cases, partial with secondary generalization in 6 cases and simple partial in 1 case. EEG showed abnormalities in 21 cases (5 with epileptiform activity and 16 with non specific disturbances). In 16 cases neuroimaging (CT and/or MRI) was normal. Five patients had cortical atrophy, 3 ischemic strokes, 2 porencephaly due to previous cranial trauma, 1 intracerebral hemorrhage, 1 subdural hematoma and 1 cerebral abscess. Based on medical history and clinical data, the EEG and neuroimaging findings, the patients were syndromic classified according to ILAE 1989 as follows:

Twelve situation-related epilepsies acute symptomatic due to alcohol, 10 localization related epilepsies, 5 generalized epilepsies and syndromes and 2 epilepsies undetermined whether focal or generalized.

Conclusions: This study demonstrates that,

1. alcohol abusers constitute a high proportion of seizures admissions (16.6 % in our study)
2. alcohol-related seizures are mostly of GTCS type but partial onset seizures (about 25 % of our cases) are more frequent in alcohol abusers than has been recognized.
3. the majority of patients are presented with situation-related seizures but more than half of them have other cerebral lesions, which are mainly responsible for the seizures. Perhaps alcohol acts as a precipitating factor.

P372
**Partial seizures after electroconvulsive therapy: an uncommon complication.** R. C. Ginestal, P. Simal, I. Corral, J. C. Martinez-Castrillo, Hospital Ramon y Cajal (Madrid, E)

Introduction: Electroconvulsive therapy (ECT) is used in the treatment of several psychiatric disorders. Incidence of adverse effects is low, with memory disturbances and confusion, often transient, being the most frequent of them. Epilepsy is an uncommon complication with only a few reports in the literature. We report the case of a patient with schizophrenia who developed partial motor seizures after the ECT. The seizures were successfully treated with valproate.

Case report: A 19-year-old woman without personal or familial medical history of epilepsy developed a schizophrenia which failed to respond to neuroleptic drugs. She was treated with ECT. After the first session, the patient developed four partial motor seizures of the right face and extremities that evolved to secondarily generalized tonic-clonic seizures and finally to status epilepticus. With intravenous diazepam and valproate, seizure control was attained. All blood and cerebrospinal fluid studies were normal. Computed Tomography and Magnetic Resonance Imaging were normal except for a little calcified meningioma next to the right frontal lobe. Valproate, with blood levels in the therapeutic range, did not prevent ECT from being effective and the patient was discharged after five ECT sessions with an important improvement of her psychiatric symptoms and without new episodes of seizures.

Discussion: Although the risk of seizures after ECT is small, the average annual incidence of new seizures is still significantly higher for these patients than for age-adjusted control population. The majority of ECT-associated seizures are of the tonic-clonic type. Partial seizures, as with our patient, are rarely reported.

Several authors have suggested that anticonvulsants used to control psychiatric symptoms and/or after-ECT seizures may prevent ECT treatment from being effective. The decision to discontinue the anticonvulsant, lower the anticonvulsant dose, or maintain the current dosage with a greater stimulus energy of each ECT session has to be made on an individual basis (risk of new seizures versus lack of efficacy of the ECT). In the patient reported herein a good psychiatric recovery after several ECT sessions was attainted with valproate blood levels in the therapeutic range and without new episodes of seizures

P373
**Motor unit number estimation in juvenile myoclonic epilepsy.** E. Aykutlu, B. Baslo, B. Baykan, C. Gurses, G. Akman-Demir, A. Gokyigit, M. Ertas, Istanbul University (Istanbul, TR)

Objective: Juvenile myoclonic epilepsy (JME) is the prototype of an idiopathic generalized epilepsy syndrome, which shows substantial clinical and genetic heterogeneity. Our aim was to disclose the subclinical involvement of anterior horn cells by motor unit number estimate (MUNE) analysis with modified McComas technique and to investigate the correlations of the MUNEs with variable clinical and EEG features in this specific syndrome.

Methods: We enrolled 75 consecutive patients with JME diagnosed according to the ILEA criteria, and 26 healthy normal subjects. All of the subjects underwent conventional motor and sensory nerve conduction studies, concentric needle electromyography and MUNE analysis with modified McComas method. In order to correlate with MUNEs, the patients' group had been evaluated regarding various clinical parameters like response to therapy, the duration of epilepsy, family history of epilepsy, febrile convulsions, focal EEG features and photosensitivity.

Results: MUNE values of the M. Abductor pollicis brevis were $54 \pm 25$ for JME patients and $109 \pm 24$ for normal controls ($p < 0.001$), and MUNE values of the M. Tibialis anterior were $35 \pm 17$ for JME patients and $80 \pm 26$ for normal controls ($p < 0.001$). There was no significant association between MUNEs and any of the investigated clinical and EEG parameters.

Conclusions: Our findings showed a direct evidence that the anterior horn cells were subclinically affected in JME patients regardless of the various clinical and EEG parameters and suggested a shared genetic background for both JME phenotype and subclinical anterior horn cell involvement.

P374
**Asymmetric EEG findings in idiopathic generalized epilepsy in adolescence.** A. Karlovassitou, E. Dimitrakoudi, P. Armentsoudis, G. Rizos, M. Arnaoutoglou, C. Goulis, P. Hamlatzis, S. Baloyannis, Aristotle University of Thessaloniki (Thessaloniki, GR)

Generalized epileptic disorders are characterized by seizures in which there are symptoms of bilateral cerebral involvement and EEG findings of synchronous abnormal activity in both hemispheres. Juvenile Myoclonic Epilepsy (JME), Generalized Tonic-Clonic Seizures on Awakening (GTCSA) and Juvenile Absence Epilepsy (JAE) are the three syndromes of Idiopathic Generalized Epilepsy (IGE) of adolescent onset currently included in the classification of epilepsy syndromes of the International League Against Epilepsy (ILAE).

The aim of our study is to investigate whether EEG features, generally considered typical for a focal seizure disorder, also occur in patients with IGE, a situation which may have as a consequence the patient to be initially misdiagnosed as having partial seizures.

We retrospectively studied the EEG of 80 patients, attending our epilepsy out-patients' clinic, with an unequivocal diagnosis of IGE (44 with JME, 34 with GTCSA and 2 with JAE). In 24 patients (10 with JME, 12 with GTCSA and 2 with JAE) (30% of the total patients with IGE) at least one EEG showed focal or lateralization abnormalities. Among these patients, 13 were untreated before the EEG and 11 were treated with valproic acid. Asymmetric EEG findings included lateralized sharp-waves, unilateral spike, or polyspike and slow-wave complexes. Typical EEG findings of IGE were revealed in 5 patients who were inappropriately treated with oxcarbazepin and in 6 patients after sleep deprivation.

In conclusion, a correct diagnosis of IGE is strictly dependent on the knowledge of the syndrome, whereas EEG is just an ancillary diagnostic tool.

P375
**Psychological repercussions and evaluation of quality of life of patients suffering from epilepsy.** P. Heras, A. Argyriou, S. Karagiannis, M. Corcondilas, D. Mitsibounas, General Hospital of Kos, University of Athens-Therapeutic Clinic (Athens, GR)

The overdose of the administered antiepileptic medication and the necessity for chronic medication often have as a result serious effects on the life quality of patients suffering from epilepsy (E).

Aim of this study was to record the repercussions in the intellectual faculty and the mental health of patients with (E). Also attempted was the recording of factors that influence the quality of life of these patients.

Patients and method: We studied 32 individuals, at the age 30–50 years with diagnosed (E) who had manifest crises from 2, 3 or more times >6 times a year. The patients were separated in 2 subgroups. 1st consisted of 24 patients who manifested simple or complex partial (E) seizures and the 2nd subgroup from 8 patients who manifested generalised epileptic seizures. Patients were called to answer in questionnaire based on the Hamilton depression scale, on the Nottingham Health Profile and Mini Mental Health Examination.

Results: A serious reduction of the comprehensive intellectual abilities of our patients was not found, only small degree disturbances of memory and concentration. Furthermore a medium or severe depression on those patients was not diagnosed.

Patients of 1st subgroup expressed minor degree of anxiety and depression and also reported small degree deterioration of their quality of life, compared with the patients of 2nd subgroup. The event of anxiety and soft depression in the patients of 2nd subgroup was related with the duration of illness and the frequency of events of epileptic generalised crises, influenced obviously negatively their quality of life.

Conclusion: The type of the (E) crisis, the duration of illness and the frequency of events of (E) crisis have direct impact on mental health of suffering patients. The event of anxiety and depression, consequently the illness, downgrades the quality of life of these patients.

P376
**Social problems in Iranian epileptic patients.** K. Gharagozli, M. Meraj Mohammadi, J. Bolhari, D. Nassabi Tehrani, H. Haghighat, Z. Keyhani Doost, M. Mohammadi, Loghman Hakim Medical Center, Shahid Lavassani Hospital, Iran Medical University, NIOC Hospital, Tehran Medical University, Iranian Epilepsy Association (Tehran, IR)

Objective: To study the prevalence of social and epilepsy induced problems among epileptic patients in Iran.

Background: More than 700,000 epileptic patients live in Iran. Majority

of them are adults between 20–55 years old. In addition to treatment difficulty and its complications, there are many problems affecting these people.

Design/Methods: We asked 217 (132 female, 85 male) epileptic patients about their important problems. The two most important troubles must be answered. Patients who had abnormal neuroimaging, history of major brain trauma, any significant physical or mental disability were excluded.

Results: Among 132 female patients, 121 persons had an unstable marriage. They were anxious about divorce and then living alone. 45 ladies have history of divorce because of inadaptable husbands. All of the ladies were anxious about having kids because of probable epilepsy. Job finding was the most important trouble among these people (181 patients). Because of dangeral of dismissal, many worker patients hide their illness. 4 male patients are drivers. Anxiety about finding antiepileptic drugs has complicated life of most patients (199 people). Cosmotic complications are troublesome for women. 2 cases had suicidal attempt.

Conclusions: Convulsive seizures are the most frequent type of seizures among epileptic patients. Stigma of the convulsion itself is a major trouble for patients. In addition to universal social views, patients of some regions have specific problems. Many Iranian patients try to shroud their seizures from other people. Cultural background causes that many female try to hide their seizures even from their relatives, because they think it is a hideous illness. Fear of being alone without any support is a big problem among females. We have many patients who have married and their husbands do not know about their seizures. They have a privacy about epilepsy and take their medicines in hiding. Having seizure is big stigma among Iranian women. Finding a job is a big problem for epileptic persons. Annually we have reports of catastrophes during work because of convulsions. Novel antiepileptic drugs are very expensive for patients. Governmental and NGO's supports are not enough for them. Car accidents because of seizure during driving are not uncommon. Social learning about epilepsy and its complication is time-consuming and needs scientific plans to obliterate stigma and troubles of epilepsy.

Study group supported by Irania epilepsy association.

## Extrapyramidal disorders

P377

**Extremely high dose of levodopa as a maintenance dose for dopamine responsive dystonia.** A. Hamad, M. Bessisso, Hamad General Hospital (Doha, QA)

Objective: To describe a patient with Dopamine responsive dystonia (DRD) who needed extremely high doses of Levodopa (12 tablets of sinemet 125) as a maintaining dose after 8 years of its initiation.

Background: DRD is a rare distinctive variety of childhood onset idiopathic torsion dystonia which can be easily diagnosed by complete and prolonged response to small daily dose of levodopa.

Case report: Twenty-year-old Qatari male patient, presented at the age of 9 years with progressive gait disturbances and frequent falls. His parents were first cousins, other siblings were normal. He was developing normally till the age of 8 years when his mother noticed right foot inversion, which progressed slowly. His examinations showed normal developed boy with normal mentality and no abnormal behavior, had spastic gait with right foot inversion. The mother noticed diurnal variation being almost normal when he woke up from sleep and worsening after exertion. His EEG, brain MRI, NCV, blood, CSF and urine tests excluded any metabolic disease. Patient was diagnosed as idiopathic dystonia. At age of 13 his left arm started to be involved. At that time he was only given sinemet 125 (levodopa 100 mg/carbidopa 25) three times/day which showed dramatic response. At age of 17 he again deteriorated and he needed to increase the dose of Sinemet 125 to six/day. One year later he again noticed wearing off effect of levodopa and increased the dose to 9 tablets daily. Patient was admitted to hospital and under EEG monitoring Sinemet was discontinued. After 90 minutes patient became trembling, his hands flexed and fisting and foot inverted, right > left, he was conscious and complained of pain, his EEG was normal. Patient was tried on clonozepam, promipexole and topiramate, which he could not tolerate and showed very mild effect. Patient increased the Sinemet 125 dose to 12 tablets/day on his own for the last 2 years. He has no motor fluctuation and he is tolerating well this high dose. His examination still showing mild RT. foot inversion and mild spastic gait.

Conclusion: Patient's clinical phenotype and initial prolonged response to small dose of levodopa in the absence of any other abnormality clinically or by investigation is diagnostic of DRD. This extremely high dose of Sinemet 125 (12 tablets daily) was not reported before to the best of our knowledge.

P378

**An Irish family with myoclonus-dystonia due to a nonsense mutation in the epsilon-sarcoglycan gene.** S. O'Riordan, L. Ozelius, T. Lynch, M. King, St. Vincent's University Hospital, Albert Einstein College of Medicine, Mater Hospital, Temple Street Childrens' Hospital (Dublin, IRL; New York, USA)

Background: Myoclonus-Dystonia (M-D) is a movement disorder characterised by proximal, bilateral, myoclonic jerks. Dystonia occurs in most affected patients and may infrequently be the only symptom. Inheritance is autosomal dominant with reduced penetrance on maternal transmission suggesting maternal imprinting. Mutations in the gene for epsilon-sarcoglycan (SGCE) have been found to cause M-D.

Objective: To describe features of an Irish family with M-D.

Methods: 6 consenting members of the family were examined and videotaped according to a standardised protocol. 5 gave blood samples for genetic analysis.

Results: There were 4 affected individuals. Three consented to venesection and each had a mutation in SGCE. This is a nonsense mutation in exon 3 at position 289 C > T, resulting in the insertion of a stop codon R97X. This mutation has been described previously in two other families.

Phenotypic Features:

II:2: The proband, a 21 year old female, had a single generalised seizure at 2 years of age followed by a transient left hemiparesis. Investigations at that time were normal. There was onset of upper limb myoclonic jerks at age 4 years. The myoclonus is alcohol-responsive. Examination revealed upper limb/truncal myoclonus with cervical and upper limb dystonia.

II:5: Her sister, aged 13 years, developed an abnormal gait at age 4 years. She developed generalised myoclonus at this time, which affected predominantly her upper limbs and trunk. On examination she also has writer's cramp, cervical and lower limb dystonia. She was diagnosed with insulin-dependent diabetes mellitus at age 5 years. Investigations including CT brain, EEG, caeruloplasmin and urinary copper, urinary amino acids and serum lipids were normal. CSF cells, protein, amino acids, amine metabolites and lactate were normal. MERRF mutations were not detected.

II:1: The oldest sibling aged 22 years has complex partial seizures treated with valproate and carbamazepine. These first occurred at 10 years of age. Interictal EEG shows a right temporal seizure focus. Examination revealed a laterocollis but no myoclonic jerks or dystonia elsewhere. He has a tendency to alcohol abuse.

II:6: Another sister, aged 11 years, had a febrile seizure at 2 years of age. She developed predominantly upper-limb and truncal myoclonus at age 3 years. Upper limb dystonia was also seen on examination. She did not consent to venesection.

Mother (I:2) is unaffected but the father (I:1), who died from complications of alcohol abuse, had an abnormal neck posture since his early twenties but apparently had no myoclonus. A paternal uncle who did not consent to examination suffers from alcohol abuse and insulin-dependent diabetes mellitus.

Conclusion: This is the third M-D family described with this mutation in SGCE. The phenotype is consistent with that previously described for M-D, although seizures and diabetes mellitus also occur in members of this family.

P379

**Stiff man syndrome in spinocerebellar ataxia type 3.** J. Berciano, J. Infante, O. Combarros, A. García, C. de Pablos, G. Amer, J. M. Polo, V. Volpini, University Hospital Marqués de Valdecilla, Son Dureta Hospital, Institut Recerca Oncologica (Santander, Palma de Mallorca, Barcelona, E)

Background: Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominant inherited ataxia with a wide range of clinical manifestations.

Objective: To describe a novel association between SCA3 and stiff man syndrome (SMS). Patients, methods and results: The studied pedigree comprises seven affected individuals in three generations with onset of symptoms between 16 and 40 years. Their clinical picture consisted of gait and limb cerebellar ataxia, dysarthria, pyramidal signs, nystagmus and ophthalmoplegia. The proband patient was a 39-year-old man in whom such semeiology, soon after onset at age 30, evolved to severe stiffness and rigidity of axial and proximal limb muscles with painful spasms, foot and hand dystonic postures, dysphagia, urinary incontinence, erectile dysfunction, leg amyotrophy and fasciculations, stocking hipoesthesia, and facial paresis and myokymias. AntiGAD and antiamphiphysin antibodies were negative. Neuroimaging studies (three cases) showed cerebellar and spinal cord atrophy. Proband's electrophysiological study revealed continuous muscular activity in proximal muscles, signs of predominantly sensory axonal neuropathy, absence of somatosensory evoked potentials from lower limbs, facial myokymias, and normal silent period, brainstem re-

flexes and central motor conduction time; neither neuromyotonia nor afterdischarges were recorded. Molecular study demonstrated the SCA3 mutation with 73 CAG repeats in all three examined patients.

Conclusion: SMS should be added to the SCA3 clinical spectrum.

P380

**Therapeutic effect of long-term creatine supplementation in patients with Parkinson's disease.** J. Bender, A. Bender, Y. Schombacher, M. Elstner, F. Gekeler, T. Gasser, K. Tatsch, T. Klopstock, University of Munich (Munich, D)

Introduction: Creatine (Cr) is a natural compound of the complex cellular energy homoeostasis system and serves in its phosphorylated form (PCr) as an energy donor in order to maintain high levels of adenosine triphosphate (ATP). There have been numerous studies investigating the potentially neuroprotective role of Cr. In animal models of various neurodegenerative disorders, such as Parkinson's syndrome, amyotrophic lateral sclerosis and Huntington's disease, Cr exerted significant neuroprotective effects. In spite of the impressive results of experimental studies, there is no clinical study so far, examining the effects of Cr on symptoms and progression of Parkinson's disease (PD) in patients. We have started a randomized controlled trial with 60 PD patients taking oral Cr for two years and report on preliminary results after 20 patients have completed the first year of the study.

Materials and Methods: 60 patients with mild to moderate PD (Hoehn&Yahr $\leq 3$) received either Cr or placebo in an initial dose of 20 g daily for 5 days followed by 2 g per day thereafter. After 1, 3, 6 and 12 months patients were clinically evaluated by physical and neurological examination and completion of the Unified Parkinson's disease rating scale (UPDRS). Quality of life (QoI) was assessed by direct questioning and the SF-36 scale. Before starting on Cr (t0) and after 12 months (t12) of treatment, standard DAT scans were performed to quantify the activity of the presynaptic dopamine transporter.

Results: By now, 20 patients have completed the first year of treatment (13 Cr, 7 controls). So far, there is no significant difference in UPDRS changes between the groups. There was also no significant effect of Cr on QoL measured by SF-36 or direct questioning. There might be a positive effect on depression: at t0, 69 % of the Cr group suffered from depression, at t12 only 54 % (placebo: t0 29 %, t12 71 %). No significant differences in DAT scan changes were found between Cr and placebo treated groups. Cr was well tolerated by all patients. No specific side effects were reported, namely there was no nephrotoxicity.

Discussion: After one year of treatment, there are no significant clinical or metabolic differences between Cr and placebo groups. Since only half of the trial time is over by now and only one third of the patients has been included in the data above, these results have to be regarded as preliminary. We hope to find more marked effects after longer treatment duration and evaluation of the complete number of patients. To avoid unblinding the study, the statistical calculation has been performed by an independent third party, therefore no subgroups (e. g. correlation of treatment effects with duration or severity of symptoms etc.) could be investigated. As Cr was well tolerated by all patients, the dosage will be increased to 4 g/d for the rest of the study duration to improve its therapeutic effects.

P381

**CD4 memory and programmed cell death in Parkinson's disease.** L. Sanvito, E. Calabrese, L. Speciale, C. Mariani, P. Ferrante, N. Canal, Don C.Gnocchi Foundation, L. Sacco Hospital (Milan, I)

Background: Parkinson's disease (PD) is a neurodegenerative disorder of unknown aetiology. Several pathogenic mechanisms have been proposed. Immune alterations have been described as involved in the pathogenesis, but the existence of immune-activation in peripheral blood remains still controversial.

Objective: To evaluate the involvement of immune system in PD, we analysed the lymphocyte subset distribution and the programmed cell death (PCD) in peripheral blood mononuclear cells of PD patients and age matched healthy controls (HC).

Methods: In the peripheral blood of 16 PD patients and 36 HC we examined, by 3-color flow cytometry, the following lymphocyte subsets: CD3 + (Total T), CD3 + CD4 + (T Helper), CD3 + CD8 + (T Citotox-Suppressor), CD4 + 45RA + (naive), CD4 + 45RO + (memory), CD16 (Natural Killer), CD19 (B lymphocyte), and the surface expression of CD25 +, CD28 +, CD95 +, CD71 +, on CD4 + and CD8 + cells. Moreover, intracytoplasmic Bcl-2 protein detection with 7-amino-actinomycin D (7-AAD) staining after PMA + IONO activation, has been performed to evaluate the amount of CD4 + and CD8 + apoptotic cells.

Results: We observed a significant increase in percentage of CD4 + T helper, and particularly CD4CD45RO + (memory cells), in PD patients in comparison to HC. Moreover, an increase in activated CD8 + cells expressing CD71 has been observed in PD versus HC. No differences were shown in CD3 +, B and NK cell percentage between PD and HC. The percentage of CD4 + 7-AAD stained cells was lower while Bcl-2 intracytoplasmic expressing CD4 + cell percentage was higher, in PD than in HC.

Conclusion: Our data indicate an immune-imbalance in PD. The increase of CD4 + T helper and particularly CD4CD45RO + and CD8CD71 + in PD suggests the presence of activated circulating T cells. These T cells alterations could be explained by the decrease of PCD observed in PD.

P382

**Ultrasound-guided versus 'blind' intraparotid injections of botulinum toxin-A for the treatment of sialorrhoea in patients with Parkinson's disease.** O. Dogu, D. Apaydin, S. Sevim, D. Talas, Mersin University (Mersin, TR)

Objective: To investigate the efficacy and safety of botulinum toxin-A (BTX-A) injections into parotid gland using ultrasound-guided versus nonguided techniques for the treatment of silaorrhoea in patients with Parkinson's disease (PD). Methods: 15 patients with PD and sialorrhoea were included and divided into two groups. Group A ($n = 8$) was injected with BTX-A using ultrasound guidance. Group B ($n = 7$) was injected with BTX-A without ultrasound guidance. Saliva secretion was assessed quantitatively at baseline and week 1, 4 and 12. Patients and/or caregivers also assessed saliva secretion using visual analog scale (VAS). Results: All patients except one reported subjective improvement in sialorrhoea at the first week. Group A showed significantly higher rate of saliva reduction at the first week, whereas in Group B the reduction was not statistically significant from baseline at first week ($p > 0.05$). Comparisons of each treatment group also showed that ultrasound guided injections were superior to blind injections for saliva reduction. VAS scores showed an improvement in the mean rate of saliva secretion in each group at first week ($p < 0.05$). Two patients suffered from dry mouth in mild severity lasting one month. Conclusion: Intraparotid BTX-A injections using altrasound guidance may be an effective, easy and safe treatment for parkinsonian sialorrhoea.

P383

**Botulinum toxin A treatment in stiff-person syndrome.** A. Szczepanska-Szerej, M. Kulka, J. Wojczal, Z. Stelmasiak, University Medical School in Lublin (Lublin, PL)

Stiff-Person Syndrome (SPS) is a very rare disorder characterised by progressive fluctuating muscle rigidity and episodic spasm. Drugs enhancing GABA neurotransmission or immunomodulatory agents provide mild relief of clinical symptoms. So far, only two reports have demonstrated a significant clinical improvement in patients with SPS when muscles were injected with BotulinumToxin A (BTA). We investigated the effectiveness of intramuscular injections of BTA in a patient with clinical, biochemical and electrophysiological evidence of SPS.

A 41-year-old woman with coexisting epilepsy and insulin-dependent diabetes mellitus was hospitalised because of stiffness and paroxysmal spasm of trunk and proximal limb muscles. Benzodiazepines and baclofen were administered. Because of insufficient results of the pharmacological treatment the injections of BTA into involved muscles were done – 1000 U BTA were injected to the pelvic girdle, thorax and lumbar paraspinal muscles. Clinical observations included measure of frequency of spasm, degree of stiffness, timed activities in 0, 1, 2, 7, 11, 16, 20 weeks. Pain and current health status of patient were assessed by Visual Analogue Scale (VAS). Significant improvement started one week after injections and lasting about 4 months was observed. Seven weeks after BTA injections the patient reported cessation of spontaneous spasm, the ability of walking increased by 23 %, distance from the top of the middle finger diminished by 52 %, hyperlordosis decreased about 30°, abduction angle in hip joints increased about 25° and patients' general health status by VAS improved about 40 %. The side effects were not observed and repeated injections of BTA were done one week ago.

Using BTA injections into involved muscles for the treatment of SPS can be followed by marked functional improvement and reduce the need for systemic drugs.

P384
**Two cases of apraxia of lid opening and reflex blepharospasm secondary to right hemispheric infarction: clinical and radiological correlations.** L. Capone, R. Gentile, F. Teatini, R. Schoenhuber, Regional General Hospital (Bolzano, I)

Introduction: Blepharospasm (BS) is a focal dystonia characterized by forceful and involuntary contracture of the orbicularis oculi muscle (OO). Apraxia of lid opening (ALO) is characterized by an inability to open the eyes at will. Reflex blepharospasm (RBS) is a forced eyes closure elicited by stretching of eyelids. ALO is sometimes associated to idiopathic BS, but cases secondary to vascular brain lesion were also documented. We report two cases of right hemispheric infarction who developed ALO and RBS. Reports: The first patient was a 83-year old woman without clinical or familiar history of neurological diseases, no referred drug intake, who developed abruptly severe left hemiparesis. On examination, her eyes were continually closed. Severe ALO made the patient functionally blind. Moreover all attempts by the examiner to open forcefully her eyes by stretching the eyelids, induced a RBS, associated with forced left rotation of neck. This phenomenon was stronger by stretching patient's right eyelids. Computer tomographic (CT) brain scan revealed an infarction of the right cerebral middle artery distribution involving basal ganglia. In the following three weeks, the patient showed a spontaneous progressive improvement. The second patient was a 73-year-old woman affected by atrial fibrillation who suddenly developed confusion and left hemiparesis. At neurological examination she held her eyes closed, she could not open her eyelids spontaneously or if asked, and RBS appeared on attempts by the examiner to forcefully open her eyes. CT brain scan showed a large right frontal hemorrhagic infarction. After few days she could newly open her eyes at will and left hemiparesis also improved.

Comments: Secondary focal dystonia often occurs in association with other neurologic signs (left hemiparesis in our cases). Upper midbrain and basal ganglia lesions are involved in developing BS, while ALO is founded also in cortical- and subcortical lesions. In our patients BS and ALO are simultaneously present after right hemispheric stroke, only in one case involving both basal ganglia and cortical areas.

P385
**The role of local eye disorders in blepharospasm: the electrophysiological findings.** S. Sahin, M. Erdemir Kiziltan, I. U. Cerrahpasa Medical School (Istanbul, TR)

Objectives: Blepharospasm is a focal dystonia characterized by involuntary contractions of eyelid muscles. Some of the blepharospasm cases are known to be associated with peripheral risk factors (PRF) such as trauma or surgery of/or near the eye, blepharitis or keratoconjunctivitis. In this study we compared blink reflex parameters and recovery curves of R2 response in blepharospasm cases with and without PRF.

Methods: Twenty-two control subjects and 39 cases with blepharospasm were included in this study; 10 of them had peripheral risk factors. Blink reflex (BR) were elicited percutaneously over the supraorbital foramen. R2 recovery yielded by the double stimuli with 200–400–600–800–1000 ms interstimulus intervals.

Results: No statistically significant relationship was found between the latency values of control and blepharospasm groups ($p > 0.05$). R2 areas were found as 8.8 Mv X ms and 6 mV X ms. in cases with and without PRF respectively but were not reflected on the statistics. The recovery curve of the R2 component of the blink reflex was enhanced in patients with blepharospasm when compared to the control group. Early recovery values which were believed to reflect the facial interneuron (FIN) excitability with a considerable importance on the segmental control of the trigeminofacial circuit in the blepharospasm cases with PRF were found meaningfully higher than the group without peripheral risk factors ($p = 0.302$).

Conclusions: Our results in blepharospasm cases with PRF show that the suppression of R2 responses of the blink reflex that normally follows a conditioning stimulus is less than blepharospasm cases without risk factors.

## General neurology

P386
**Necroapoptotic cell death in NMDA-induced toxicity.** M. Kilinc, Y. Özdemir-Gürsoy, A. Can, T. Dalkara, Baskent University, Hacettepe University, Ankara University (Ankara, TR)

Two mechanically distinct but related forms of neuronal death have been identified, and take the form of necrosis or apoptosis. Both forms of cell death have been detected after NMDA toxicity in the brain.

Caspase-3 is a cystein protease that acts as both an initiator and an executer of the apoptotic process. Immunohistochemical studies have shown that activity of this enzyme increases after NMDA toxicity.

Cathepsin-B is a lysosomal protease, related with necrotic cell death. When the enzyme is activated, this can be detected first by the appearance of dense granular cathepsin-B immunoreactivity within the cells, and later by diffuse staining in the extracellular space consistent with degranulation of the lysosomes. Showing the colocalization of caspase-3 activity along with cathepsin-B may help to demonstrate that necrotic and apoptotic processes do take place in the same cells, at the same time, after NMDA toxicity.

We produced excitotoxic lesions by intrastriatal stereotactic N-metil-D-Aspartate (NMDA) microinjections. Mice were anesthetized with chloral hydrate, and body temperature was kept at 36.9°C with a thermostatic heating pat. The head was fixed to a sterotaxic frame. A burr hole was drilled, and an injection needle (26 ga) was lowered into the right striatum (anterior 0.5 mm, lateral 2.5 mm, ventral 2.5 mm, from bregma). Drug was injected in a volume of 0.3 μ, over 2 min, and the needle was left in place for an additional 8 min. Mice were killed at 1–6 hours after injection. After perfusion with saline followed by a formalin solution, the brains were extracted and formalin fixed. Then 20-μm-thick coronal cryostat sections were taken. Every 10th section was stained by hematoxylin and eosin. The remaining sections were stained by double immunofluoresence histochemistry using an antibody to caspase-3p20 and later another antibody to cathepsin-B. The antibody to caspase-3p20 detects the active form of caspase 3 while the cathepsin antibody detects mature and precursor forms of cathepsin-B.

We observed that intrastriatal microinjections of NMDA consistently produced well delineated lesions. The lesions were usually located approximately at the level of bregma and confined to the striatum along with the adjacent cortex. NMDA-induced striatal cell loss and pallor were visible at 1 hour. We determined at the light microscopic level that pynotic neurons could be detected as early as 2 hours. We also observed that both caspase-3 and cathepsin-B activity increased together after subcortical NMDA injection. The density of caspase3p20 positive cells was more prominent in the periphery, while cathepsin-B positive cells were robust in the center of the lesion.

In conclusion our study demonstrates that excitotoxic cell death has a mixed (necroapoptotic) phenotype.

P387
**Diagnostic pitfalls in patients with hypoxic brain damage.** V. C. Zingler, B. Pohlmann-Eden, Klinikum Grosshadern, LMU, Toronto Western Hospital (Munich, D; Toronto, CAN)

Introduction: The two destruction proteins, neuron-specific enolase (NSE) and protein S-100B (S-100B), have been reported to be significantly elevated in patients who remain unconscious after cardiac arrest (CA) and cardiopulmonary resuscitation (CPR) during the first 3 days after CPR. The bilateral loss of cortical responses (BLCR) pattern exhibited by somatosensory evoked potentials (SEPs) is usually considered a reliable sign of poor outcome in these patients. The aim of this case series is to present the partly contradictory biochemical and electrophysiological data of three patients who were in a persistent vegetative state (PVS) due to severe hypoxic brain damage after CPR in order to discuss the possible diagnostic pitfalls.

Methods: Serum concentrations of NSE and S-100B were measured on days 1, 2, 3, and 7; SEPs were also recorded within 48 hours and on day 7 after CPR.

Results: Two patients had significantly increased concentrations of NSE and S-100B during the first 3 days after CA, a finding that indicates ongoing neuronal destruction. However, the SEPs of these patients were either normal or only showed a diminished amplitude configuration. The SEPs of the third patient repeatedly exhibited a BLCR pattern, but both destruction proteins were only mildly increased above the upper normal values on all study days.

Discussion: Previous neuropathological studies showed that diffuse hypoxia due to CA can cause different or combined neuropathological fea-

tures: [1] diffuse neocortical damage, [2] subcortical white matter damage, and [3] damage to the major relay nuclei of the thalamus. The preservation of cortical responses in the two patients with elevated levels of both destruction proteins might have been caused by the random multifocal laminar cortical and subcortical injuries, whereas the neurons that generated the N 20 SEP-components were preserved. The history of the third patient who had a BLCR pattern and only mildly increased protein values suggests a strategic lesion in the major relay nuclei of the thalamus. The extent of cortical brain damage and the amount of destruction proteins released were minor.

Conclusions: In light of our partly conflicting data for the three PVS patients, we conclude that only a multimodal approach combining biochemical and electrophysiological tests with clinical data makes it possible to reliably assess the prognosis of these patients.

**P388**

**Typical electrophysiological impairment in case of Wilson's disease.** W. Hermann, P. Günther, T. Villmann, A. Wagner, Universitätsklinik und Poliklinik für Neurologie (Leipzig, D)

Objective: In addition to hepatic and extrapyramidal motor clinical symptoms, Wilson's disease patients also exhibit subclinical disorders of other central nervous pathways.

Methods: In this study, an impairment profile is described by means of 8 simultaneous electrophysiological tests (EAEP/early acoustically evoked potential, MSEP/median nerve somatosensory evoked potential, TSEP/tibial nerve somatosensory evoked potential, T-VEP/transient visually evoked potential, MEP/motor evoked potential, EEG, heart frequency variability, SSR/sympathetic skin response) for 37 patients (28 patients with the neurological form, 9 patients with the non-neurological form) undergoing long-term medicamentous therapy.

Results: The 64.3 % occurrence of a delayed wave III and/or IPL III-V prolongation in patients with the neurological form makes pathological FAEP the most common form of disorder, followed by impairment in MSEP, TSEP, MEP and T-VEP. Patients with the non-neurological form usually have normal values, although latency prolongations occur in isolated cases. The range of findings of evoked potentials is primarily characterised by latency prolongations, i. e. a demyelinising impairment type, and significant losses of potential hardly occur (except in the MEP). The electrophysiological impairment profile does not include EEG changes or vegetative disorders. Regarding the severity of nervous pathway disorder, the MEP shows the most pronounced latency delays.

Conclusions: Observing this characteristic electrophysiological impairment profile is useful for both monitoring therapy and differential diagnosis.

**P389**

**Vitamin B12 deficiency can mimic multiple sclerosis – report of two cases.** W. Wicha, I. Kurkowska-Jastrzêbska, A. Czlonkowska, Institute of Psychiatry and Neurology (Warsaw, PL)

Because of heterogeneous manifestations of MS incorrect diagnosis is not uncommon. Numerous disorders can potentially mimic multiple sclerosis (MS). The diagnosis of MS requires certain clinical features. Tests supporting the diagnosis of MS, including magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) examination help to make diagnosis as early as possible, however, they are not specific for MS. One of presently uncommon diseases that should be considered in differential diagnosis of MS is Vitamin B12 deficiency. We would like to present two cases of Vitamin B12 deficiency that was misdiagnosed as MS. In the first case, 38 years old woman revealed paresthesia and gait disturbances. Neurologic examination revealed minor spastic paraparesis, diminished tendon reflexes, disturbation of superficial and vibration sense in lower limbs and trunk and slight ataxia. MRI scan showed multifocal demyelinating lesions periventricular and affecting corpus callosum, no foci were found in spinal cord. Oligoclonal bands were positive in CSF examination. Because she did not improve after methylprednizolone treatment she was diagnosed for vitamin B12 deficiency, and showed very low vitamin B12 level. Gastroscopic examination established diagnosis of Addison-Biermer disease. After administration of Vitamin B12 patient gradually improved and became asymptomatic. In the second case, 32 year old woman with a history of recurrent anemia, presented dizziness, weakness of legs, urinary urgency and Lhermitte's sign. On neurologic examination she had slight spastic paraparesis. MRI changes consisted of small, periventricular lesions and large, non-homogenous lesion at the level C2 – C5. Oligoclonal bands were negative. After administration of Vitamin B12 neurologic symptoms gradually improved. After a year she was hospitalized with dizziness and gait disturbances and depression that started after disruption of the supplementation of Vitamin B12. MRI showed periventricular lesions, while spinal cord appeared to be normal. Vitamin B12 treatment improved neurological and psychiatric disturbances. No further deterioration was seen. In both presented cases macrocytic anemia was present. Good response to Vitamin B12 supplementation allowed us to establish diagnosis of Vitamin B12 deficiency. Therefore Vitamin B12 deficiency must be considered if there is atypical response to treatment or hematologic abnormalities.

**P390**

**Are mirror movements influenced by handedness?** I. Uttner, E. Kraft, D. A. Nowak, F. Müller, J. Philipp, A. Zierdt, J. Hermsdörfer, University Ulm, Städtisches Krankenhaus, Neurologische Fachklinik, Ingenieurbüro Dr. Philipp, Krankenhaus München-Bogenhausen (Ulm, Munich, Bad Aibling, Berlin, D)

Execution of bimanual antiphasic movements is characterized by a strong tendency of interlimb coupling. In right-handers (RH), dominant hands seem to act as leading hands, whereas in left-handers (LH) no such clear dominance of one limb has been reported (Swinnen et al. (1996) Neuropsychol, pp. 1203–1213). This could also be true for the physiological phenomenon of the mirror movements (MM), involuntary co-activation of symmetric muscles opposite to intended movements of one hand, which is possibly based on control of both right and left limbs by one cerebral hemisphere.

To objectify effects of handedness on MM, we measured grip forces of 17 LH and 17 RH matched by age, gender, and motor practice. Using force transducers which had to be held in a grip between thumb and the other fingers of each hand, subjects had to repetitively change the grip force in one hand ("voluntary hand") while the other ("mirror") hand just had to prevent the transducer from dropping. The task was performed with slow (1.5 Hz, triggered by acustical signal) and maximal speed. To detect MM, we identified the maxima and minima of the voluntarily produced force amplitudes and looked for corresponding peaks in the "mirror hand". Mirror activity was then quantified as the amplitude ratio of the "mirror" and the "voluntary" hand. In addition, cross-correlations and time lags (i. e. time interval between the corresponding maxima) of the force amplitudes were computed.

MM amplitude ratios reached from at mean 0.31 % (fast, voluntary activation of the non-dominant hand) to a maximum of 0.41 % (slow, non-dominant hand) for the LH and from 0.26 % (fast, dominant hand) to 0.46 % (slow, dominant hand) for the RH. In LH, time lags ranged from 6.12 ms and 29.94 ms (fast/slow, dominant hand) to 0.29 ms and 37.71 ms (fast/slow, non-dominant hand). The cross-correlations measured in this group ranged from at mean $r = 0.31$ (fast, non-dominant hand) to $r = 0.43$ (slow, non-dominant hand). For the RH, we found time lags of 10.76 ms, 35.82 ms, 6.71 ms and 25.53 ms. Cross-correlations ranged from $r = 0.30$ (fast, dominant hand) to a mean maximum of $r = 0.39$ (slow, non-dominant hand). Statistical comparisons showed no significant differences between the two groups (range: $p = 0.057–0.97$; U-test). In sum, our results indicate no substantial influence of the handedness on extent and temporal synchronization of MM.

**P391**

**Evaluation of neuro-otology patients in a general neurology office.** J. Porta-Etessam, A. Martínez-Salio, A. Berbel-García, A. Ramos, J. Millán, R. Garcia-Ramos, V. Gonzalez-Martinez, Hospital Universitario (Madrid, E)

About 30 % of neurology consults in a general neurology unit are due to dizziness, vertigo or disequilibrium. The objective is to determine the aetiology and prognosis of the dizziness and vertigo syndromes in 160 patients seen in a general neurology office.

Prospective study including 160 (96 female and 64 male) consecutive patients suffering from dizziness or vertigo seen in a general neurology office. The patients belonged to the same health area of 80,000 covered by "Centro de Especialidades Médicas de Aranjuez" (Hospital Universitario "12 de octubre". All the patients were examined by the authors and have a complete neurological history and examination. Supplementary examinations such as CT scan of the head and IAC, brain MRI, electronistagmography, and brain stem evoked potential, routine blood (including ESR, VDRL, vasculitis screening) and urine analyses were carried out in some of the patients.

About 60 % of all patients presenting with dizziness, vertigo or disequilibrium suffered one of the following syndromes: bilateral neuropathy (20 %), vestibular neuritis (19 %), Psychophysiologic dizziness (17 %), benign paroxysmal positioning vertigo (12, 5 %) and benign recurrent ver-

tigo (9%). Most of the patients improved with treatment. Neuroimaging revealed only two patients suffering from cerebellar stroke and one with an acoustic neurinoma.

Most of the patients suffer from: Bilateral vestibulopathy, phobic postural vertigo, psycho-physiologic dizziness and benign paroxysmal positionating vertigo. With a correct diagnostic and therapeutic approach the Diagnosis and management of vertigo always require interdisciplinary approach, and history and examination are still more important than neurophysiological techniques.

P392

**Cerebrotendinous xanthomatosis: a case report.** R. Gürer, N. Isik, H. Celebi, S. Erdogan, Z. Yilmaz, T. Seleker, SSK Goztepe Educational Hospital (Istanbul, TR)

Cerebrotendinous Xanthomatosis is a rare autosomal recessive lipid storage disease with prominent neurological features.We report the physical, radiological and biochemical findings of a patient diagnosed as Cerebrotendinous Xanthomatosis.

A 24 year-old woman was referred for evaluation of progressive dementia and abnormal gait. Physical examination showed nodular thickening of both achilles tendons and high arched feet. Neurologic examination showed mild spasticity in the lower extremities, normal power, diffuse hyperreflexia, moderate truncal ataxia, mild lower extremity ataxia, stocking distribution sensory loss to light touch and loss of proprioception in the legs.

Her gestation, birth and early development were normal but she had intractable diarrhea during childhood. She attended school education only up to fifth class of primary school because of learning problems.

Xanthomas appeared at the age 7 on the achilles tendons bilaterally. They were grayish yellow in colour and they were painless.Cataracts were found at age 10. Her walking became unsteady at age 22.

Her neuropsychological tests showed widespread neurocognitive deficits with poor attention and concentration and seriously impaired recent memory. Laboratory tests gave normal total plasma cholesterol, LDL cholesterol and triglycerides levels. Cardiological examinations including echocardiography and electrocardiography were normal. Her EEG was normal. Her brainstem auditory evoked potentials and visual evoked potentials were normal but bilateral P40 responses were prolonged in her somatosensory evoked potentials with tibial nerve stimulation. Cranial MRI showed periventricular and subcortical T2 hyperintensities in cerebral hemispheres. Her MR Spectroscopy was reported as normal. MR study of achilles tendons showed a diffuse enlargement of the tendon from musculotendinous junction to calcaneal insertio, and also showed heterogenous hypointensity signal in T1 and T2 images. Cystic degeneration area in the right achilles tendon was named as xanthoma. We are not able to detect cholestanol level in our country.

Early diagnosis and treatment is imperative in Cerebrotendinous Xanthomatosis to slow or even reverse the progression of the disease.We began the treatment with chenodeoxycholicacid. Her gait had been improved minimally so that tandem walking was steadier but no improvement was observed in her intellectual functions.

P393

**Reversible corpus callosum lesions due to metronidazole toxicity.** V. I. Meire, J. L. De Bleecker, University Hospital (Ghent, B)

Background: Focal imaging abnormalities of the corpus callosum are rare in non-multiple sclerosis patients. We report a rare case of drug-induced corpus callosum lesions by metronidazole. Recognition is important because of the reversibility of both the clinical and the MRI abnormalities after discontinuation of the drug.

Patient and methods: We investigated a 20-year-old man with Becker muscular dystrophy and ulcerative colitis. He presented with extremity dysesthesia, dysarthria and decreased visual acuity. He had been taking metronidazole for two years. The family history was negative. The patient was investigated by MRI scans of the head, blood analysis, lumbar puncture, multimodal evoked potentials and electromyography (EMG).

Results: MRI studies of the head showed prominently increased signal intensity within the genu and the splenium of the corpus callosum on T2-weighted images. Extensive laboratory investigations only showed increased creatine kinase due to the muscular dystrophy. The cerebrospinal fluid was normal with no signs of intrathecal immunoglobulin synthesis. Pattern reversal visual evoked potentials showed increased latencies on both sides. EMG confirmed a distal sensory neuropathy, probably due to metronidazole toxicity. All clinical symptoms and signs improved dramatically 2 months after metronidazole was stopped, and gradually normalized thereafter. Control MRI after two months showed significant improvement of the abnormalities in the corpus callosum. A final MRI after eight months showed almost complete resolution.

Conclusion: Drug toxicity should be included in the differential diagnosis of possible demyelinating lesions of the corpus callosum. Our case confirms metronidazole toxicity as a reversible cause of increased signal intensity in the corpus callosum on T2-weighted images.

P394

**Intravenous immunoglobulin as long-term treatment in young patients with myasthenia gravis after thymectomy.** G Rizos, C. Tsalamas, D. Xafenias, R. Lampousis, A. Karlovasitoy, P. Xamlatzis, S. Baloyannis, Ahepa University Hospital (Thessalonica, GR)

Objective: To study the effectiveness and safety of intravenous immunoglobulin (IVIG) as long-term therapy in patients with myasthenia gravis (MG) after thymectomy.

Methods: 5 patients with MG after thymectomy, group A (4 females 1 male, main age 24 ± 6 years) received IVIG initial dose of 400 mg/kg per day for five days, followed by 400 mg/kg per day for 2 days every 4 months. As control group (group B) we study 5 patients (aged 34 ± 8) who received prednisone 15 mg per os (3 patients) every alternate day or azathioprine (2 patients) 2.5 mg/kr per day. All of the patients were in class IV according to clinical classification of myasthenia gravis foundation, had positive acetylcholine receptor antibody titres and were positive in repeated stimulation test (Desmet). All of the patients were re-examinated every 3–4 months for at least 2.5 years. The disease severity and progression was tested using the Quantative MG score and the activities of daily living (ADL).

Results: Patients in group A, anticholinergic drug (pyridostigmine) was gradually diminished (300 mg to 180–240 mg). All of them had improvement in Quantitative MG score and in ADL in the first 18 months but in the last 1 year of the study they were stable exept 1 patient who died from pulmonary infection at the end of the study. The acetylcholine receptor antibody titres were diminished but not statistically important according to group B. There were no serious side effects related to IVIG.

In control group B, the anticholinergic drug was diminished for only a short time period (at the end of the study all patients received 300 to 360 mg pyridostigmine) and had fluctuations in Quantitative MG score and in ADL (the mechanical ventilation was necessary in 2 patients).

Conclusions: In spite of the small number of patients of the study it seems that IVIG can be used as a chronic maintenance therapy for treatment of MG on a long-term basis without any significant side effect and may be able to stabilize chronic patients mainly in the first 18 months, but there were not any important differences for longer time period compared to more "classical" treatments.

P395

**Osmotic myelinolysis: case report.** S. Eryilmaz, M. Kilinc, S. Benli, N. Ozdemir, M. Teksam, Baskent University (Ankara, TR)

Myelinolysis is a neurologic disorder that can occur after rapid correction of hyponatremia. Initially named "central pontine myelinolysis", this disease is now known to also affect extrapontine brain areas. Manifestations of myelinolysis usually evolve several days after correction of hyponatremia. Typical features are disorders of upper motor neurons, spastic quadriparesis, pseudobulbar palsy, and mental disorders ranging from mild confusion to coma. Death may occur.

We treated a 40 year old woman, who was a chronic renal failure patient. She had presented with severe headache, nausea, and vomiting. As her blood urea nitrogen (BUN) and creatinine (Cr) levels were high she was treated with haemodialysis. Although this was her first haemodialysis session her BUN and Cr levels were lowered near normal in an outpatient haemodialysis center. After dialysis her blood Na level was found to be 123, and K 2.1 mEq/L, which was addressed by Na replacement therapy. During this replacement therapy she experienced a tonic-clonic seizure, and she was referred to our hospital. On neurological examination she was awake, she could cooperate to verbal stimuli by eye movements, and was quadriplegic. The first Magnetic Resonance Imaging revealed nothing abnormal. The MR repeated one week later revealed hyperintense areas in the pons, bilateral caudate and lentiform nucleuses, and parietal cortex on T2W images. Perfusion MRI and MR Spectroscopy findings were compatible with osmotic myelinolysis. Her neurologic status improved very little in two months' time.

We wanted to present this case to emphasize the importance of slow correction of hyponatremia, in clinically suspected cases repeated neuroradiologic examinations should be performed and to demonstrate the evalution of the myelinolytic lesions of osmotic myelinolysis.

P396
**Headache in hospitalized patients.** S. Santos, T. Casadevall, O. Fabre, M. Garces, C. Tejero, L. Pascual, J. López Del Val, P. Larrode, C. Iñiguez, University Clinical Hospital Lozano Blesa (Zaragoza, E)

Objective: to study the prevalence of headache in hospitalized patients and analyse the differences between the symptomatic and primary types.

Methods: the database of patients admitted to our hospital because of their headache during the last four years.

Results: the study included 412 patients. There were 171 men and 239 women, ranging in age from 14 to 95 years with a mean age of 56.97 years (SD 19.86). Among the 127 primary headache cases (42.04 years SD: 19.1), the following diagnoses regarding the headaches subtypes were made: 53 cases had migraine, 40 had tension-type headache, 30 had chronic daily headache (15 transformed migraine, 13 chronic tension headache and 2 continua hemicrania), while 4 patients had trigemino-vascular headaches (1 SUCNT syndrome, 3 cluster headache). In the whole group of secondary headaches (63.97 SD: 18.3) the more frequent etiologies were stroke (150 cases) and acute post-traumatic headache (10 cases).

Conclusion: in our study, the most common type of headache was the one due to stroke whereas tension-type headache is the more prevalent in the group of primary headache. Patients with primary headache were significantly younger ($p < 0.05$).

P397
**Clinical improvement after coenzyme Q 10 treatment in a late onset cerebellar ataxia.** M. Gironi, C. Lamperti, R. Nemni, M. Moggio, F. Guerini, P. Ferrante, N. Canal, Fondazione D.Gnocchi, Ospedale Maggiore Milano (Milan, I)

The unique combination of late onset cerebellar ataxia, hypergonadotropic hypogonadism, motor-sensory neuropathy and sensory-neural deafness due to CoQ10 deficiency is described.

Primary CoQ10 deficiency has been described associated with a childhood onset of cerebellar ataxia and cerebellar atrophy (Musumeci et al. (2001) Neurology 56:849–855; Di Giovanni et al. (2001) Neurology 57:515–518).

Two brothers, 38 and 33 years old, out of seven sibs of non consanguineous parents, had a normal development through childhood and adolescence. Family history was negative for neurological and endocrinological disease. At 30 years of age they started complaining progressive dysarthria, walking imbalance and azospermia. On examination, scanning speech, broken pursuit movements, unsteady balance and ataxic gait were evident. Blood tests including Vitamin E and anti-gliadin were normal, patients were screened for Friedreich ataxia, SCA 1–6. Endocrinological screening showed decreased testosterone and increased gonadotropin. Brain NMR showed severe cerebellar atrophy. Instrumental tests found out mild sensorineural deafness, moderate sensory-motor neuropathy, predominantly of axonal type, cerebellar and pons atrophy, mild cognitive impairment. Muscle biopsy was normal as well as the mitochondria enzymes. No mutations in the mtDNA were found. CoQ10 concentrations in frozen muscle were tested by reverse-phase high-performance liquid chromatography and COQ10 level in muscle was 15.8 μg/mg tissues and 13.5 μg/mg tissues (normal range $27 \pm 5$ μg/mg tissues).

International Cooperative Ataxia Rating Scale (ICARS) was carried out before and monthly after Coq 10 treatment (750 mg/die and 1200 mg/die) showing a clinical response in postural and gait stability, speech articulation and kinetic functions. Primary CoQ10 deficiency is a mitochondrial encephalomyopathy, of which ataxia can be considered the cornerstone syndrome. Our patients are similar to the ones previous described with CoQ10 deficiency, nevertheless they are peculiar because of the adult onset and the severe endocrinological involvement.

We suggest that CoQ10 deficiency must be considered as a possible differential diagnosis of adult onset cerebellar atrophy and cerebellar ataxia, when other causes of ataxia are ruled out. CoQ10 administration seems to be efficacious also in adults.

## Genetics

P398
**Clinical and genetic investigations in families with benign familial infantile convulsions (BFIC).** Y. G. Weber, A. Berger, N. Bebek, S. Karafyllakes, G. Kurlemann, S. Maier, C. Hang, C. Laux, D. Rating, B. Püst, A. Halbach, U. Stephani, B. A. Neubauer, Y. Fukuyama, M. Osawa, K. Saito, F. Lehmann-Horn, K. Jurkat-Rott, H. Lerche, University of Ulm, Pediatric Hospital, University of Munster, University of Heidelberg, University of Kiel, University of Giessen, University of Tokyo (Ulm, Deggendorf, D; Istanbul, TR; Munster, Duisburg, Heidelberg, Hamburg, Kiel, Giessen, D; Tokyo, JP)

Benign familial infantile convulsions (BFIC) is a rare form of idiopathic epilepsy with an autosomal dominant mode of inheritance. It is characterized by series of partial afebrile seizures occurring around the 6th month of life. The disease has a benign course with a usually normal development and only rare seizures in adulthood. Linkage analysis could define three susceptibility loci on chromosomes 19q, 16p12-q12 and 2q23–31. Up to now, no responsible gene has been found. We collected 17 BFIC families of German, Turkish or Japanese origin with 2–10 affected individuals. Partial seizures and generalized convulsive seizures occurred, in some families generalized tonic clonic seizures were predominant. Ictal EEGs were not available. In 10 of the families analyzed up to now, linkage to the previously described locus on chromosome 19 and 2 was excluded, as well as to the known loci for benign familial neonatal convulsions, BFNC, on chr 8q and 20q. The chromosome 16 BFIC locus could be confirmed in 8 of the 10 analyzed families with a maximum Lod score of 2.8. Complete linkage results for these 5 loci for all families will be presented.

P399
**Mutational analysis of exons 5 and 6 of PTEN gene in patients with malignant glioma.** J. Abdullah, N. Zainuddin, H. Jaafar, M. N. Isa, University Sains Malaysia (Kubang Kerian, Kelantan, MY)

Mutations of PTEN gene have been reported previously in malignant gliomas, with frequent occurrence in glioblastoma multiforme (GBM). In this study, we analyzed PTEN status in DNA samples of malignant gliomas from patients of the East Coast of Peninsular Malaysia using PCR-SSCP method. PCR analysis was performed and the amplified products were subjected to SSCP analysis. Out of 20 glioma cases, two cases of glioblastoma multiforme, three cases of anaplastic astrocytoma, a case of anaplastic pleomorphic xanthoastrocytoma and a case of anaplastic ependymoma showed SSCP band shifts. DNA sequencing analysis of these samples revealed missense and nonsense mutations, with cluster of mutations in the region 5' to the core phosphatase motif of exon 5 and the 5'-end of exon 6. Our findings demonstrated that exons 5 and 6 of the PTEN tumour suppressor gene contribute to the progression of malignant glioma.

P400
**Clinical course and HLA in Parkinson's disease.** G. Gossrau, A. Kempe, M. Füssel, B. Herting, U. Sommer, R. Koch, H. Reichmann, J. B. Lampe, Institute of Reconstructive Neurobiology Bonn, University of Dresden, Schering AG (Bonn, Dresden, Berlin, D)

Idiopathic Parkinson's Disease (PD) is a common progressive neurodegenerative disorder, characterized clinically by a combination of motor symptoms and postural instability. Pathological features include degeneration of dopaminergic neurons in the substantia nigra pars compacta coupled with eosinophilic inclusions. It is widely believed that a combination of interacting genetic and environmental causes may be responsible for the majority of PD-cases. The role of the immune system in PD is still a matter of controversy. Immunological abnormalities have been reported in various brain areas and in peripheral immune parameters in PD patients.

A different clinical course of PD may be a result of spatiotemporal distinctions in disease-related pathogenic cascades. This could have one reason in a specific pattern of immunological competent molecules within the affected tissue.

Therefore, we investigated 37 unrelated German PD-patients for certain clinical features and the corresponding HLA genotype. The diagnosis was made on the basis of clinical criteria for definite PD and none of the patients exhibited family members with a movement disorder. Clinical parameters assessed in this study include the dominant type of PD symptoms, the age at disease onset and the presence of dyskinesias. The typing of HLA class I and II alleles was systematically performed using polymerase chain reaction-sequence specific oligonucleotides (PCR-SSO) and polymerase chain reaction-sequence specific primer (PCR-SSP).

As published elsewhere, the PD patients showed a statistically signifi-

cant increase of the HLA-A*68 allele (Bonferroni's-corrected chi-square: $p < 0.0004$) and the HLA-DQB1*06 allele (Bonferroni's-corrected chi-square: $p < 0.02$) when compared to normal controls.

Among other things, the HLA Cw*07 allele is present in 50 % of PD patients with earlier disease onset (between age 25 and 50 years) compared to 25 % of patients with later disease onset (51 years and older) and normal controls (25.6 %).

This difference results in Mantel-Haenszel corrected p value $p = 0.05$ which is after Bonferroni adjustment not statistically significant. Nevertheless it is a first clue for varieties in HLA molecule pattern between PD patients of different ages at disease onset. At least this observation has to be validated with a larger patient group.

Our results may indicate an involvement of the HLA molecules in the pathogenesis of idiopathic Parkinson's disease.

P401

**Tau P301L mutation and non-Alzheimer dementias in Italy.** M. Mancuso, M. Leone, M. Filosto, G. Tognoni, A. Rocchi, G. Siciliano, P. Nichelli, L. Murri, Neurological Institute (Pisa, Modena, Verona, I)

Background: Mutations in the tau gene are responsible for familial frontotemporal dementia (FTD), for some case of progressive supranuclear palsy (PSP) and cortico-basal degeneration (CBD). The Proline-to-Leucine mutation at codon 301 (P301L) is considered the most common tau mutation in FTD. Most of the patients harboring this amino acid change share similar presenting symptoms such as aggressive behavior, disinhibition, irritability and language deficits.

Aims: To assess the frequency in Italy of the P310L mutation and the possible genotype/phenotype correlations.

Patients and Methods: We have studied 48 unrelated patients (mean age 55.3 + 8.11 yrs). 29 were diagnosed as FTD (60 %), 12 (25 %) as PSP and 7 (14 %) as CBD. A family history was present in the 35 % of FTD and in 25 % of PSP cases. All the other cases in whom was not observed or reported a first-degree relative with dementia were considered sporadic. Total DNA from patients' blood was extracted using standard protocols. The P310L mutation was studied by PCR-RFLP analysis.

Results and Conclusions: We did not observe the P301L mutation in any case. Our study indicates that the frequency of the P301L mutation in the Italian population with non-AD dementia is probably low. Furthermore, it is unlikely that P301L mutation has a distinct clinical phenotype because our cases, in whom a phenotype suggestive of P301L mutation was observed, did not carry this change.

P402

**Regulation of Socs-1 and Gerp/TRIM-8 expression on multiple sclerosis patients undergoing interferon beta-1 treatment.** E. Toniato, L. Bonanni, G. Vianale, R. Grande, L. Liberati, R. Muraro, S. Martinotti, D. Gambi, University of Chieti (Chieti, I)

The pathogenesis of Multiple Sclerosis (MS), the most common demyelinating disease of the Central Nervous System (CNS), is known to involve T and B lymphocyte-mediated autoimmunity processes. However, the mechanism that regulates lymphocyte activity and cytokine secretion in MS is poorly understood. Type I (IFN-a/b) and II (IFN-g) interferons are a class of cytokine mediating the antiproliferative and immunomodulatory function on different cell types. IFN-a/b mostly controls antiproliferative pathways, whereas IFN-g is more prone to regulate immuno functions. Recent advances in therapy for multiple sclerosis (MS) have pointed out the use of IFN-b on therapeutic protocols. Several clinical trials suggest that IFN-b, and in particular IFN-beta 1a, may have important advantages in the treatment of relapsing-remitting MS (RRMS). The mechanism by which the IFN-beta exerts its action on the SM is still unclear. Our main goal was to investigate its role in order to identify a possible molecular marker for monitoring the patient's responsiveness to therapy. We analysed two important proteins involved in the IFN-g signaling: SOCS-1 and the recently SOCS-1 associated protein TRIM-8/Gerp. SOCS-1 is a member of the suppressor of cytokine signaling (SOCS) family of transducers that negatively regulate the activation of cytokine signaling. TRIM-8/Gerp is a conserved RING finger tri-partite motif protein interacting with SOCS-1 in vitro and in vivo, whose expression can be induced by interferon-g in lymphoid as well as epithelial cells. In addition co-expression of TRIM-8/Gerp with SOCS-1 decreases SOCS-1 levels by regulating protein stability. On this report we present for the first time the modulation of SOCS-1 and TRIM-8/Gerp expression in vivo on patients undergoing IFN-beta1a treatment. Functionally, expression of TRIM-8/Gerp decreases the repression of interferon-g signaling mediated by SOCS-1. By analyzing RNA expression of both proteins through quantitative PCR, we found an inverted correlation between SOCS-1 and Gerp/TRIM-8 expression on patients responding or not responding to therapy. Furthermore correlation of SOCS-1/TRIM-8 expression with cytokine release will be shown and discussed.

P403

**Analysis of tumour suppressor gene p16 and telomerase activity assay among the central nervous system tumour patients in Malaysia.** J. Abdullah, S. Sulong, M. N. Isa, University Sains Malaysia (Kubang Kerian, Kelantan, MY)

Our objectives are to investigate the possibility of p16 gene alterations via homozygous deletion and mutation analysis and to detect telomerase activity in central nervous system tumours. 50 tumour tissues collected between 1997 to 2002 from the Brain Tumour Tissue Bank of Neuroscience Unit in USM were used in p16 gene analysis and 23 of these were also utilized in telomerase analysis. Single-Strand Conformation Polymorphism analysis was performed to screen for p16 gene mutation at exon 1 and 2, which was confirmed by DNA sequencing analysis. Telomerase activity was detected based on a PCR-Telomeric Repeat Amplication Protocol using a Telomerase Detection Kit (Intergen, Co). The PCR-products were electrophoresed on a 12.5 % polyacrylamide gel and stained with silver staining. Positive amplification of p16 gene and GAPDH gene were observed in all samples, suggested no homozygous deletion occurred. Mutation screening analysis of p16 gene indicated that there was no mobility shift and suggested no mutation at exon 1 and 2 had occurred in all samples. DNA sequencing results on all high-grade tumours showed that all base sequences of exon 1 and 2 were normal. The activity was detected in 26.1 % of the tumour samples and mostly present in high-grade tumours (66.7 %). There was a significant association (Fisher's exact test) between telomerase activity status with tumour grade ($p = 0.008$). Alteration of the p16 gene was not identified in all the samples and suggested that the gene may not play a major role in tumourigenesis of these tumours in Malaysia.

P404

**Genetic variation at the apolipoprotein E (ApoE) locus and the prevalence of risk factors for ischaemic stroke (IS) in men and women patient populations.** M. Baranska-Gieruszczak, G. Gromadzka, A. Ciesielska, I. Sarzynska-Dlugosz, A. Czlonkowska, Institute of Psychiatry and Neurology, Medical Academy (Warsaw, PL)

Background: ApoE is encoded by the ApoE gene located on chromosome 19. Three alleles of the ApoE gene encode three ApoE isoforms in humans: E2, E3, E4. Although the main role of ApoE is the participation in lipids metabolism, ApoE is called the protein of still increasing role in cell biology. The influence of the carrying of allele 4 on increased risk of heart diseases in smokers was independent from serum cholesterol levels – it suggests that ApoE plays an important role not only in lipids metabolism – it probably may increase the risk of vascular diseases on the way on other mechanisms. In vivo and in vitro studies indicate that the expression of ApoE may be regulated by estrogens – so the influcence of ApoE on lipids metabolism and on the risk of vascular diseases could be gender-dependent.

Aim of Study: to assess the relationship between ApoE genotypes and the prevalence of dyslipidemias and other risk factors for IS in men ($n = 120$) and women ($n = 124$) patients.

Methods: The analysis of ApoE genotypes was performed using polymerase chain reaction- restriction fragments length polymorphism (PCR-RFLP) method. IS risk factors, such as hypertension, atrial fibrillation, heart failure, coronary heart disease, transient ischemic attacs (TIA), stroke, cigarette smoking, diabetes mellitus were noticed. Serum lipids concentrations were measured with standardized procedures.

Results: The distribution of ApoE genotypes in women and men patients populations (respectively): E3/E3: 68.54 %, 64.17 %; E2/E3: 16.13 %, 13.33 %, E3/E4: 12.90 %, 21.67 % ($p = 0.07$); E2/E4: 2.42 %, 0.83 %. The frequency of the most analysed stroke risk factors was similar in men and women patients populations. However, as compared to the carrying of allele 4, the carrying of allele 2 of ApoE gene was associated with significantly ($p < 0.05$) lower frequency of: hypercholesterolemia (HCh): only in women; hyperLDL (HLDL): only in men, and hypertension: only in women. As compared to the homozygotic carrying of allele 3/3, the presence of allele 2 of the ApoE gene was associated with significantly ($p < 0.05$) lower frequency of HCh: only in women; HLDL: in women and in men, and hypertension: only in women.

Conclusion: Our data suggest that genetic variation at the ApoE locus in Polish IS patients population is a genetic factor that is associated with different frequencies of dyslipidemias and hypertension. This association seems not to be identical in men and women.

## Multiple sclerosis

**P405**

**Pyramidal tract damage and clinical impairment: do they modulate fmri cortical activation during simple motor tasks in multiple sclerosis patients?** A. Gallo, M. Rocca, B. Colombo, A. Ghezzi, A. Falini, G. Tedeschi, G. Scotti, G. Comi, M. Filippi, Neuroimaging Research Unit, Department of Neurology, Ospedale di Gallarate, Department of Neuroradiology (Milan, Gallarate, Naples, I)

Introduction: Using functional magnetic resonance imaging (fMRI), cortical functional changes have been shown to occur in patients with MS and different clinical phenotypes. These changes have been related to the extent of brain and cervical cord tissue damage. In this study a large sample of MS patients underwent fMRI during a simple motor task to assess if pyramidal lesions and clinical impairment can modify the pattern of cortical brain activations.

Patients and methods: We investigated 76 right-handed patients with established MS divided into four groups according to the presence (+)/absence [-] of clinical impairment of the right hand and the presence (+)/absence [-] of lesions along the left pyramidal tract: group 1, les+/clin+ (m/f = 5/17; mean age = 46 years; median EDSS = 5.5; mean disease duration = 12 years); group 2, les-/clin+ (m/f = 3/7; mean age = 45 years; median EDSS = 5.5; mean disease duration = 12 years); group 3, les+/clin- (m/f = 8/13; mean age = 43 years; median EDSS = 1.0; mean disease duration = 10 years); group 4, les-/clin- (m/f: = 12/11; mean age = 45 years; median EDSS = 1.5; mean disease duration = 10 years). In each subject, the following sequences were acquired: 1) fMRI during the performance of a simple motor task consisting of repetitive flexion-extension of the last four fingers of the right hand, 2) dual-echo turbo-spin echo sequence. FMRI data were analyzed using SPM99 and cluster analysis. On dual-echo scans, lesions along the left pyramidal tract, from cortex to brainstem, were identified.

Results: To evaluate the effect of lesions alone, we compared group 4 vs. group 3, and group 1 vs. group 2; these comparisons showed a posterior displacement of the center of activation of the ipsilateral primary sensorimotor cortex (SMC) ($p = 0.02$) in group 3 compared to group 4, whereas no difference was found with the second comparison. To evaluate the effect of clinical impairment alone, we compared group 3 vs. group 1, and group 4 vs. group 2; these comparisons showed a posterior displacement of the center of activation of the contralateral primary SMC ($p = 0.04$) in group 1 compared to group 3, while a rostral displacement of the ipsilateral primary SMC ($p = 0.01$) was seen in group 2 vs. group 4. Finally, to investigate whether lesions and clinical impairment might have a synergic effect on fMRI activations, we compared group 1 vs. group 4. In group 1, we found an increased activation of the ipsilateral primary SMC ($p = 0.007$) which was also displaced medially and rostrally.

Conclusions: The movement-associated recruitment of the contra- and ipsi-lateral primary SMC is influenced by the presence of lesions along the pyramidal tracts and the severity of clinical impairment. However, specific patterns of cortical reorganization related either to injury or to clinical impairment were not identified.

**P406**

**Neutralizing antibodies during treatment of relapsing multiple sclerosis patients with three interferon betas: correlation with clinical outcomes.** D. Paolicelli, C. Avolio, V. Lavolpe, M. Ruggieri, P. Livrea, M. Trojano, University of Bari, University of Foggia (Bari, Foggia, I)

Background: Evidence of reduction of interferon beta (IFNb) efficacy in about 25 % of treated multiple sclerosis (MS) patients led to extensive studies of the incidence of neutralizing antibodies to IFNb (NABs) and their influence on therapeutic benefits.

Objectives: To compare the incidence of NABs to three IFNb products in patients with MS and to investigate the relationships between NABs, clinical and demographic outcomes in a follow-up study population.

Design/methods: Five-hundred and seventy five relapsing MS were treated with IFNb (160 with Betaferon; 203 with Avonex; 212 with Rebif 22) at standard dosage and administration routes. Sera were longitudinally obtained from all patients before and quarterly during treatment for up to 24 months; the presence of NABs was tested by cytopathic effect (CPE) assay. A titer equal or higher than 20 neutralizing units (NU)/mL was considered the cutoff value for positivity; patients with two or more consecutive positive samples 3 months apart were considered NAB+. Clinical outcomes (relapses and EDSS) every 3 months were also assessed.

Results: Five-hundred and twenty nine patients resulted NAB- and 46 NAB+. Betaferon group showed a higher (16,2 %, $p < 0.00001$) NABs incidence than Avonex (2 %) and Rebif 22 (8 %) groups. The peak of appearance of NABs was observed between the 4th and the 6th quarter of treatment in all groups, then the NABs positivity declined. At baseline, NAB+ patients showed a longer ($p = 0.02$) disease duration and a lower ($p = 0.01$) progression index (EDSS/disease duration) in comparison with NAB- group. The relapse rate reduction resulted significantly ($p < 0.05$) higher in NAB- patients (56 %) than in NAB+ patients (50 %). Moreover the NAB+ patients showed a significant ($p = 0.002$) increase of the mean relapses number after seroconvertion ($0.26 \pm 0.44$) in comparison with that observed before ($0.68 \pm 1.1$).

Conclusions: These results confirm that IFNb-1b is higher NABs inducer than IFNb-1a, Rebif is higher than Avonex. The highest incidence period of NAB production is after one year of treatment, observed in a follow up study of 24 months. A longer disease duration before treatment seems to have influence on the development of NABs, supporting the decision to treat early. NAB+ patients experience a reduction of treatment effect on relapses compared with NAB-.

**P407**

**Increase of matrix metalloproteinase-9 in peripheral blood of multiple sclerosis patients treated with high doses of methylprednisolone.** D. Mirowska, W. Wicha, A. Czlonkowska, A. Czlonkowski, F. Weber, Institute of Psychiatry and Neurology, Medical University of Warsaw, Max-Planck-Institut (Warsaw, PL; Munich, D)

Increased blood-brain-barrier (BBB) permeability during relapses of multiple sclerosis (MS) depends on several factors. Among them matrix metalloproteinases (MMPs) are of great interest - especially MMP-9 and MMP-2 and the natural tissue inhibitor of MMP-9 (TIMP-1). Intravenously applicated methylprednisolone (MP) accelerates the recovery from relapse in MS and stabilizes the BBB, as shown by a decrease of gadolinium enhancement in MRI. The mechanism, however, is poorly understood. One of the current concepts is that MP may stabilize the BBB by altering the balance of MMP-9 and TIMP-1.

The aim of our study was to investigate, whether different doses of intravenously given MP has any short and/or long term effect on MMP-9, MMP-2 and TIMP-1 plasma levels.

30 patients with relapsing-remitting MS were treated with 1000 mg ($n = 17$, group I) or 500 mg ($n = 13$, group II) MP intravenously for five consecutive days. EDSS score was evaluated before starting therapy, after 7 days and at 3 months. MMP-9, MMP-2 and TIMP-1 levels were determined before treatment, after 3, 7, 14 days and after 3 months.

During therapy with MP we observed a statistically significant decrease in median EDSS score after 7 days and after 3 months compared with the state before treatment. Improvement of EDSS was similar in group I treated with higher and group II treated with lower dose of MP and comparable to that seen in the whole patients population. There was, however, no significant improvement between day 7 and month 3.

Before therapy baseline TIMP-1 and MMP-2 levels were significantly lower in MS patients than in healthy controls. In addition MS patients showed higher levels of MMP-9 than healthy controls. The difference, however, was not statistically significant. There was a statistically significant increase of MMP-9 plasma levels at day 7 after starting therapy followed by a decrease at day 14 and month 3 in group I. A similar trend was obvious in group II. No significant effect of MP treatment, however, was noted on TIMP-1 and MMP-2 plasma levels.

In conclusion, our results demonstrate that a double dose of MP might not be superior with respect to clinical improvement and that MP increase MMP-9 levels in peripheral blood. Therefore the well known effect of MP in restoring the BBB may possibly be related rather to some other mechanism than suppression of MMP-9 - e. g. to suppression of adhesion molecules or pro-inflammatory cytokines.

**P408**

**Evaluation of mitochondrial manganese in leukocyte of multiple sclerosis patients.** M. A. Cucci, A. Nonnato, P. Barbero, L. Branciforte, A. Ricci, E. Verdun, M. Clerico, A. Pipieri, A. Caropreso, G. Pescarmona, L. Durelli, University of Turin (Turin, I)

Objective: To measure leukocyte mitochondrial manganese (Mn) in patients with relapsing-remitting (RR) or secondary progressive (SP) MS and in healthy controls.

Background: Mitochondrial manganese is mainly associated with superoxide dismutase (SOD) activity. SOD converts $O_2$- in $H_2O_2$, buffering the concentration of toxic free radicals like $O_2$-, NO, $H_2O_2$ and peroxy nitrate that with cytokines, chemokines and proteases are candidate mediators of demyelination and axonal loss.

Material and methods: Thirty-one MS patients (20 women, aged 22–62

years) affected with SP (15 cases), or RR MS (16 cases) and 15 control subjects (8 women, aged 22–51 years) were included in the study. Leukocyte mitochondrial fraction was separated after cell lysis by means of differential centrifugation in buffer. Protein mitochondrial fraction was determined by microplate spectrophotometry. Mn concentration was analyzed in atomic absorption spectroscopy with graphite furnace atomizer (GFA-AAS) after mitochondria mineralization in $HNO_3$ (65%) at 100° C (1 hour).

Results: All healthy controls had intramitochondrial Mn concentration > 10 microg/g protein, and the mean concentration was 91.9 ± 72.5. In the RR MS patient group, 87.5% had an Mn concentration < 10 microg/g protein, and the mean concentration was 16.4 ± 39.7. In the SP MS patient group, only 20% had an Mn concentration < 10 microg/g protein, and the mean concentration was 139.7 ± 178.2. The intramitochondrial Mn concentration of RR MS patients was significantly lower than that of healthy control ($p = 0.004$) and of SP MS patients ($p = 0.01$). SP MS patient concentration was not significantly different from that of controls ($p = 0.27$).

Conclusion: A reduced availability of mitochondrial Mn could increase free radical concentration. Free radicals could induce the peroxidation of neuronal cell membrane lipids and lead to demyelination or axonal loss. Further studies are in progress to correlate Mn reduction with clinical, immunological, and MRI markers of disease activity in MS.

**P409**

**Longitudinal study of ex-vivo spontaneous and induced apoptosis in peripheral blood lymphocytes from multiple sclerosis patients treated with interferon beta-1b: a 12 month follow-up.** A. Garcia-Merino, D. Diaz, H. Barcenilla, A. Prieto, C. Castrillo, M. Alvarez-Mon, Clinica Puerta de Hierro, Universidad de Alcala, Schering España on behalf of the GENIO II Group

Objectives: To quantify and describe changes in apoptosis occurrence in peripheral blood lymphocytes (PBMCs) from multiple sclerosis (MS) patients before and after interferon beta-1b (IFN beta-1b) therapy.

Background: In MS, it is believed that there exists an aberrant systemic activation of the immune system which involves myelin specific T-cells. Lymphocyte apoptosis is a homeostatic mechanism downregulating lymphocyte activation. There is evidence of an abnormal regulation of apoptosis in MS, but interpretation of results is controversial. To some authors, an abnormally low apoptosis of autoreactive T cells would be a pathogenic trait in MS; others have found an increased lymphocyte apoptosis as a consequence of the high lymphocyte activation seen in these patients.

Design/Methods: Blood samples from 45 patients with relapsing-remitting and secondary-progressive MS were analyzed in a central laboratory at baseline and after 1, 6 and 12 months of IFN beta-1b therapy. PBMCs were purified and characterized using monoclonal antibodies and the FACScalibur analyzer. Apoptotic index (AI) was calculated for T-cells expressing CD3, CD4, CD8 and CD45RO/RA antigens, and for B-cells expressing CD19 and CD5 antigens. AI was determined after a 24 hour-culture under spontaneous conditions and after polyclonal induction by mitogen (PHA).

Statistical analysis: A variance analysis and the Student's t test for multiple comparisons were applied.

Results: Both spontaneous and mitogen-induced AI showed a significant reduction ($p < 0.01$) in all subpopulations except for CD19 + cells. Changes in spontaneous AI were already significant from month 1 in all but CD3 + CD8 + and CD45RA + CD8 + (both significant from month 6) and CD45RO + CD8 + and CD5CD19 + subpopulations (significant after 12 months). Changes in PHA-induced AI were significant on month 1 in all but CD3 + CD8 + cells (significant after month 6).

Conclusions: Our results show that IFN beta-1b therapy not only does not increase lymphocyte apoptosis in MS but, instead, causes a robust, steady and consistent reduction of the proportion of lymphocytes that suffer apoptosis in this ex-vivo short term culture. This reduction is observed for both spontaneous and mitogen induced apoptosis. Our interpretation is based on two facts: 1- abnormally activated T cells have increased susceptibility to apoptosis; 2- IFN beta-1b normalizes this activated state. Such normalization results in a reduction of susceptibility to ex-vivo apoptosis of T lymphocytes from MS patients.

**P410**

**Anti-thyroidal antibodies in multiple sclerosis: Is there a therapeutical consequence?** U. Kullik, F. Hoffmann, S. Schueler (Halle, D; Namsos, N)

Background: Despite the increasing knowledge about the immunopathogenesis of multiple sclerosis (MS) it is far from being completed. A comorbidity with other autoimmunological disorders has been reported. The appearance of several organ- and non-organspecific antibodies such as anti-thyroidal peroxidase (anti-TPO) or anti-thyroideaglobuline (anti-TG) is annotated pluraly. The rating of these findings is not clear yet. Considering the disease modifying therapy in MS it has to turn one's attention to other chronic inflammatory disorders of the central nervous system after identifying. In attention of all these facts there is a need for notice the hashimoto's encephalopathy (HE) during diagnostical process. The incidence of HE is possibly higher than assumed since there is more consideration to this syndrome and more case reports were found in the literature. The occurrence of antithyroid antibodies in MS indicates the possibility of a coincidence with HE. Eventually it may be an "epiphenomenon" within the changed immunopathogenesis. Possibly there is a therapeutical consequence.

Methods: Since December 2001 we have chosen 15 patients with MS (McDonald-criteria applied) and high titers of thyroid-antibodies (anti-TPO and/or anti-TG). These patients were examinated with respect to the clinical course during several drug modifying therapies and response of the antibody-titer. Thyroid hormones and thyroid antibodies were analyzed in a 3-month-period, at least for 18 months. At this fixed date EDSS, 25-feet-test and clinical neurological status were examined. The mean duration of illness was 60 months [1–204]. At the beginning the mean value of EDSS was 5.5 (2.0–7.0), after one year 5.0. The course modifying therapy contains glatirameracetate (3 patients), interferon beta-1b (4 patients), interferon beta-1a (3 patients), azathioprin (1 patient) and mitoxatrone (4 patients).

Results: There are now provisional results after one year of investigation. Patients who had glatirameracetate injection show unaltered values in 2 of 3 cases, one patient displayed with lowering anti-TG-titers. Among interferon beta-1a all values relapsed (3/3 patients). During treatment with interferon beta-1b one patient displayed with unchanged values, two patients showed rising values and two patients had falling values, respectively. Immunosuppressive therapy with mitoxantrone led to subsiding titers except in 3 of 4 patients. The patient undergoing oral therapy with azathioprin showed low values after this period.

Conclusion: The importance of anti-thyroidal antibodies in MS-patients is still not clear. Because of the small amount of long-term investigations there is no possibility to estimate therapeutical consequences. Further investigation is needed to clarify the role of thyroid antibodies whether being a side effect in changed immunopathogenicity or a sign of special pathogenic entity.

**P411**

**The multiple sclerosis functional composite and cognitive function.** S. B. Ritter, G. Ladurner, Department of Neurology, Christian Doppler Klinik (Salzburg, A)

Background: The Multiple Sclerosis Functional Composite (MSFC) was recommended by a task force of the National Multiple Sclerosis Society as a new clinical outcome measure for clinical trials. The MSFC consists of three quantitative tests, assessing leg (Timed 25-Foot Walk [25FW]), arm (9-Hole Peg Test [9-HPT]) and cognitive function (Paced Auditory Serial Addition Test (PASAT-3.0). The PASAT is a commonly used procedure in neuropsychology to detect deficits in information processing speed and working memory. In Multiple Sclerosis (MS), several authors report that patients are not able to perform the PASAT due to severe cognitive deficits or they refuse to perform this test. Whereas the MSFC showed concurrent and predictive validity against the Expanded Disability Status Scale (EDSS), the task force recommended that the MSFC may not be suitable in all disease severity ranges.

The goal of this study was to assess the relation between cognitive performance, anxiety and depression, assessed with a comprehensive neuropsychological test battery and the overall MSFC score and its components in MS patients.

Methods: The study sample consisted of 42 MS patients (64% female) receiving IFNB-1b for a mean of 33.95 (SD, 20.75) months. Mean age was 42.6 years (SD, 8.48), mean disease duration was 9.98 years (SD, 7.97), mean education time was 12.10 years (SD, 3.35) and mean EDSS-Score was 2.55 (SD, 1.77). Patients suffered from either relapsing-remitting (85.7%) or secondary progressive disease course.

Results: The MSFC overall score ($r = -0.613$, $p = 0.000$) and its components (leg: $r = 0.745$, $p = 0.000$; arm: $r = -0.512$, $p = 0.001$; cognitive: $r = -0.434$, $p = 0.004$) correlated significantly with the EDSS, thus providing strong evidence of face validity as well as convergent and divergent validity. One third of the study sample was not able to perform the PASAT-3.0, whereas only one patient was not able to reach a score on Symbol Digit Modalities Test (SDMT). The MSFC overall score showed weak to strong correlations with all cognitive test scores and correlated moderately with depression. In stepwise linear regression analysis, visual memory (Rey Complex Figure) was the strongest predictor for MSFC overall score, fol-

lowed by divided attention (SDMT). Both tests accounted for 54.3 % of the variance.

Conclusion: We conclude from our findings that it would be useful to add a measure of visual memory and/or attention to the MSFC to improve its clinical significance.

**P412**

**Paroxysmal symptoms in a series of 107 consecutive multiple sclerosis patients.** C. Zuliani, M. Fabbri, C. S. Fattorello, Civil Hospital (Mirano, I)

Paroxysmal symptoms (PS) do not occur frequently in the course of Multiple Sclerosis (MS), but are relatively typed. They could be recognized since they can serve as clinical indicators of the disease and can be treated efficiently. The medical records of 107 consecutive patients with definite MS, 38 males and 69 females, aged between 20 and 70 years (mean age 38.63 years), admitted to the Department of Neurology of Mirano-Ve (Italy) from January 1994 to January 2003 (for a total of 330 hospitalizations) were reviewed to study the incidence and characteristics of PS. We found 27 subjects (25.23 %), 10 males and 17 females, aged from 24 to 70 years (mean age 39.34 years): 11 experienced trigeminal neuralgia, 7 on the right and 4 on the left side, as the initial symptom of MS in 2 (in 1 with associated signs of brain-stem involvement) and as a relapse in the others (in 1 of them neuralgia always manifested itself some days before every relapse). Most patients had a good result from medical treatment (in 3 prolonged use of carbamazepine, in the others steroid therapy for relapses), while in 2 was necessary transcutaneous ablative procedure. Epileptic seizures occurred in 4 patients, in everyone as the presenting feature of MS, generalized in 2, partial with secondary generalization in 1, and simple partial, representing like Clinical Isolated Syndrome (CIS) in the last; only in 1 there were EEG abnormalities. 2 cases showed short episodes many times a day of left arm paroxysmal dystonia, as the first manifestation of MS in 1 of them, secondary in both to a demyelinization lesion in posterior arm of right internal capsule detected by Magnetic Resonance Imaging (MRI); these episodes disappeared spontaneously in one case, with carbamazepine at low dose for a few months in the other. 3 subjects, 1 of them many years before MS diagnosis, complained of spontaneous Lhermitte' s sign, and 2, with a Secondary Progressive MS (SPMS) and a high grade of disability and severe cognitive impairment, a pathological spastic laughing. At last 5 cases showed a variety of pain syndromes: 2 with SPMS acute paroxysmal pain, in upper limbs in one and in lower limbs in the other; 3, in whom radicular compression was ruled out by imaging techniques, acute cervical radicular pain, on the right side in 2 and on the left in 1, in one of them in close relationship to trauma.

The present data suggest that PS are surprisingly common in MS (25.23 % in our series), often represent the presenting feature of disease and are probably caused by ephaptic transmission due to myelinoaxonal dissociation within the demyelinated plaque. The most common of them, in accordance with the literature, is also in our study trigeminal neuralgia (40.74 %).

**P413**

**Trigeminal neuralgia as onset symptom of multiple sclerosis.** G. Santuccio, R. Nemni, L. Sanvito, N. Canal, Don Gnocchi Foundation, University of Milan (Milan, I)

Background: Trigeminal Neuralgia (TN) is characterised by paroxysms of facial pain lasting up to two minutes and with pain-free intervals, occurring in the territory of one or more trigeminal nerve branches. TN is caused in about 80 % of cases by a focal compression of the trigeminal nerve root close to its entry zone into the pons, due to an aberrant loop of artery of vein. Rarely, also compressive space-occupying masses in the posterior fossa can cause TN. TN is reported to occur with a low prevalence in Multiple Sclerosis (MS), about 2–3 % of cases, usually in the late phases of the disease, but in some cases it can be the presenting symptom of MS. These reports do not provide information about clinical course of MS patients with TN at onset. Among autoimmune diseases, Systemic Sclerosis and Sjogren Syndrome can cause TN.

Objective: To evaluate the incidence of TN as presenting symptom in 40 newly diagnosed consecutive MS patients.

Methods: We collected the clinical data of 40 MS patients who were admitted in our centre for the diagnosis and treatment of the disease. We followed for up to 2 years 16 men and 24 women with defined diagnosis according to Poser criteria. 30 of them underwent interferon or copolymer treatment, 5 immunosuppressive drugs and 5 were without therapy. 4 of them, 3 women and a man, had an onset with TN, age ranged from 30 to 40. One patient man started at age 19 with unilateral trigeminal hypoesthesia (TH).

Results: We report the data of a small cohort of MS patients followed in our center. Unilateral TN was the first symptom in 4 patients (10 %), while unilateral TH was in another (3 %). None of our patients developed TN in the late stages of the disease. 3 of the 4 patients with TN had a complete remission of pain, one spontaneously and 2 after carbamazepine therapy, while one patient still complains the presence of paroxysms despite of medical treatments, 2 gamma-knife applications and a recent surgical approach. All 5 patients have a benign disease course to date after a 2 years follow-up. All of them are undergoing interferon treatment.

Conclusions: TN is well described in MS patients and usually it occurs in the late phases of the disease. Here we report additional data concerning the relationship between MS and TN. In contrast with data reported in the literature, we found a high incidence of TN at onset. Our results suggest that TN may be an early symptom of MS more frequently than expected and should not be necessarily considered a negative prognostic factor.

**P414**

**Cost-benefit and cost-effectiveness analyses of interferon beta-1a therapies for multiple sclerosis.** A. Beresniak, P. Coyle, T. Vollmer, P. O'Connor, M. Martin, Serono International Sa, Barrow Neurological Institute, MS Clinic St. Michael's Hospital, Innovus UK (Geneva, CH; New York, Phoenix, USA; Toronto, CAN; London, UK)

Objective: To evaluate the cost-benefit and cost-effectiveness of the two IFN beta-1a therapies currently licensed for RRMS.

Background: Relapses have a detrimental impact on patients' quality of life and incur important healthcare costs. Evidence suggests that early treatment with higher, more frequent doses of IFN beta-1a is beneficial in RRMS, but decision-makers need to know the economic consequences of each regimen.

Methods: Rebif (Serono), IFN beta-1a 44 mcg subcutaneously three times weekly, was shown to be more effective at delaying the time to first relapse and reducing relapse rate than Avonex (Biogen), IFN beta-1a 30 mcg intramuscularly once weekly, in the EVIDENCE study. Taking account of this, cost-benefit and cost-effectiveness analyses were performed. Effectiveness was defined as number of relapses (classified according to severity) and time to first relapse. Cost evaluation accounted for mean cost per relapse (evaluated according to specific level of resource utilisation and assessed via a specific cost study), cost of care (estimated at USD2520/year for patients with stable MS) and treatment cost (calculated from average wholesale price). Cost-benefit (total cost per patient/costs avoided per patient) and cost-effectiveness (treatment cost/time to first relapse for 39th percentile, 48 weeks) ratios were determined for each regimen.

Results: Rebif had a superior cost-benefit ratio (21.57) to Avonex (30.50), and was more cost-effective in terms of delaying time to first relapse (USD1100 per month for Rebif and USD1400 per month for Avonex, over 24 weeks). Concerning incremental cost-effectiveness ratios (cost per additional unit of effectiveness), the cost saving per relapse avoided using Rebif versus Avonex was USD3600, if only patients who experienced relapses were considered. If the entire study population was included and all costs (treatment, care, and relapse-related costs) were taken into account, the cost saving per relapse avoided with Rebif versus Avonex increased to USD28100. Using the number needed to treat (10.39), the cost per patient to avoid one relapse was USD12460.

Conclusion: The unit cost of Rebif is greater than that of Avonex, but this transparent analysis shows that Rebif is associated with a cost saving after only 24 weeks' treatment. While cost to avoid a relapse seems expensive, it does not take into account indirect relapse costs (e. g. lost work time, impact on caregivers, etc).

**P415**

**Leptin as a marker of clinical outcome in secondary progressive multiple sclerosis patients.** M. Rotondi, M. Caggiula, G. Frisullo, F. Odoardi, V. Nociti, C. Carella, P. A. Tonali, M. Mirabella, Institute of Endocrinology II University, Institute of Neurology University of Rome (Naples, Rome, I)

Leptin is a hormone synthesised almost exclusively by adipose tissue with an important role in regulating immune and inflammatory responses. Leptin up-regulates the production of Th1 type cytokines and it is required for the induction and progression of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS).

In relapsing-remitting MS patients under interferon beta (IFN beta)–1a therapy we found that serum leptin levels correlate with disease activity.

In this study, we determined serum leptin levels in secondary progressive (SP) MS patients being treated with IFNbeta-1b, at baseline and seri-

ally throughout therapy and correlated leptin levels with patient clinical outcome, in order to evaluate whether changes in this hormone are predictive for IFN beta-1b efficacy.

We studied 20 patients affected by SP-MS, who underwent IFN beta-1b therapy. Case report documentation, body mass index (BMI) and blood samples were obtained at baseline and after 2, 6, 12 months of treatment. Serum leptin levels were measured by radioimmunoassay (RIA).

Serum leptin level significantly decreased in patients who did not show EDSS progression up to the end of the study since two months after starting therapy.

Our data suggest that serum leptin level can be a useful predictive marker of clinical outcome and response to therapy also in SP-MS patients under IFN beta treatment.

**P416**

**Interleukin 1 receptor antagonist genotypes in Greek patients with multiple sclerosis.** K. Aggelakis, E. Dardiotis, D. Papadimitriou, P. Kolia, K. Kletsas, A. Papadimitriou, G. Hadjigeorgiou, University of Larissa (Larissa, GR)

The interleukin-1 (IL-1) family of cytokines encompasses three members; IL-1a, IL-1b, and IL-1 receptor antagonist (IL-1ra). IL-1ra is a pure antagonist of the other two members and prevents activation of the target cell by IL-1a and IL-1b. A penta-allelic polymorphism of a variable number of an 86-bp identical tandem repeat (VNTR) within the fourth intron of IL-1ra gene has been described and associated inconsistently with MS phenotypes and severity. Hereby, we present data concerning the putative association of the 86-bp VNTR polymorphism in IL1-ra gene with susceptibility to or age-at-onset of MS. Using standard molecular methods we analyzed DNA from 116 Greek patients (80 women and 36 men) with clinically definite MS and from 105 age- and sex-matched controls. Patients and controls were from Thessaly district (Central Greece). Thirty-five patients with relapsing remitting and 57 patients with secondary progressive MS were classified as bout-onset ($n = 92$) whilst 24 patients referred progressive-onset. The possible association of the IL-1ra polymorphism with susceptibility to MS was tested using the Fisher s exact test whilst the differences in age-at-onset were tested with the non-parametric Mann-Whitney U test.

Results: The distribution of genotypes was as follows: a) Patients: 1/1 = 56, 1/2 = 42, 1/3 = 6, 2/2 = 8, and 2/3 = 2; b) Controls: 1/1 = 54, 1/2 = 39, 1/3 = 6, and 2/2 = 6. There was no association between the IL-ra polymorphism and disease susceptibility ($p = 0.59$) or age-at-onset ($p = 0.79$). After stratification of patients according to the type of onset we found that the distribution of genotypes was statistically different among patients with bout-onset and progressive onset ($p = 0.03$). This difference was due to an increased frequency of allele-2 carriers in the group of patients with progressive onset. Moreover, the distribution of genotypes in patients with progressive onset was statistically different from controls ($p = 0.03$).

Conclusions: No significant differences in genotype frequencies were found between MS patients and healthy controls. Dividing patients according to the type of onset we found an increased frequency of allele-2 in MS patients with progressive onset. Due to the small number of patients with progressive onset, our results seek verification in a larger group of patients.

**P417**

**Epidemiological analysis of multiple sclerosis in Szczecin region, 1960–2000.** D. Nocon, PAM (Szczecin, PL)

Background: Epidemiological analyses of multiple sclerosis (MS) have been done only in some Polish regions and in short follow-up. We have therefore made an attempt to recognize the problem of the distributions of MS in the Szczecin Region (north-western part of Poland).

Methods: The epidemiological studies of MS were carried out from 1.01 1960 to 31. 12. 2000 in 1034 patients with multiple sclerosis in Szczecin area (9 982 km$^2$) with population 998,972 (men-484,394, women-514,578). With relapsing-remitting type of course were 384 (37.14%) patients, with secondary progressive 368 (35.59%) and with primary progressive 282 (27.27%). Cases were ascertained from all outpatients neurological clinics, departments of neurology and special MS hospital outpatient clinic (since 1981).

Results: Prevalence on 31. 12. 2000 was 58.46/100,000 inhabitants and was statistically higher ($p = 0.01$) in women-70.54, in men-51.40. Incidence in 10-year periods was 4.40 [1960–69], 3.13[1970–79], 2.51 [1980–89] and 2.46 [1990–2000] per 100 000 inhabitants.The mean age at onset was 33.99 in men and in women increased from 24.85 in 60th's until 37.22 in 90th's. The survival increased and was in 31. 12. 2000–56.68, life expectancy increased from 6.88 in 60th's until 23,07 in 90th's. An area 1202 km$^2$ (south of Szczecin Region) with 45 231 inhabitants with prevalence 110.54 (rest of region-52.89) we noticed in our survey as a focus of MS.

Conclusions: Szczecin Region has a high MS prevalence. Late onset (especially in women) and great proportion of progressive forms are noteworthy in our series. This may explain poor prognosis (short life expectancy and survival in our study).

**P418**

**Interobserver reliability in the diagnosis of multiple sclerosis in a clinical setting: a comparison between the Poser's and the new McDonald's criteria.** V. Zipoli, E. Portaccio, G. Siracusa, G. Pracucci, S. Sorbi, M. P. Amato, University of Florence (Florence, I)

Objective: To assess the interobserver reliability on the diagnosis of Multiple Sclerosis (MS) using the Poser's and the new McDonald's criteria.

Background: An accurate and reliable diagnosis since the beginning of the disease is essential for both clinical practice and research purposes. Published data until now have dealt with diagnostic criteria developed before 1983.

Materials and Methods: A designated neurologist, who did not participate in the reliability assessment, selected the patient records among the cases consecutively admitted to our Department between September 2001 and June 2002 for their diagnostic work-up. The study sample consisted of 44 patients, including 41 MS and 3 non-MS cases. Clinical information as well as results of laboratory and instrumental tests were recorded in standardized forms. Four neurologists were asked to make a diagnosis according to the Poser's and the McDonald's criteria and to assess MRI scans scoring the number of lesions according to the McDonald's guidelines. The level of interobserver reliability was measured using the kappa statistic (k).

Results: We found a moderate reliability on the overall diagnosis using both the Poser's and McDonald's criteria (k respectively 0.57 and 0.52). Analysing the reliability for distinct diagnostic categories, we observed a moderate to substantial reliability for the three McDonald's categories (range of k values 0.49–0.64) and a fair to substantial reliability for the nine Poser's categories (range of k values 0.37–0.67). We further measured the reliability level on specific diagnostic items. Taking into account clinical information, the reliability on dissemination over time was substantially higher ($k = 0.69$) than that found on dissemination over space ($k = 0.46$). On the contrary, for MRI assessment, the reliability for spatial dissemination was substantial ($k = 0.74$) compared to the fair reliability ($k = 0.25$) yielded by dissemination over time.

Conclusions: In comparison with the Poser's classification, the new McDonald's criteria allow us to anticipate the diagnosis yielding a good diagnostic reliability. Moreover, they compare favourably to the previous ones in terms of reliability on distinct diagnostic categories.

**P419**

**The event related potential P-300 and Wisconsin card sorting test in multiple sclerosis patients treated with interferon beta-1b: an open label, prospective, one year study.** S. Flechter, L. Pollak, J. Vardi, J. M. Rabey, The MS Clinical Research and Therapy Services, Assaf Harofeh Medical Center (Zerifin, IL)

Cognitive decline in multiple sclerosis (MS) patients may have a negative impact on quality of life and affect all active daily living domains. To evaluate the effect of interferon (IFN) beta-1b (Betaferon®) treatment on cognitive function and Event Related Potential (ERP) compared to the clinical course (assessed by EDSS score) in MS patients a one-year, prospective, open label study was initiated. Assessments of cognitive function were performed using the parietal lobe ERP P-300, which has demonstrated a relationship to cognitive function, and the Wisconsin Card Sorting Test (WCST) (Perseverative Response) score, which measures frontal lobe function.

Sixteen patients with clinically definite relapsing-remitting MS were enrolled from the MS outpatient clinic. Prior to the onset of treatment the mean EDSS score, the WCST score and the mean ERP P-300 (amplitude and latency) were assessed. These assessments were repeated after one year of treatment, and the mean difference calculated for each parameter. Three values were obtained for the WCST score – the uncorrected raw score, an age- and education-corrected score, and a US census age-matched score. The changes in P-300 and the WCST score compared to baseline were analysed using the paired T-test.

Significant reductions when compared to baseline values were observed in both P-300 amplitude ($20.3 \pm 8.3$ microvolts vs $13.1 \pm 10.6$ microvolts; $p = 0.026$) and latency ($312.9 \pm 15.6$ msec vs $302.0 \pm 17.0$ msec; $p = 0.002$) after one year of treatment with IFN beta-1b. Significant im-

provements after one year of treatment were also observed in both the raw and US census age-matched scores for the WCST relative to baseline ($20.7 \pm 30.7$ vs $13.1 \pm 10.6$; $p = 0.001$ and $96.7 \pm 15.7$ vs $100.1 \pm 11.1$; $p = 0.0025$, respectively). In addition, a small, non-significant improvement in the EDSS score was observed ($2.9 \pm 0.5$ vs $2.8 \pm 1.1$).

The use of simple and inexpensive tools including neuropsychological tests and ERPs (for example, P-300) may help detect early cognitive impairment. IFN beta-1b, an immunomodulatory therapy, has a positive therapeutic effect on cognitive dysfunction, independent of its effect on disease course as assessed by EDSS score. This effect may lead to improvements in patient quality of life.

**P420**

**Tiagabine for treating painful tonic spasms in multiple sclerosis: a pilot study.** C. Solaro, P. Tanganelli, Ospedale P. A. Micone (Genoa, I)

Objective: Tiagabine is a new antiepileptic medication (AED). It is used for treating partial seizure; recently it was reported to be effective for treating infantile spasms and stiff man syndrome. In multiple sclerosis (MS) patients painful tonic spasms (PTS) are common affecting about 10% of patients and they are usually treated with GABAergic medication such as baclofen or gabapentin.

The aim of the study was to evaluate the efficacy of tiagabine in treating PTS in multiple sclerosis subjects.

Materials and Methods: We have performed a pilot study on a group of MS pz with PTS, not responder or intolerant to conventional medication, using Tiagabine at dosage range 5–30 mg/die.

Seven MS patients followed as out patients at Department of Neurology PA Micone Hospital in Genova, suffering of PTS, previously treated with medication such as gabapentin, baclofen, diazepam or clonazepam.

The subjective level of the symptom was scored with a three point scale already described by the same author. Tiagabine was started at the dosage of 5 mg daily and the dosage increased until symptoms relief or to the maximum dosage (30 mg). No other medication with potential action on spasticity were allowed during the study period. 3 were male and 4 were female, the mean age was 45.1 years, mean disease duration 7.1 years, mean EDSS 4.0, 4 relapsing remitting, 1 secondary progressive, 2 primary progressive; mean dosage of Tiagabine 12.8 mg daily.

Results: Two patients drop out from the study due to side effects: 1 case nausea and dizziness, 1 case drowsiness and weakness. In 4 cases reported almost a reduction of two points at the utilised scale. The two subjects with side effects had longer disease duration and higher EDSS score; over a period of three months efficacy was maintained.

Conclusions: The study suggests a new medication for treating a paroxysmal symptom in MS. The frequency of side effects and the efficacy should be confirmed in larger studies.

**P421**

**Gradual introduction of interferon beta-1b prevents flu-like symptoms in patients with multiple sclerosis.** E. Alexiou, I. E. Markakis, M. Xifaras, M. Ioannidou, T. Kostis, A. Tsakiris, Piraeus General State Hospital (Nikaia, GR)

Background: Flu-like symptoms are common in multiple sclerosis (MS) patients receiving immunomodulatory therapy with interferon beta. They consist in fever, headache, myalgias and malaise and are more apparent during the first weeks of treatment. Paracetamol and non steroidal anti-inflammatory agents have been proposed for the management of these side effects.

Objectives: We used gradual dose escalation of interferon beta-1b in order to prevent flu-like symptomatology in MS patients.

Methods: We studied 10 patients with definite relapsing-remitting MS characterized by 3 relapses and deterioration by at least 0.5 points on the Expanded Disability Status Scale (EDSS) in the two previous years. All of them had an entry disability score on EDSS of $< 6$. Patients were treated with interferon beta-1b according to the following schedule: 2.5 million IU (0.5 ml) three times weekly for 2 weeks, 4 million IU (0.75 ml) three times weekly for two weeks, 4 million IU every second day for 4 weeks and finally the standard dose of 8 million IU (1.5 ml) every second day. The primary outcome measure was the proportion of patients with flu-like symptoms.

Results: The gradual dose escalation of interferon beta-1b was well tolerated and no patient suffered from flu-like symptoms. There was no need for non steroidal anti-inflammatory agent administration. Haematological and biochemical parameters remained normal.

Conclusions: Gradual introduction of interferon beta-1b minimizes side effects, prevents acute liver dysfunction, encourages long-term therapy and does not seem to interfere with the clinical course of MS.

**P422**

**Mitoxantrone safety in secondary progressive multiple sclerosis patients.** E. Verdun, P. Barbero, L. Manneschi, G. Salemi, A. Pipieri, M. Clerico, A. Ricci, M. A. Cucci, F. Battaglieri, E. Cammarata, I. Pesci, G. Savettieri, E. Montanari, L. Durelli, University of Turin, Civil Hospital, Department of Neuropsychiatry (Turin, Fidenza, Palermo, I)

Background: Mitoxantrone (MITO) was developed as an antineoplastic drug but its several immunosuppressant properties provided a rationale for use in Multiple Sclerosis (MS). MITO has been, in fact, recently approved for treatment in MS. The side effects described are heart failure, alterations of the blood cell counts, of the liver, and of the kidney, alopecia, and clinical infections.

Objective: To determine safety of MITO in the treatment of secondary progressive (SP) MS.

Design and methods: In 3 Italian MS centers (Fidenza, Palermo, Torino), 93 SPMS patients were treated with MITO 5 mg or 8 mg/m$^2$ monthly or every 3 months administered intravenously (i. v.). The treatment mean duration was 16 months at the mean cumulative dose of 120 mg. Thirty-four patients completed treatment, 22 discontinued and 37 are still on treatment. The patients have a neurological assessment every 3 months. Echocardiograms are done every 6 months, ECG every month, and laboratory tests before each administration and 12 ($\pm 3$) days thereafter.

Results: Twenty-two of 93 patients in MITO discontinued treatment: 5 patients for effects recognized as complication of MITO therapy (1 for alopecia, 1 for allergic reactions, 3 for decreased left ventricular ejection fraction), 5 for response failure, 6 for patient decision, 1 for blood hypertension, 1 for development of gastric carcinoma, 1 for car accident, 1 acute myocardial infarction, 1 for pulmonary thrombo-embolism, 1 for lower limb thromboarteritis. We present the preliminary safety data about the 34 SPMS patients who already completed the treatment at the mean cumulative dose of 120 mg. Baseline patients characteristics were: gender, women 18 (53%); mean age, $41 \pm 10$; mean disease duration, $8.4 \pm 5.9$; mean duration of progressive phase, $2.6 \pm 2.7$; mean EDSS score, $5.9 \pm 1.2$. During MITO infusion were observed more frequently: nausea (38.2%), epigastralgy (26.5%), menstrual disorder (27.7%), amenorrhoea (16.6%), and mild thinning of hair (23.5%). These adverse events usually graded mild or moderate. There was no evidence of clinical infections, liver, kidney alterations and cardiac dysfunction. Blood cell count dropped in almost all patients in the days after the infusion returning to baseline value in less than one month; only 1 patient had a transient leukopenia.

Conclusion: In the 34 SPMS patients who completed the treatment, MITO seems to be well tolerated. However it is necessary to end the observation in all patients and to start a longer follow-up.

**P423**

**Safety and tolerability of mitoxantrone therapy in secondary progressive multiple sclerosis: preliminary results of five patients.** B. Bastan, B. I. Cikrikci, A. Kurne, R. Karabudak, Hacettepe University (Ankara, TR)

The immunomodulating therapies have been shown to be reducing the relapse rate, disability progression and new magnetic resonance imaging (MRI) lesions in multiple sclerosis (MS). While these therapies have demonstrated substantial progress in altering the natural course of MS; certain number of patients still shows disease activity and progression of disability. Compared to a single agent, combining immunomodulators and/or immunosuppressive drugs may be another approach to improve therapeutic efficacy.

Mitoxantrone (MTX) is an anthracenedione antineoplastic agent that intercalates with DNA and is a potent inhibitor of both DNA and RNA synthesis. The effects of MTX in reducing the relapse rate, MRI activity and disease progression in secondary progressive MS (SPMS) have been shown promising. The FDA has approved the drug for the treatment of worsening relapsing-remitting (RR) or SPMS. However there is controversy concerning the potential myelosuppressant and cardio toxic adverse effects.

In this single-center, open label, prospective safety and tolerability study, we report the preliminary treatment results of five MS patients showing disease progression despite their immunomodulating ($n = 3$) or immunosuppressive ($n = 2$) drug therapies. The mean age of patients was 33.8 years [24–41], with the mean duration of disease 9.8 years [3–21]. The mean Expanded Disability Status Score (EDSS) was 5.7, ranging between 3.5 and 6.5. The patients were receiving immunomodulatory/immunosuppressive treatment for at least 3 years. All patients received a standard intravenous dose of MTX every 3 months, with close monitorization of complete blood count and left ventricular ejection fraction. The treatment was well tolerated, with the most common side effect being mild nausea. None of the patients had significant decrease in the parameters monitored. Cumulative MTX doses were lower than 140 mg, which is considered to be the

safety margin. While the results of this present study are preliminary, the drug and the combination were well-tolerated. The patients with aggressive forms of RR and SPMS may benefit from MTX.

The safety profile, the adverse events as well as the EDSS and MRI follow-up will be presented.

## Neurorehabilitation

**P424**

**Botulinum toxin A for the treatment of upper limb spasticity after stroke.** E. Cardoso, B. Rodrigues, M. V. S. Barroso, A. M. Souza, N. Fonseca, G. Pedreira, R. Lucena, A. Melo, D. Farias, M. Brandao, C. Menezes, UFBA Division of Neurology and Epidemiology - DINEP

Background: Stroke is the most important cause of neurological sequelae in adults. A common complication is severe hypertonia of upper limb muscles, which can cause pain, discomfort and psychological disturbance. Botulinum toxin treatment may reduce disability blocking acetylcholine release at neuromuscular junctions in these patients.

Objective: To determine if the use of botulinum toxin type A (BTX-A) in the upper limbs is an adequate treatment for spasticity after stroke.

Method: Systematic review of literature selecting all double-blind, placebo-controlled, randomized clinical trials (RCT) evaluating the safety and efficacy of BTX-A for the treatment of spasticity in upper limbs after stroke. Only data presented as mean ± standard deviation, or allowing transformation, were considered for analysis. The search was performed in databases up to 2003, using the words "botulinum toxin" and "spasticity". Then, references of the selected studies were checked for identifying others. The studies were selected and the quality of the RCT evaluated by three independent reviewers using the method proposed by Jadad et al. The measured outcomes were any change in the Modified Ashworth Scale (MAS) or in the physician's and patient's Global Assessment Scale (GAS).

Results: From the 98 selected papers, 5 fulfilled all inclusion criteria. Although one study presented the described criteria, it was excluded from the meta-analysis because it did not present comparable data to be analyzed. Clinical improvement was tested using the MAS (WMD = 0.95, 95 % CI = 0.74 to 1.17). Mean clinical improvement was higher in the botulinum group ($Z = 8.57$, $p < 0.00001$) between the 4th and 6th week of the study. Mean MAS score change was also higher when wrist joint was included at the same endpoint ($Z = 8.44$, $p < 0.00001$). The mean GAS scores improvement were significantly higher in all parameters in the BTX-A group (WMD = 1.11, 95 % CI = 0.81 to 1.41, $Z = 7.32$, $df = 1$, $p < 0.00001$). Related to the GAS parameters, 2 among 3 studies included did not show differences in the BTX-A response.

Conclusion: The outcomes show that treatment with BTX-A in patients with post-stroke upper limb spasticity resulted in statistically significant reduction in muscle tone. Its overall effect in functional disability was minimal.

**P425**

**Botulinum toxin A for the treatment of the spastic equinus foot in cerebral palsy.** M. V. S. Barroso, E. Cardoso, A. M. Souza, N. Fonseca, B. Rodrigues, G. Pedreira, N. Ribeiro, R. Lucena, A. Melo, M. Brandao, C. Menezes, D. Farias, UFBA (Salvador, BR)

Background: Cerebral Palsy (CP) is the main cause of motor disability in children, affecting 1 in 500–1000 new born. Among the patients with CP, 70 % have spasticity and the equinus foot is the main cause of this condition, determining variable functional disability. Botulinum toxin treatment may reduce disability blocking acetylcholine release at neuromuscular junctions in these patients.

Objective: To determine if botulinum toxin type A (BTX-A) is an adequate treatment for spasticity associated with equinus foot in cerebral palsy.

Method: Systematic review of literature selecting all double-blind, placebo-controlled, randomized clinical trials (RCT) evaluating the safety and efficacy of BTX-A for the treatment of equinus foot due to spasticity in cerebral palsy. Only data presented as dichotomous, or allowing transformation, were considered for analysis. The search was performed in databases up to 2003, using the words "botulinum toxin", "spasticity", "equinus foot" and "cerebral palsy". Then, references of the selected studies were checked for identifying others. The studies were selected and the quality of the RCT evaluated by three independent reviewers using the method proposed by Jadad et al. The measured outcomes were Gait Improvement (GI), Subjective Assessment (SA) and Adverse Effects (AE).

Results: From the 33 selected papers, 6 fulfilled all inclusion criteria. Gait improvement was tested using the Physician Rating Scale (PRS) and Video Gait Analysis (VGA) (Peto OR = 3.99, 95 % CI = 2.20 to 7.22). Mean GI was higher in the BTX-A group ($chi^2 = 1.67$, $p = 0.00001$). Subjective assessment was tested using scales and questionnaires filled in by the patient's parents or guardians (Peto OR = 3.99, 95 % CI = 1.89 to 8.44). Mean SA rating was higher in the BTX-A group ($chi^2 = 1.52$, $p = 0.0003$). Adverse effects were observed in both groups with a predominance in the BTX-A group (Peto OR = 2.62, 95 % CI = 1.47 to 4.67). In both groups, all AE were rated as mild to moderate; most of them were mild, self limiting and none of them were severe.

Conclusion: The outcomes show that treatment with BTX-A in patients with spastic equinus foot in cerebral palsy resulted in statistically significant gait improvement. Also observed was an overall clinical benefit. The observed adverse events were mild and moderate and were not related with withdrawal of treatment.

**P426**

**Supporting means for the best rehabilitation of patients suffering from stroke in Greece: opinions of patients.** P. Heras, A. Argyriou, M. Corcondilas, D. Mitsibounas, General Hospital of Kos, University of Athens (Athens, GR)

Aim of this study was to register the opinion of stroke patients regarding their needs for rehabilitation with particular emphasis on sources of psychosocial support in a rural area of South-west Greece.

Patients and methods: In this study conducted by the department of internal medicine of the general hospital of Kos, the Hippocrates Island, 81 patients, 44 men and 37 women aged 55–75 years hospitalized with a definite diagnosis of stroke have been evaluated. Sixty-eight of them suffered an ischemic stroke and 13 of them a hemorrhagic stroke. An anonymous questionnaire consisting of 14 questions, was given to the patients, three months after their discharge from hospital, by home visit. Each patient was asked to complete the questionnaire and mailed it to the authors.

Results: The type of stroke, severity and also the demographic characteristics don't have a statistical impact on the patients' answers regarding the sources of psychological and sentimental support accepted ($\chi^2$ test, $p > 0.8$). According to the patients' answers resulted that family represents the main supporting source that contributed to a faster and more extensive recovery of psychological and functional status after a stroke incident.

Conclusion: The present study clearly confirmed that there is an increasing need for professional psychological and emotional support of stroke patients that could be best provided in the context of organized rehabilitation centers.

**P427**

**Association of somatic neurological status and neurogenic bladder dysfunction in patients with spinal cord injury in rehabilitation phase.** A. Belova, N. Lebedeva, Institute of Traumatology (Nizhny Novgorod, RUS)

We aimed to study the relationships between somatic neurological deficit and urinary tract dysfunction in patients with spinal cord injury (SCI). 22 SCI patients (aged from 15 to 66, spinal injury level C5-Th7, mean duration of trauma 3–8 months) hospitalized to the regional rehabilitation center (N. Novgorod, Russia) in 2001 were studied by neurological and urological (including urofluorometrogram, sphincter electromyography) examination. 15 patients had spastic paralysis and 7-flaccid paralysis of extremities. Patients showed following degrees of spinal cord injury: ASIA level A (complete lesion)–3 cases; level B (sensation but no motor function)–9 cases; level C (muscle grade < 3 score)–7 cases; level D (muscles grade ≥ 3 score)–3 cases. None of the subjects with spasticity had detrusor areflexia, whereas among patients with flaccid paralysis 5 demonstrated it. None of the subjects with flaccid paralysis had detrusor hyperreflexia without vesicosphincter dyssynergia, but 8 patients with spastic muscle hypertonia had. Detrusor hyperreflexia with vesicosphincter dyssynergia presented in 7 patients with spasticity and in 2 patients with flaccid paralysis; according to the Fisher Method, the incidence of it was not significantly different in patients with flaccid and spastic paraplegia. Among patients with moderate motor dysfunction (levels C and D) 4 had slight bladder impairment (independent voiding, post-voiding residuals less than 50 ml) and 10 had severe vesiculomotor dysfunction (intermittent catheterisation). Among patients with severe motor dysfunction (levels A and B) slight bladder impairment was discovered in 2 cases and severe vesiculomotor dysfunction in 10 cases. The degree of vesiculomotor dysfunction had no significant difference in patients with severe and slight motor deficit ($p > 0.05$).

Conclusion: In cases of spinal cord injuries somatic nerve function and

autonomic nerve function may suffer in different fashion. Patients with flaccid paraplegia may have detrusor hyperreflexia with bladder/sphincter dyssynergia. Severe vesicomotor impairment may exist in patients with slight motor dysfunction. It is therefore important to evaluate all these patients using urological examination to choose corresponding rehabilitation methods.

P428
**Lymphocyte apoptosis in patients during rehabilitation period after ischaemic stroke.** A. Akhmetzyanova, National Center of Invalid Rehabilitation (Tashkent, UZB)

Ischemic stroke due to damage of hepatoencephalic barrier induces specific immune response, changes immunocompetent cell functions, provides shift in the humeral immunity, natural defensive factors. Apoptosis, that is, programmed cell death, is found to be important for development as of ischemic brain tissue damage so for pathogenesis of many other diseases.

We have investigated 85 patients with ischemic stroke with duration of disease of one year. The patients' age fluctuated from 33 to 65 years. Etiological pathogenic factors included cerebral atherosclerosis, arterial hypertension and their associations. The study patients included 50 males (64.7%) and 30 females (33.5%).

Investigation of apoptosis among lymphocytes of peripheral blood was performed by determination of receptor Fas/APO1 (SD95). There was found growth of lymphocytes with increased expression of SD95 receptor in study persons (29,8"b1, 1) in comparison with controls. Analysis of data obtained in relation to hypertension duration with presence of concomitant diseases revealed that reliably high indicators of SD95 lymphocytes were noted in patients with disease duration over 5 years ($p < 0.05$) and in associated diseases. However, it is necessary to note that SD95 lymphocyte number was also found reliably higher in the group of patients with duration of disease less than one year.

Thus, increase in SD95 lymphocytes in patients during rehabilitation period after ischemic stroke indicates presence of factors stimulating programmed death of immunocompetent cells and may be predictive for prognosis of disease outcomes.

## Pain and headache

P429
**Effects of Topiramate on quality of life in migraine prevention: a double-blind, placebo-controlled trial.** H.-D. Diener, W. Neto, D. Jacobs, S. Bhattacharya, G. Papadopoulos, University of Essen, Germany, J&J PRD, USA and TOPMAT-MIGR-002 Investigators Group

Objective: To assess the effect of topiramate (TPM) on migraine frequency and associated health-related quality of life (HRQOL).

Background: In 3 placebo(PLA)-controlled trials (MIGR-001,–002,–003) treating over 1500 patients, TPM significantly reduced migraine frequency and migraine days. In MIGR-001, TPM 100 mg/day improved some HRQOL measures. We analysed these parameters in the present study MIGR-002.

Methods: In a 26-week double-blind trial, patients with > 6 months of 3–12 migraine periods/month (±aura) received PLA or TPM 50, 100 or 200 mg/day after 2 weeks wash-out and 4 weeks baseline. TPM was escalated + 25 mg/day weekly within 8 weeks (split doses) to target dose, subsequently sustained till trial end. Mean monthly migraine period rates, responder rates (at least 50% reduction in mean monthly migraine period rates) and mean monthly migraine days were evaluated. Patients' well-being was assessed with the Migraine-Specific Quality-of-Life questionnaire (MSQ) and Medical Outcomes Study Short-Form 36 (SF-36). MSQ was analysed a priori for Role Restriction (RR) and Role Prevention (RP), SF-36 for Role Physical (RP) and Vitality (VT).

Results: Of 693 patients, 483 were randomised, 468 analysed (intent-to-treat). Although all 3 TPM dosages resulted in significantly more responders vs PLA (39–49% vs 23%; $p < 0.001$), only 100 and 200 mg TPM significantly decreased mean monthly migraine period rates and monthly migraine days (respectively 4.1–3.5*–2.9* vs 4.5, and 4.8–4.0*–3.5* vs 5.4, for 50–100–200 mg TPM vs PLA; change vs PLA *$p < 0.001$). On the MSQ, all 3 dosages significantly improved the RR and RP subscales vs PLA ($p \leq 0.019$). TPM 100 and 200 mg/day significantly improved the EF subscale ($p < 0.001$) and the SF-36 RP subscales ($p \leq 0.022$). Changes in MSQ-RP and MSQ-RR significantly correlated with changes in monthly migraine frequency, migraine duration, days on acute medications, migraine days, number of migraines, migraines with aura, and migraines without aura ($p \leq 0.002$). Correlations of the SF-36 RP and VT subscales were generally weaker. There was a dose-dependent drop out with TPM (17–26–21% vs 12% with PLA). Adverse events were consistent with its current label (most common: paraesthesia, and fatigue).

Conclusions: TPM, in particular 100 and 200 mg/day, showed significant benefits for the well-being of migraine patients, as measured by the MSQ and SF-36. The HRQOL changes correlated with improvements in migraine parameters.

P430
**One-year prevalence of chronic daily headache in a population of German and Turkish adults.** I. Kavuk, Z. Katsarava, M. W. Agelink, V. Limmroth, A. Stang, H. C. Diener, University of Essen (Essen, Gelsenkirchen, D)

In this study we wanted to investigate the prevalence and the risk factors of chronic daily headache (CDH) in a population with Turkish immigrants and German adults. Employees of a big textile company in a little township in Germany were asked to participate in the study. A total of 523 people were interviewed. Participants with headache were clinically examined by a neurologist. Diagnostic criteria for each headache type were based on those of the International Headache Society (IHS). CDH was diagnosed if the subjects had headache = 15 days/month with a duration of = 4 hours each day. Also CDH was further classified into chronic tension-type headache (CTTH) and CDH with migrainous features (CDH-MF). 35 participants (7.4%) fulfilled the criteria of CDH. The prevalence of CDH was higher in Turkish immigrants (10.7% versus 3.6% in the German group). The prevalence of CDH with medication overuse was significantly higher in the group of Turkish immigrants (7.9% versus 0.9%). Only 1.7% of the Turkish people had consulted a neurologist or a physician for their headaches in the previous year (German people: 24.2%). A pharmacological treatment in headache prophylaxis was higher in German migraine sufferers (14.7% of migraine sufferers). None of Turkish headache patients had got a prophylaxis. A further risk factor for developing a CDH was that Turkish headache sufferers had often consulted a hoca (an islamic religious) for their headaches in the previous year. We could show three main predictors (low consulting rate to a neurologist or a physician; no preventive therapy and the higher non-medical treatment rate with consulting a hoca as a non-specialist for headache) for the reason of a higher prevalence of CDH in Turkish immigrants.

P431
**Migraine without aura as a sign of covert neurological involvement in Behçet's syndrome? A case-control study.** C. Camarda, R. Monastero, C. Pipia, L. Camarda, M. Mannino, M. Francolini, G. Lopez, G. Didato, D. Raimondo, R. Camarda, University of Palermo (Palermo, I)

Background: It is not clear whether or not headache represents an early sign of neurological involvement in the course of Behçet's disease (BD).

Aims: To evaluate the prevalence of headache and the frequency of different headache syndromes in patients with Behçet's Disease without neurological involvement. Furthermore, to investigate the relationship with other clinical, and behavioural variables.

Methods: Twenty-seven BD patients and 27 control subjects, matched for age and gender, underwent a validated semistructured questionnaire based on the International Headache Society criteria. Levels of anxiety and depression were measured with the Zung Anxiety and Depression scales. BD Current Activity Form, a standardised index scoring major clinical features of the disease in the month preceding the examination, was used to assess disease activity. Detailed information on current medication was collected.

Results: Headache occurred in 88.9% of BD patients. There was no difference in the prevalence of the different headache syndromes between BD patients and controls. Only migraine without aura (MwA) was significantly more frequent in BD patients than controls (44.4% vs. 11.1%, respectively, $P = 0.013$). No relationship was found between MwA and clinical, and behavioural variables.

Conclusions: Patients with BD without neurological involvement showed an higher prevalence of MwA than controls. The high frequency of migraine cannot be explained in terms of demographical, clinical, and behavioural variables, probably accounting for a vascular or neuronal subclinical dysfunction as recently described by brain metabolic studies. We suggest to include a careful interview for migraine in the diagnostic work-up of BD.

P432
**Quality of sleep in migraine: comparisons to multiple sclerosis and to the general population.** K. Aydinkoc, C. Wöber, E. Geuder, K. Schmidt, R. Zingerle, N. Hattinger, J. Holzhammer, G. Klösch, S. Asenbaum, K. Vass, I. S. Lobentanz, J. Zeitlhofer, University of Vienna (Vienna, A)

The quality of sleep has been recognized as important component of the quality of life in chronic diseases. Nevertheless, there is still a considerable lack of information. Therefore, we were interested to examine the quality of sleep in migraine and to compare the findings with those in multiple sclerosis (MS) and in the general population, respectively.

We investigated a total of 914 subjects. Out of these, 214 had migraine, 163 had MS and 537 were controls. The patients were volunteers recruited via articles in newspapers. The controls were randomly selected from the general population. To assess the severity of the two disorders the Headache Impact Test (HIT) and the Expanded Disability Status Scale (EDSS) were applied. The quality of sleep was assessed in all subjects by means of the Pittsburgh Sleep Quality Index (PSQI).

The total PSQI-score was significantly higher in patients than in controls (migraine: $5.3 \pm 2.5$ vs. $4.4 \pm 3.5$, $p < 0.001$; MS: $6.6 \pm 4.0$ vs. $4.4 \pm 3.5$, $p < 0.001$) and it was significantly higher in patients with multiple sclerosis than in those with migraine ($p = 0.02$). The time until falling asleep was significantly longer in patients with MS than in the two other groups. The sleep duration, however, was shortest in migraineurs and compared to the controls this difference was statistically significant ($p = 0.035$). Among 14 further items of the PSQI, patients with MS scored highest in 10, migraineurs scored highest in 3 and one score was identical in the two groups. Comparing migraineurs to controls showed statistically significant differences in 8 out of 16 items. The score for the use of sleep medication was higher in controls, whereas the other 7 scores were higher in patients with migraine. Comparing migraineurs to patients with MS showed statistically significant differences in 6 items. A sleep disturbance caused by pain was more common in migraineurs, the other 5 items showed higher scores in subjects with MS.

In conclusion, the quality of sleep in patients with migraine is significantly poorer than that of the general population and significantly better than that of patients with MS. The management of patients with migraine and MS requires increased attention to the quality of sleep.

P433
**Medication compliance in painful diabetic neuropathy patients.** S. Giannopoulos, M. Kosmidou, S. Markoula, C. Mokou, I. Sarmas, A. Kyritsis, Medical School of Ioannina, Medical School of Thessaloniki (Ioannina, Thessaloniki, GR)

SSRI's and anticonvulsants are widely used for treatment of painful diabetic neuropathy. However, the majority of patients are hesitant to use these drug groups, thus their compliance remains an issue.

The objective of this study was to estimate the compliance of patients with painful diabetic neuropathy to two SSRI's (paroxetine or sitaloprame) and one anticonvulsant (gabapentin).

This was a 6 month prospective trial in 101 patients with painful diabetic neuropathy and minimum score of 2 on a pain intensity scale ranging of 0 to 4. Compliance was assessed with patient interviews and pill counts. Adverse events, early discontinuation or satisfaction with treatment were also evaluated.

Patients receiving SSRI's reported greater satisfaction with their treatment ($p < 0.05$) compared to the patients taking the anticonvulsant. Overall, there was statistically significant greater improvement in mood in the SSRI group ($p < 0.05$). The anticonvulsant group of patients was more concerned of the efficacy and the side effects of their treatment compared to the SSRI group. For vasomotor symptoms and sexual behaviour the SSRI's group reported significantly greater mean improvements ($p < 0.05$). Overall, 43% of those taking SSRI's noticed no effect, 51% felt better and 6% felt worse. Among the patients taking the anticonvulsant, 50% felt better, 38% noticed no effect, but over 10% felt worse. Finally, on the pill count more patients on SSRI's (94%) than on anticonvulsant (84%) were taking over the 75% of their medication ($p < 0.05$).

The lack of negative effects on quality of life and the better compliance of SSRI's suggests that these medicines may be the drugs of choice in painful diabetic neuropathy.

P434
**Pharmacokinetics of pregabalin in patients with chronic pain.** H. N. Bockbrader, P. Burger, B. W. Corrigan, Pfizer Global Research and Development (Ann Arbor, USA)

Purpose: To describe the pharmacokinetics of pregabalin (P) following single and multiple doses in chronic pain patients (CPP), and to identify concomitant medications that impact P pharmacokinetics in these populations.

Methods: Data from 1202 P plasma concentration observations in 974 subjects from 9 studies in adult patients with chronic pain, were pooled with 4381 observations from healthy volunteer and renal impaired patients (HV) and epilepsy patients (EP) to develop a population pharmacokinetic model. CPP model parameter estimates were then obtained using CPP data alone. The effects of concomitant oral antidiabetic agents, diuretics, and insulin on P disposition in CPP were also determined.

Results: P pharmacokinetics were well described in CPP, HV and EP by a one-compartment P pharmacokinetic model with first order absorption and a lag time. After inclusion of covariates, no significant differences in P disposition between CPP, EP, and HV were observed. P oral clearance (CL/F) in CPP increased proportionally with creatinine clearance (CLcr) from 0 up to 105 ml/min, above which P CL/F was independent of CLcr. P volume of distribution (Vd/F) in CPP was dependent on body weight, and was approximately 19% higher in females. Administration of P with food in CPP decreased the rate of drug absorption but not to a clinically significant extent. The ratios of CL/F values of CPP taking concomitant medications, expressed as a percentage of CL/F for CPP not receiving the concomitant medication was 110%, 93%, and 102% for oral antidiabetics, diuretics, and insulins respectively.

Conclusion: P demonstrates linear pharmacokinetics in CPP. P CL/F is related to CLcr and this relationship is similar between CPP, HV and EP. P dosage adjustment in CPP is not required for concomitant administration of insulins, diuretics, or oral antidiabetics.

P435
**Hemicrania continua and chronic paroxysmal hemicrania responsive to cyclo-oxygenase (COX)-2 specific inhibitors.** J. Porta-Etessam, A. Martínez Salio, A. Berbel Garcia, D. Perez Martínez, R. Saiz Diaz, M. Toledo Heras, Hospital Universitario 12 de Octubre (Madrid, E)

To report two cases of hemicrania continua and a patient suffering chronic paroxysmal hemicrania completely responsive to new non-steroidal anti-inflammatory drugs: refecoxib and celecoxib.

Case 1: 34-year-old woman had sudden onset of short-lived pains on the left side of her head. She suffers 10 to 18 attacks per day associated with autonomic features. We put her on Indomethacin 25 mg three times a day, and in 24 hours her hemicranial pain completely disappears. Because of oedema we discontinued Indomethacin and one week later the pain returned. We introduced refecoxib 25 mg qd with total recovery.

Case 2: A 56-year-old man present with an 8-month history of a continuous left sided headache strictly unilateral. The pain was moderate but fluctuating. He had not phono-, photofobia, nausea or vomiting. An MTI was normal and Indomethacin 25 mg three times a day was started with complete recovery. Two months later he started having gastric symptoms, indomethacin was discontinued and the hemicrania returned. With celecoxib 200 mg twice a day the hemicrania disappears.

Case 3: A 78-year-old woman with 1-year history of continuous left sided headache, fluctuating and accompanied by autonomic disturbances. With indomethacin 50 mg three times a day the hemicrania disappears, however she developed tinnitus one month later. We discontinued indomethacin and introduced refecoxib 25 mg qd with complete recovery.

The potential role of COX-2 inhibitors opens a wide range therapeutic window for indomethacin-responsive headache.

The cyclooxigenase (COX)-2 could be implicated in the pathogenesis of indomethacin-responsive headache.

P436
**Painful ophthalmoplegia due to actinomycosis of the cavernous sinus.** J. Mandrioli, P. Sola, M. Leone, G. Collina, G. Frank, P. Cortelli, University of Modena, Bellaria Hospital (Modena, Bologna, I)

Tolosa-Hunt syndrome (THS) is characterized by ophthalmoplegia associated to unilateral, severe retro-orbital pain. The etiology is unknown. The cavernous sinus or the superior orbital fissure are occupied by a low grade granulomatous inflammatory process. Differential diagnosis includes neoplasms (primitive or metastatic), infectious and inflammatory diseases, trauma, and aneurysms. Steroid therapy is usually resolutive. In refractory cases other etiologies should be considered.

We report a case of previously healthy man, who developed painful ophthalmoplegia, caused by actinomycosis of the cavernous sinus. Actinomycetes include gram-negative and gram-positive coccobacilli, transmitted by contiguous spread, and producing suppurative and granulomatous inflammatory reaction, multiple abscesses, and sinus tracts discharging sulfur granules. It causes subacute-to-chronic infection in man, with a wide spectrum of clinical manifestations, mostly involving the cervicofacial, thoracic, abdominal, and pelvic regions.

A 43-year-old man was admitted because of a one month history of acute onset left-sided retro-orbital pain, followed by left sixth cranial nerve palsy. Magnetic resonance imaging (MRI), initially normal, three months after the onset, revealed the presence of soft tissue in the left cavernous sinus, isointense to the gray matter in the T1-weighted sequences, hypointense in the T2-weighted images and with homogeneous enhancement after gadolinium administration. THS was suspected, and the patient was successfully treated with high-dose prednisolone. However, ocular pain and sixth cranial nerve palsy recurred few days after therapy discontinuation. Two months later the patient developed fever and bilateral pneumonia, and was successfully treated with antibiotic therapy. For the persistence of the ophthalmoplegia and the worsening of the neuroradiological picture, an endoscopic transphenoidal biopsy was performed. Histopathological analysis revealed a granulomatous aspecific inflammation containing actinomycetes colonies. The patient was treated with intravenous Penicillin G followed by amoxicillin p. o., with rapid improvement of pain and of cranial neuropathy. A control MRI showed a consistent reduction of the enlarged cavernous sinuses.

Central nervous system involvement in course of actinomycosis infection is uncommon, in particular, the involvement of the cavernous sinus is very rare. The present case report suggests that in patients with painful ophthalmoplegia resembling THS, resistant to steroid therapy, antibiotic therapy should be taken into account, and other possible causative agents should be investigated. Surgical exploration was crucial for the diagnosis, even if surgery in this region carries a significant potential for neurological morbidity.

## Peripheral neuropathy

### P437

**Hereditary motor and sensory neuropathy type 1A with central nervous system involvement in two generations.** M. Panas, G. Karadima, P. Floroskoufi, N. Kalfakis, A. Kladi, K. Spengos, D. Vassilopoulos, University of Athens (Athens, GR)

Introduction: Hereditary motor and sensory neuropathy type 1A (HMSN1A) is the most common form of inherited demyelinating neuropathy. The mode of transmission is autosomal dominant and the genetic defect, in most cases, is a large segmental duplication within band 17p11.2, involving 1.5 Mb of DNA, which includes the gene for peripheral myelin protein 22 (PMP22). There are some reports of HMSN1A with central nervous system (CNS) involvement. These cases are usually due to spinal cord compression by hypertrophied nerve roots or to the co-existence of chronic inflammatory demyelinating neuropathy (CIDP).

Patients and methods: Two parent-offspring pairs from two unrelated families are presented. All four patients presented with muscle weakness and atrophy, steppage gait and pes cavus, as well as bilateral pyramidal signs. The laboratory investigation included electrophysiological evaluation, brain and spinal cord MRI and molecular genetic analysis.

Results: The electromyographic study showed findings of chronic demyelinating neuropathy, with no conduction block. The visual and brainstem auditory evoked responses and the central motor conduction times were abnormal. The spinal cord MRI imaging was normal, while the brain MRI showed foci of demyelination in two patients.

Discussion: The diagnosis of HMSN1A in our patients was confirmed by molecular analysis. The CNS involvement was not the result of a detectable cause, such as spinal cord compression or CIDP. The above findings, in conjunction with familial nature of the CNS involvement, suggest that some cases of HMSN1A may be complicated by primary CNS demyelination.

### P438

**Anti-GFAP IgG antibodies in Guillain-Barré syndrome.** I. Basta, S. Allaria, B. Cavanna, M. Carpo, E. Nobile-Orazio, Belgrade University, Milan University (Belgrade, YU; Milan, I)

Antibodies to different glycolipids have been reported in patients with Guillain-Barré syndrome (GBS) but their pathogenetic role is still debated. In order to determine whether antibodies against protein antigens may be also involved in this disease we tested by immunoblot IgG and IgM reactivity with human cauda equina and spinal cord in the sera from 30 patients with GBS, 16 of whom were also tested during the recovery phase. As controls we examined sera from 14 patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), 11 with multifocal motor neuropathy (MMN), 11 with amyotrophic lateral sclerosis (ALS), 68 with other neurological and non-neurological diseases (OND) and 28 normal subjects. We found high titers (> 1/800) of IgG antibodies to some protein bands of the approximate molecular weight (MW) of 45–50 kDa in spinal cord but not cauda equina, in 20 patients (67 %) with GBS, and 1 each with MMN and CIDP (9 % and 7 %, respectively)(GBS vs. MMN/CIDP $p < 0.05$). In all 16 positive GBS patients re-tested during recovery this reactivity was reduced. A similar IgG reactivity with the 45–50 kDa bands was found in 2 patients (18 %) with ALS (GBS vs. ALS $p < 0.05$), 15 (23 %) with OND (GBS vs. OND $p < 0.001$) and in no normal subject. Since the MW of reactive bands corresponded to that of Glial Fibrillar Acidic Protein (GFAP), we tested sera from 6 positive GBS patients (titers of 1/6400 up to 1/12800) for IgG reactivity with purified GFAP by immunoblot. In four of them IgG bound to GFAP with the same pattern obtained with a commercial anti-GFAP antibody. This reactivity was abolished in one patient by serum preincubation with purified GFAP. Immunofluorescence staining of neonatal rat Schwann cells with sera from two positive patients gave the same strong cytoplasmatic pattern observed with a commercial anti-GFAP antibody. This study shows that IgG antibodies to GFAP are significantly increased during the acute phase of GBS and decrease during recovery. Further investigation is necessary to clarify their possible pathogenetic role in the disease.

Acknowledgement: This work was supported by an ENS fellowship stipend to I. Basta.

### P439

**Clinicopathological pattern of neuropathy associated with IgG or IgA monoclonal gammopathy.** M. Hézode, D. Adams, C. Théodore, C. Lacroix, G. Saïd, Centre Hospitalier Universitaire de Bicêtre, Institut Gustave Roussy (Le Kremlin-Bicêtre, Villejuif, F)

Background: Spectrum of peripheral neuropathy associated with IgG or IgA monoclonal gammopathy (IgGA-MG) is not clearly known because essentially based on small series which focused on either MGUS, solitary plasmacytoma, multiple myeloma or POEMS syndrome.

Objectives: Aim of the study was to determine the clinical presentation of neuromuscular diseases and the type of nerve injury associated with IgGA-MG.

Methods: In this retrospective and monocentric study, we reviewed the consecutive files of patients who have been referred for a peripheral neuropathy associated with monoclonal IgG or IgA gammopathy over the past 24 years. All the patients underwent clinical examination, electrophysiological studies and nerve biopsy when necessary, and appropriate hematological investigations looking for plasma cell dyscrasia.

Results: One hundred and ten patients were included, with a mean age of 62.7 years (extremes: 27–87) and a sex ratio (male/female) of 1.5.

Patients were initially classified according to clinical, electrophysiological and CSF data as: CIDP (n = 41/37 %), Guillain-Barré syndrome (n = 1), distal symmetrical polyneuropathy (DSP) (n = 39/35 %), mono or multiplex neuritis (n = 16/14 %), motor neuropathy (n = 3), ALS (n = 6/5 %) and atypical neuropathy (n = 4). The disability was mild (no walking difficulty) in 58 pts (52 %), moderate (walking difficulty) in 40 pts (36 %) and severe (bedridden) in 12 pts (8 %).

Among the 94 patients who underwent nerve biopsy, axonal pattern was found in 39 pts, mixed axonal and demyelinating pattern in 30 pts, demyelinating pattern in 13 pts and a normal nerve in 12 pts. Specific lesions were found in 17 patients with either DSP or neuritis multiplex (n = 14) or multifocal neuropathy with conduction blocks (n = 3). They include amyloidosis (n = 4), plasmacytic or lymphomatous infiltration (n = 3), granuloma (n = 3), vasculitis (n = 7). In 12 cases (13 %) NB findings modified the presumed pathological (axonal or demyelinating) mechanism suggested by electrophysiological study.

The monoclonal gammopathy was an IgG in 87 pts and IgA in 23 pts. Light chain type was kappa in 51 pts, lambda in 56 pts, unknown in 3 pts. MG was associated with a myeloma in 25 pts which included stage I myeloma in 16 pts (solitary plasmocytoma in 6) and stage III myeloma in 8 pt, lymphoma in 1 pt, a light chain (AL) amyloidosis in 5 pts (including 1 with myeloma) (4.5 %). At referral, 15 patients fulfilled criteria for POEMS syndrome. MG was of undetermined significance (MGUS) in 73 pts (66 %).

Conclusion: This study underlines the heterogeneity of peripheral neuropathy pattern and plasma cell dyscrasia associated with IgG or IgA mon-

oclonal gammopathy, the high incidence of POEMS syndrome and the usefulness of nerve biopsy in progressive or severe axonal neuropathy.

**P440**

**Polymorphisms in LPS-signaling genes related to the development of Guillain-Barré syndrome.** K. Geleijns, B. C. Jacobs, J. D. Laman, W. van Rijs, P. A. van Doorn, Erasmus Medical Center (Rotterdam, NL)

Objective of study: Antecedent infections play a role in the pathogenesis of Guillain-Barré syndrome (GBS), a polyneuropathy leading to a flaccid paralysis. Campylobacter jejuni, the predominant pathogen in GBS, is a Gram-negative bacterium with an outer membrane consisting mainly of lipopolysaccharide (LPS). C. jejuni infected GBS patients are characterized by an aberrant immune response to LPS which induces cross-reactive antibodies to peripheral nerve gangliosides. LPS induced host cell activation is mediated by polymorphic receptors, such as CD14 and Toll-like receptor (TLR4). We postulate that GBS patients have an altered immune response to LPS due to polymorphisms in these LPS-signaling genes and we investigated these polymorphisms in relation to GBS and its clinical and immunological subgroups.

Methods: Genomic DNA of GBS patients (n = 178) and healthy controls (n = 209) was analysed for the C[-260] T polymorphism in the promoter region of the CD14 gene, by using restriction fragment length polymorphism (RFLP) analysis. Two co-segregated polymorphisms in the fourth exon of the TLR4 gene were analysed by using the LightCycler technique, a PCR-based technology in which specific single base mismatch (SNP) can be detected. Statistical analysis was performed by using a chi-squared test.

Results: At position -260 of the CD14 gene, the CC genotype was found in 21.9 % of the GBS patients and in 23.9 % of the controls. The CT genotype was found in 50.6 % of the patients and in 44.5 % of the controls. The TT genotype was found in 27.5 % of the patients and 31.6 % of the controls. The following genotypes were found at position + 896 of the TLR4 gene: 88 % of the GBS patients and 86 % of the controls were AA homozygotes, 12 % of the patients and 14 % of the controls were heterozygotes. Only one of the controls was GG homozygote.

In a small group of GBS patients (n = 68) we studied the relation between antecedent infection and the genotype distribution; no association was found.

Conclusion: No significant difference was found in the genotype distribution of the polymorphisms in CD14 and TLR4 gene between healthy controls and the whole group of GBS patients. Preliminary studies show no association between antecedent infection and the CD14 and TLR4 genotype distribution.

**P441**

**Toxic polyneuropathies due to glue sniffing – clinical, electrophysiological and nerve biopsy studies.** M. Zouari, I. Turki, M. Kefi, S. Belal, F. Hentati, Institut National de Neurologie (Tunis, TN)

Objective: To report the clinical findings, electrophysiological, and nerve biopsy studies of 38 juvenile patients who had a toxic polyneuropathy due to glue sniffing.

Background: Progressive sensorimotor neuropathy developped after glue sniffing was described. The neurological picture consisted of a symmetrical progressive, ascending, mainly motor, polyneuropathy with muscle atrophy. Electrophysiological and nerve biopsy studies consisted in an axonal neuropathy with axonal degeneration and presence of giant axons due to accumulation of neurofilaments on electron microscope. It is concluded that n-hexane inhalation produces a toxic effect with axonal changes.

Methods: Thirty eight patients admitted for a progressive sensorimotor neuropathy due to glue sniffing in the National Institute of Neurology were selected. All patients had clinical examination, electrophysiological and sural nerve biopsy studies.

Results: The mean age at examination was 18 ± 2,5 years (range: 12 to 24 years). The mean duration of the exposure to the toxic with prolonged inhalation of n-hexane (glue sniffing) was 2.1 ± 2.1 years (range: 3 months to 8 years). All patients developped weakness and paresthesia mainly in lower limbs at a mean duration of 4 months after inhaling the glue. All patients were men and had similar clinical features characterized by a symmetrical progressive, ascending, sensorimotor polyneuropathy with pronounced amyotrophy and severe motor handicap leading to wheelchair bound in 10 patients (26 %). Electrophysiological studies showed an axonal sensorimotor neuropathy predominantly in lower limbs. Nerve biopsy study showed an axonal neuropathy with axonal degeneration, and presence of giant axons in 15 patients (39 %). Muscle biopsy found a severe neurogenic atrophy with target fibres in 23 patients (60 %).

Conclusion: These findings seem to indicate the pathological process of axonal swellings and confirm the toxic mechanism with primary axonal changes due to the n-hexane inhalation.

**P442**

**Long-term effectiveness of steroid injections and splinting in mild and moderate carpal tunnel syndrome.** S. Sevim, O. Dogu, H. Kaleagasi, M. Aral, H. Camdeviren, A. Milcan, C. Bagdatoglu, Mersin University (Mersin, TR)

Background: There is no consistent non-surgical treatment program for mild and carpal tunnel syndrome (CTS) at this time. Although splinting and local steroid injections into, and more recently, proximal to the carpal tunnel have been reported to be effective non-surgical treatment options, long-term effectiveness of these methods remains controversial. A possible several month honeymoon period of symptoms is suggested for local steroid injections indicating long-term follow-up studies.

Objective: To compare the detailed electrophysiologic and clinical outcomes of steroid injections and splinting in mild and moderate CTS.

Method: In the first phase of this long-term, prospective, randomised and blinded study 120 patients with clinical symptoms and electrophysiologic evidence of CTS were included and assessed by a structured historical questionnaire, a severity scale and by detailed electrophysiologic examination. 60 patients were instructed to wear splints every night, 30 were injected with betamethasone 4-cm proximal to the carpal tunnel and 30 into the carpal tunnel. At the end of 1 year totally 108 patients qualified and enrolled in the study. All of these patients were re-evaluated by the same methods used at baseline and at the end of one year.

Results: Significant improvement for splint group was present in clinical symptom severity in the follow-up examination ($p < 0.001$) but not for injection and control groups. Highly significant reduction was found in median-versus-ulnar digit IV antidromic sensory distal latency difference, median second lumbrical-versus-ulnar interossei distal motor latency difference and median nerve motor distal latency and highly significant increase in the mean antidromic median sensory conduction velocity for the splint group between the baseline and the follow-up examination values ($p < 0.001$ in all 4 parameters). None of the parameters of any other group showed statistically significant difference at the follow-up study when compared with those at baseline.

Conclusion: There can be some short-term beneficial effects of steroid injections in mild and moderate CTS; and even if there were in our study, we demonstrated that these benefits do not last one year. On the other hand, when it was properly used, splinting provided symptomatic relief and improved sensory and motor conduction velocities in our patients in the long-term follow-up.

**P443**

**Risk factors for the development of peripheral neuropathy in type I diabetes mellitus.** W. Fadel, M. Rowisha, M. Gabre, H. Mourad, M. El Batsh, Tanta University Hospital (Tanta, EGY)

Object: this study was done to investigate the role of some risk factors for development of peripheral neuropathy in children and young adolescents with type I diabetes mellitus (type I DM).

Materials: diabetic patients were divided into three groups: group I included 10 diabetic patients with clinically and electrophysiologically evident peripheral neuropathy, group II included 20 diabetic patients with subclinical peripheral neuropathy evident only by measuring the motor nerve conduction velocity (MNCV) and group III included 30 diabetic patients without any evidence of peripheral neuropathy. Ten normal healthy individuals with matched age and sex served as a control group (group IV). All the studied groups were subjected to full clinical and neurological examination, measuring (MNCV) of the common peroneal and median nerves, estimation of glycosylated hemoglobin level (HbA1c), assay of erythrocyte superoxide dismutase (SOD) level as well as molecular genetic study of the manganese superoxide dismutase (Mn-SOD) gene polymorphism.

Results: showed that there was a significant increase in HbA1c level and a significant decrease in both SOD level as well as a significant decrease in (MNCV) of common peroneal and median nerves in group I and group II as compared with group III and group IV. There were significant negative correlations between HbA1c when compared with SOD and MNCV as well as significant positive correlations between SOD levels and MNCV in all the diabetic groups. The frequencies of Ala allele and the Ala/Ala genotype of the Mn-SOD gene were significantly lower in diabetic patients with neuropathy compared with diabetic patients without neuropathy. In contrast, the Val allele and Val/Val genotype were significantly more frequent in diabetic patients with neuropathy than in control group.

Conclusion: poor glycaemic control, low levels of the key antioxidant enzyme SOD, and the Ala[−9]Val genotype of the Mn-SOD gene were significant risk factors for the development of diabetic neuropathy in type 1 diabetic patients which may necessitate appropriate support for enhancing antioxidant supply to act against the rapid onset and progression of peripheral neuropathy whereas the Ala allele and Ala/Ala genotype are associated with low risk of neuropathy in patients with type 1 DM.

**P444**

**Acute polyneuropathy induced by aminolevulinic acid.** V. E. Drory, N. Scheinfeld, C. Sylantiev, G. B. Groozman, Tel-Aviv Sourasky Medical Center, Rabin Medical Center (Tel-Aviv, Petach-Tiqva, IL)

Background: Photodynamic therapy (PDT) is used with increasing frequency for treatment of low-grade malignancies. Aminolevulinic acid (ALA) is administered as a photosensitizer in the tissues before PDT.

Case report: An 82-year-old male with Barrett's esophagus was started on PDT. After the first procedure he developed transient abnormal liver function. After the second dose of ALA, before PDT, the patient developed vomiting, abdominal pain, numbness and weakness in his legs, areflexia and ambulation difficulties. He had a slight increase of liver enzymes, his CSF protein was 66 mg/dl (N < 45), without cells, the EMG showed a mainly axonal motor and sensory polyneuropathy. Within the subsequent days he deteriorated gradually, with hoarseness and difficulty swallowing, urinary retention, impotence, sinus tachycardia, postural hypotension and hyponatremia, his forced vital capacity was reduced.

Porphyrin levels were: urine ALA 1 mg/24 h (N < 5 mg), porphobilinogen 4.3 mg/24 h (N < 2 mg), uroporphyrinogen 83 mg/24 h (N < 30 mg), coproporphyrinogen I 138 mg/24 h (N < 60 mg), coproporphyrinogen II 347 mg/24 h (N < 189 mg), blood porphobilinogen deaminase 66 mmol/ml (N 25–45 mmol/ml). Feces coproporphyrinogen was normal. He recovered gradually. Six months later he was able to walk independently, with slight proximal weakness. Repeated EMG showed late axonal changes. The patient is followed up for three years without any further change in his neurological or electrophysiological examination.

We suppose that this patient had a genetic predisposition for porphyria, revealed by the exposure to ALA, which has a main role in the porphyrin metabolism.

Conclusion: This is the first report of a porphyric predisposition revealed by PDT. Although this complication is probably very rare, it has to be taken into account and a detailed family history regarding porphyric symptoms should be obtained before PDT.

**P445**

**CIDP refractory patient treated with interferon beta 1b.** E. Cocco, M. G. Mascia, G. L. Floris, P. Marchi, M. G. Marrosu, University of Cagliari (Cagliari, I)

Background: Chronic inflammatory demyelinating polyneuropathy (CIDP) is a progressive or relapsing remitting immune mediated polyneuropathy. Conventionally this condition is treated with corticosteroids, intravenous immunoglobulins (IVIg), plasma exchanges and immunosuppressive drugs like azathioprine and cyclophosphamide, but some patients could be unresponsive to these therapies.

Case report: We present the case of a 41-year-old woman, who in July 1997 experienced progressive weakness in both lower limbs and sensory symptoms. On the basis of electrophysiological and clinical examination diagnosis of CIDP was made. After the first cycle of IVIg with a good response, she became refractory to this treatment. She presented new relapses treated with plasma exchanges with temporary improvement of the symptoms, but other relapses occurred within few months despite the specific drugs used (prednisone, IVIg, azathioprine, cyclophosphamide and cyclosporin). In total the patient presented 9 relapses during 27 months. In July 2001 she started therapy with Interferon (IFN) beta 1b (Betaferon) subcutaneous injections every alternative days. No other relapses occurred from beginning of Betaferon treatment and she is relapse free for 19 months and shows a marked improvement of clinical condition. In April 2002 sierological examination showed presence of antithyreoglobulin and antithyreoperoxidase antibodies, with increase of free triiodothyronine and free thyroxine. Autoimmune thyreopathy was diagnosed and the patient started treatment with antithyroid drug.

Conclusion: IFN beta 1 b could be considered as alternative therapy in CIDP patients refractory to conventional therapy, particularly in patients with relapsing remitting course of the disease.

Acknowledgement. A special thanks to Dr. Ecari and Dr. Ermini from Schering Italia for providing Betaferon.

**P446**

**Seizures associated with hereditary sensory motor neuropathy type I (HSMN I).** V. Milic-Rasic, V. Brankovic-Sreckovic, S. Todorovic, N. Jovic, J. Jancic-Stefanovic, Clinic for Child Neurology and Psychiatry (Belgrade, YU)

Background: Type I HSMN or Charcot-Marie-Tooth type 1 (CMT 1) is the most frequent inherited peripheral neuropathy. It represents disease of myelination genes mostly PMP22, Po and Cx32. "Central" manifestation of the disease, except subclinical signs, are uncommon in typical autosomal dominant (AD) CMT 1, but can be present in the rare autosomal recessive (AR) CMT 1 phenotypes.

Patients and Methods: We investigated 52 CMT 1 patients for the associated central nervous system manifestations. All patients underwent clinical, electrophysiological and genetical tests for the confirmation of the disease. Additionally to electromyoneurography (EMNG) studies, in all patients electroencephalography (EEG) and evoked potentials (VEP, SSEP, AEP) were done.

Results: Seven (13.4%) of 52 CMT 1 patients had associated seizures. Febrile seizure was detected in two and epileptic seizures in five patients. Partial seizures with secondary generalizations were diagnosed in one patient with 17p11.2 non-duplicated AR CMT 1. Generalized seizures were detected in three patients: in one with AR CMT 1, in another patient with AD CMT 1B (mutation in Po gene-Arg98His) and in the third patients with X-linked CMT 1 (possible Cx32 mutation). Posttraumatic epileptic seizures were diagnosed in the case with 17p11.2 non-duplicated AD CMT 1. The seizures were not found within the patients with classical form of CMT 1A and 17p11.2 duplication. EEG and EP abnormalities were compared in the subgroup of patients with and without associated seizures.

Conclusion: Seizures associated with CMT 1 were found in patients within the other than AD CMT 1A typical form of the disease. The diagnostic value of EEG and EPs in CMT 1 patients is emphasized.

**P447**

**Fatal peripheral neuropathy: a case report of neurolymphomatosis?** F. Arslan, G. Karlikaya, C. Orken, A. P. Hays, Y. Parman, H. Tireli, Haydarpasa Numune Research Hospital, Columbia University, Istanbul University (Istanbul, TR; New York, USA)

A 23 year old man had been in good health until August 2002 when he was admitted with acute painful progressive polyneuropathy restricted to his lower extremities.

Neurophysiological investigation showed an acute axonal and demyelinating sensorimotor polyneuropathy. Albuminocytologic dissociation was found in his CSF examination and he was diagnosed as AIDP.

The disorder stabilized, but after two weeks the patient began to deteriorate rapidly.

Following a sural nerve biopsy, he had a wound infection followed by fever, pancytopenia, severe weight loss, mild nephropathy, progression of polyneuropathy, right facial nerve palsy, diplopia and mental confusion. A repeat CSF examination exhibited a lymphocytosis of 50 cells/mm$^3$.

Cranial MRI showed multiple contrast enhanced lesions in frontal and occipital lobes and cerebellum. There was leptomeningeal contrast enhancement in his lumbosacral MRI.

The sural nerve biopsy specimen demonstrated marked lymphocytic inflammation, mainly T cell type in chiefly the perineurium and adjacent epineurium (perineuritis). Bone marrow biopsy was normal. Lymphocytic infiltration was seen in renal biopsy.

With all the above findings, even though we could not confirm it pathologically neurolymphomatosis was suspected. After chemotherapy he had temporary clinical improvement.

Because of severe pancytopenia another bone marrow biopsy was performed and displayed nodular T cell infiltration without clear evidence of malignancy.

The rapid detorioration continued, with severe pancytopenia, fungal sepsis and the patient needed mechanical ventilation. He died 4 months after his admission, presumably as a result of neurolymphomatosis, a rare type of non-Hodgkin's lymphoma that is confined to the nervous system.

Most patients with neurolymphomatosis present with an acute, subacute or chronic painful progressive sensorimotor polyneuropathy but it rarely affects the central nervous system.

**P448**

**Numerous episodes of facial palsy: case report and literature review.** N. Yardimci, S. Benli, U. Can, T. Zileli, Baskent University (Ankara, TR)

Regardless of the trigger, peripheral facial paresis is best described as an event-trauma to the nerve and determining the cause is a clinical challenge because of the diversity of disorders that affect the nerve.This case report describes a patient who suffers excessive preauricular pain followed by peripheral facial paresis on the left side of his face once a week or so lasting for one to four hours.Between the attacks he had no pain and no paresis.Occurring once a week these symptoms progressed to every other day in two months' time and he had had a gradually increasing left facial paresis in the silent periods.His clinical examination revealed a grade 3/6 left facial paresis with relative sparing of the forehead and eye.The remainder of the cranial nerves were normal. No masses were palpable in the parotid bed or neck. Results of otologic and audiologic examinations were normal. A magnetic resonance imaging scan demonstrated an enhancing 2x1,5 cm mass in the inferior region of the left parotid gland profound lobe. A total left parotidectomy with the involved portion of the facial nerve was performed. Pathologic evaluation confirmed the presence of an adenocarcinoma with the invasion of the facial nerve main truncus. Facial nerve dysfunction is a clinical sign that denotes malignancy in the evaluation of a parotid gland mass. Malignant tumors may produce facial paresis via extrinsic mechanical forces on the nerve, producing either compression (if the nerve is confined by surrounding structures, as by the fallopian canal) or stretch (if the nerve is allowed to elongate, as in its extratemporal portion) or by directly infiltrating the nerve or by local inflammation from infection or tumor necrosis (as in Warthin's tumor predisposed to inflammatory degeneration). Inflammation may act with the effects of extrinsic compression and inflammatory mediators may also directly precipitate ischemia via mivrovascular thrombosis, edema formation, and possibly neurotoxic effects. A comprehensive review of the literature on recurrences (either ipsilateral or contralateral) for Bell's palsy, the percentages range from 2.6 % to 19.5 %. The mean percentage is approximately 7.6 %. About 70 % of patients have their first recurrence within ten years after their first palsy. More than one recurrence is approximately 1.08 %. In this unusual case we report facial palsy recurrences more than once a week for two months' time due to a parotid mass for the first time in the literature.

**P449**

**Sensory Guillain-Barré syndrome with anti-GQ1b antibodies: case report and relation with Miller-Fisher syndrome.** G. Galassi, M. Leone, A. Barbieri, University Hospital (Modena, I)

Guillain Barré syndrome (GBS) is characterized by ascending motor paresis, hypo/areflexia, minimal objective sensory symptoms, electrophysiological evidence of demyelination and cerebrospinal fluid (CSF) albuminocytologic dissociation. Several related syndromes are regarded as variants being the pathogenesis considered an autoimmune attack on peripheral nerve antigens. A 42 year old veterinary was admitted because of severe myalgias following one day high fever. Twelve hours later, he experienced dysphonia, dysphagia, difficulty in chewing. Clinical examination showed normal mental state and eye movements, bilateral facial weakness, tongue paralysis, limb and trunk sensory ataxia. Muscle strength was minimally affected and deep jerks were weakened. Sensory testing revealed distal loss for pin-prick, vibration, position in the four extremities. Normal laboratory included emato-urinary tests, creatine kinase, rheumatology and endocrine profiles, microbiological and viral screenings. Search for enteric Campylobacter jejuni was negative. CSF protein was 160 mg/dl (normal < 45). In serum, antiGQ1b immunoglobulins G (IgG) were elevated. On admission, electrophysiology was normal. Twenty days later, evidence of demyelination was limited to prolonged F-waves and terminal latencies. Intravenous immunoglobulin (IVIg 0.4 g/kg/daily for five days) had transient improvement. Because of relapse of sensory symptoms and ataxia, 10 days later a course of plasmapheresis was performed with benefit. Patient was followed clinically during six months showing almost complete recovery. In conclusion, we report an acute neuropathy with benign course, high protein in CSF, predominant involvement of sensory fibers, electrodiagnostic evidence of demyelination. The nosologic position of this case seems to fit the criteria of sensory variant of GBS.The detection in serum of antiGQ1b immunoglobulins G (IgG) proposes similarities with Miller Fisher syndrome and with ataxic form of GBS suggesting the possibility of a common pathophysiology. Previous authors proposed the term of "anti GQ1b IgG antibody syndrome" including in the spectrum also patients with brainstem symptoms such as Bickerstaff encephalitis. Clinical recognition of such cases raises the challenge of identifying the antigen that focuses an autoimmune attack predominately on the Schwann cells and myelin sheath of sensory rather than motor axons.

**P450**

**Acute polyneuritis cranialis post-vaccination: a rare variant of Guillain-Barré syndrome.** P. Alla, J. P. De Jauréguiberry, G. Leyral, E. Kaiser, J. Valance, Sainte-Anne Hospital (Toulon, F)

Polyneuritis cranialis is a syndrome of multiple cranial nerve involvement. In Guillain-Barré syndrome (GBS) a limited cranial nerve involvement is usual. In Miller Fisher variant ophthalmoplegia is constant. Clinical findings confined to involvement of lower cranial nerves are rare. We report a case of acute pure polyneuritis cranialis associated with antiganglioside antibodies after vaccination in a young girl.

Case report: a 16 year old girl with a past of hay fever was vaccinated in January 2001 against diphtheria, lockjaw, poliomyelitis and whooping cough. Twelve hours after she was admitted in urgency for an acute respiratory distress with diffuse erythrodermia, diarrhea, vomiting and fever. Neurologic exam showed a bilateral symmetrical involvement of lower cranial nerves (IX, X, XI, XII), a facial diplegia with Bell's phenomenon, normal tendon reflexes in four limbs, no sensitive disorder. General symptom decreased rapidly and polyneuritis cranialis persisted. Cerebral and spinal MRI was normal. Three studies of CSF were normal. Standard biological analysis was normal. Various serological analyses were negative as HIV, cytomegalovirus, campylobacter jejuni, Lyme. The research of antiganglioside antibodies was positive for anti-sulfoglucuronyl paraglobo-side (SGPG) antibodies. Electroneuromyography showed no abnormality on all limbs but a demyelinisation on facial nerves. On day 12 she was treated by intravenous immunoglobulins (400 mg/kg/day during five days). She recovered progressively a good speech and deglutition. On day 26 she quitted intensive care and was addressed in rehabilitation. One year after neurological exam was normal.

Discussion: this patient presents a general immuno-allergic acute reaction post vaccination accompanied by polyneuritis cranialis. It is also an immunological complication post vaccination with presence of antiganglioside anti-SGPG antibodies. This case is characterised by bilateral, symmetrical and exclusive lower cranial nerve and facial nerve palsy. Rare similar cases are reported in the literature (Polo et al. (1992) J Neurol Neurosurg Psychiatry 55:398). First Van Bogaert and Moore in 1938 (J Bel Neurol Psychiatrie, 38: 275) described three cases of GBS confined to the cranial nerves. Various elevated serum antiganglioside antibodies can accompany the polyneuritis cranialis as variant of GBS but antiGT1 antibodies are detected in a higher percentage in the GBS with bulbar palsy (Yoshino et al. (2000) J Neuroimmunol 105:195). The anti-SGPG antibodies have affinity for epitopes in both myelin and axon of peripheral nerves and may cause nerve damage by different pathogenic mechanisms, leading to different clinical presentations (Kohriyama et al. (1987) J Neurochem 48:1516).

Conclusion: acute polyneuritis cranialis with bilateral symmetrical involvement of lower cranial nerves and facial diplegia is a possible variant of GBS post vaccination.

**P451**

**Multifocal enlargement of spinal roots in chronic inflammatory demyelinating polyradiculoneuropathy.** I. E. Markakis, E. Alexiou, P. Davaki, I. Kambas, E. Kalamboki, A. Tsakiris, Piraeus General State Hospital, Eginition Hospital (Nikaia, Athens, GR)

Background: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a disorder of probably immunological cause, with a progressive or relapsing-remitting course but typical clinical and laboratory findings. In cases of chronicity, repeated episodes of segmental demyelination and remyelination can result in macroscopic spinal root and plexus hypertrophy.

Objects: We present a case of CIDP with marked bilateral and symmetrical tumorous enlargements of the spinal roots along the neuraxis, resembling multiple neurofibromas.

Case history: A female patient, aged 38 years, presented at the age of 36 with a 5-year history of progressive stocking-glove sensory impairment and a 6-month history of generalized weakness of the lower limbs. On examination she had a poor balance, marked weakness of proximal leg muscles and bilateral drop feet. Tendon reflexes were absent. Nerve conduction studies and cerebrospinal fluid analysis were characteristic of CIDP. Sural nerve biopsy revealed mononuclear cell infiltration and segmental demyelination in fibers of all calibers. The patient was treated with oral prednisolone and monthly trials of intravenous immune globulin at a dose of 0.4 g/kg and had a rapid clinical improvement. Six months after her admission a computed tomography scan of the chest and abdomen revealed multiple paraspinal nodular masses in the cervical, thoracic and sacral regions. Magnetic resonance imaging demonstrated that the lesions corresponded to massive multifocal enlargements of the spinal roots that were radiologically indistinguishable from neurofibromas.

Discussion: The diagnostic dilemma in this case was the distinction of CIDP from neurofibromatosis. Our patient had none of the known stigmata or manifestations of the latter. Moreover peripheral neuropathy complicating neurofibromatosis is extremely rare and is usually of the axonal type.

Conclusions: Neglected or prolonged cases of CIDP can rarely mimic neurofibromatosis. The absence of typical manifestations of neurofibromatosis and the pathological demonstration of peripheral nerve demyelination provide the correct diagnosis.

**P452**

**Clinical findings and treatment in 7 patients with CIDP.** S. Cagirici, S. Ilhan, U. Turk Boru, Dr Lutfu Kirdar Kartal Education Hospital (Istanbul, TR)

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is an acquired polyneuropathy in which there is steady progression, intermittent progression or relapsing-remitting course over a period of years. CIDP is presumed to occur because of immunologic antibody-mediated reaction. The affected nerves showed interstitial and perivascular infiltration of the endoneurium with inflammatory T cells and macrophages. The consequence is a segmental demyelination of spinal roots and peripheral nerves. It usually responds to plasma exchange, intravenous gammaglobulin (IVIG) or corticosteroids.

We reviewed here clinical features and response to treatment in 7 patients with CIDP investigated in our clinic in last two years.

All 7 patients were examined clinically, had electrodiagnostic, cerebrospinal fluid (CSF) and serum testing. The pretreatment clinical states and improvement following immunotherapy in the patients were assessed Rankin disability score. The clinical features, treatment and outcome of 7 CIDP patients (2 female, 5 male) are shown in the table. All patients had sensory-motor polyneuropathy syndromes. CIDP followed a relapsing remitting course in three; a progressive course in three and a monophasic course only one of the patients. On neurological examination, all patients had symmetric weakness predominantly in distal muscle groups.

They had predominantly distal, relatively severe or moderate loss of most sensory modalities, including vibration, pain and temperature. The results of nerve conduction studies showed evidence of demyelination polyneuropathy in six patients. One patient had axonal and demyelinating polyneuropathy findings. There were conduction block in 4 patients and conduction slowing in the others. Only 4 of 7 patients had accepted sural nerve biopsy. The nerve specimens were found chronic demyelinating lesion.

We had treated monthly prednisolone in 2, IVIG in 1 or combined immunotherapy (IVIG and prednisolone; prednisolone and azothiopurine) in 4 patients. One patient who started IVIG treatment was added to prednisolone and azothiopurine. Our patients had similar response rates to prednisolone; IVIG and prednisolone; azothiopurine and prednisolone.

The clinical pictures of CIDP vary and are heterogenous in course, distribution of lesion and response to immunotherapies. We summarized here; the clinical findings, treatment and outcome of 7 patients with CIDP.

**P453**

**Very late-onset familial amyloid polyneuropathy type 1 (TTR Met 30) in Catalonia (Spain): clinicopathological features in three families.** A. Pou-Serradell, M. Téllez, F. Alameda, V. Planté-Bordeneuve, Hospital del Mar, Hôpital de Bicêtre (Barcelona, E; Paris, F)

Background: Typically, familial amyloid polyneuropathy (FAP) TTR Met30, the most common type of FAP in western countries is characterized by onset in the second or third decade of life, a high penetrance rate, marked autonomic dysfunction, loss of superficial sensation and progression of the disease over 10 to 15 years.

Objective: To compare clinical and pathological (nerve biopsy) features of FAP TTR Met30 between patients with onset before and after 65 years of age.

Patients and Methods: Patients in this study were from Catalonian families [2] and Majorcan family [1] with late-onset (66 years or older) FAP TTR Met30. They were seen in our Service of Neurology from 1997 to 2003. Inclusion criteria were polyneuropathy, Met30 transthyretin mutation and onset at age 65 or older. Clinical routine examinations, nerve conduction studies and nerve biopsy were performed using standard methods.

Results: In these patients with a very late-onset (at 66, 68, 75 and 78 years of age respectively), the male-to-female ratio was extremely high [4:0], a family history was evident in only 1 (with two brothers affected) of the 3 families, the rate of penetrance was very low, the most common initial symptom was paraesthesias in the legs (with disturbances in both superficial and deep sensations), autonomic dysfunction was generally mild, the range for surviving after onset of symptoms was 4 years and the symptomatic sibling of one familial case showed similar clinical features to those of the proband. Deposits of amyloid in sural nerve biopsy was not present in one patient.

Conclusions: Cases of very late-onset FAP TTR Met 30 show clinical features distinct from those of typical early-onset FAP TTRMet30, they may be more prevalent and widespread than previously believed and factors responsible for this clinicopathological differences still need to be identified.

**P455**

**Intramyelinic oedema: an elementary pathological lesion in CIDP.** F. Madia, M. Sabatelli, L. Quaranta, G. Lippi, A. Conte, M. L. Mereu, P. Tonali (Rome, I)

Intramyelinic edema (IE) is a well recognized elementary pathological lesion of the peripheral nerve consisting of collection of fluid with proteinaceous material splitting the myelin sheath at the level of dense major line or intraperiodic line. This peculiar abnormality has been described in several experimental neuropathies induced by toxic, such as triethyltin, hexaclorophene, tellurium and 2',3'-dideoxicytidine, and by mechanical injury. It has also been described in experimental allergic neuritis induced by systemic passive transfer of human IgM anti-myelin-associated glycoprotein.

On the contrary, IE is not usually observed in human nerve biopsies. This abnormality has been described only in three reports by Waddy el al. (J Neurol. 1989, 236: 400) in 5 patients with idiopathic chronic inflammatory demyelinating polineuropathy (I-CIDP), Sabatelli et al. 1996 (Clin Neuropathol 15:17) in one patient with I-CIDP and Cai et al. 2001 (JPNS 6:95) in a patient with CIDP-MGUS.

Thus IE is not usually listed among elementary pathological lesions in human peripheral nerve diseases.

However the occurrence of IE in only chronic inflammatory demyelinating neuropathies raises the question whether this abnormality is specific of CIDP and whether it may play some role as a mechanism of loss of function in this disease.

We reviewed morphological data of sural nerve biopsies from 46 CIDP patients (with or without MGUS) admitted in our Institute from 1988 to 2002.

IE was observed in 11 patients with CIDP, but in none of the 580 patients affected by other neuropathies.

Three out of the 11 patients had CIDP associated with IgM-paraprotein, without anti-MAG activity, the other 8 patients were affected by idiopathic CIDP. Only in one patient IE was a prominent feature while in the remaining patients it was confined to sporadic fibers. In three patients, with a mild form of CIDP, IE was the only pathological finding, in the remaining patients it was associated with segmental demyelination and axonal loss. In all fibers disclosing IE, the axons showed markedly shrunken profiles.

Our findings show that although IE is observed only in a minority of CIDP patients (23 % of our series), this pathological finding may be considered a specific abnormality of inflammatory demyelinating neuropathies. We suggest that axonal shrinkage, which is invariably associated to IE, may represent a mechanism of loss of function in CIDP, in addition to segmental demyelination and axonal loss.

# Poster Session 3

## Cerebrovascular disorders

**P456**

**Early asymptomatic recurrence of cervical artery dissection: 3 cases.** E. Touzé, M. Zuber, C. Oppenheim, E. Méary, J. F. Meder, J. L. Mas, Hôpital Sainte-Anne (Paris, F)

Cervical artery dissection (CAD) recurrences are rare but it has been suggested that the risk could be higher during the first month. Nevertheless, early recurrences have been rarely documented and nearly all the cases were diagnosed in patients with recurrent ischaemic event. We report 3 patients with early asymptomatic recurrence of CAD involving an initially apparently unaffected vessel.

From January 2000 through December 2001, 27 consecutive patients (mean age: 45,6 years, 16 men) were admitted in our stroke unit for an acute CAD and were investigated according to standardized radiological workup including duplex sonography, cervical gadolinium-enhanced MR-angiography and axial cervical plane T1 and T2 weighted MRI. A first follow-up radiological workup including the same techniques (neck MRI if necessary) was performed in all patients, 70.2 days (range: 15.8–183.3) on average after the diagnosis of CAD. Two independent neuroradiologists reviewed MRA and MRI and compared the initial and the first follow-up radiological examination.

An asymptomatic recurrent dissection involving an initially apparently unaffected vessel was observed in 3 patients between 45 and 55 days after the initial CAD (carotid artery in all cases; 2 stenotic + aneurysmal and one stenotic only). None of them had traumatism during the follow-up. The 3 patients did not have morphological distinguishing features suggestive of connective tissue disease nor biological abnormalities on usual tests. They were under oral anticoagulants (as were 23 of the patients without CAD recurrence) with INR in therapeutic range when the first radiological follow-up was performed and this treatment was continued in all patients. A second follow-up radiological examination was performed between 26 and 48 days after the first one. This new control showed that all stenoses had improved or disappeared and all aneurysms had persisted.

Our results suggest that early CAD recurrences could have been underestimated as most of them seem not to result in stroke in patients under anticoagulants. Neurologists in charge of patients with CAD should be aware of such early radiological modifications, although early recurrences of CAD do not worsen the usual good prognosis of these patients and do not justify a change in antithrombotic therapy.

**P457**

**The atherogenic effect of smoking is not necessarily mediated via inflammation.** F. Weber, German Air Force Institute of Aviation Medicine (Furstenfeldbruck, D)

Background: Smoking is an important risk factor of atherosclerosis. It is a subject of debate, whether the atherogenic properties of smoking can be mediated by subclinical or clinical infections. The present study was undertaken to address this issue.

Methods: A cohort of 483 military aviators, officially considered as fit for flying and therefore free of infection, was included in the study. All attended baseline examination with measurement of cardiovascular risk factors (body mass index, blood pressure, physical working capacity, cholesterol, high-density lipoprotein cholesterol, triglycerides, gamma glutamyl transferase, cigarette smoking) and of markers of inflammation (white blood cell count, percentage of neutrophils, erythrocyte sedimentation rate). All underwent carotid ultrasonography at the endpoint after 10 years of follow-up.

Results: Age, cigarette smoking and blood pressure were independent long term predictors of intima-media thickness (IMT). After adjustment for age, cigarettes smoked daily at baseline, categorized in units of five cigarettes each, (0.057, regression coefficient in mm [95% CI 0.029 to 0.085]; $p < 0.0001$) and systolic blood pressure (0.007 [95% CI 0.003 to 0.010], $p < 0.0001$) were the only predictors of IMT. White blood cell count, percentage of neutrophils, erythrocyte sedimentation rate were not associated with IMT.

Conclusion: Even in smokers, early carotid atherosclerosis can be independent of infection.

**P458**

**New approach to the problem of impaired carbohydrate metabolism in ischaemic stroke.** D. Koziarskia, P. Nowacki, L. Majkowska, Pomeranian Medical University (Szczecin, PL)

The aim of our prospective analysis was to verify the hyperglycaemia of acute phase of stroke in the 6-months follow-up study. Material and methods: the study was carried out on 220 patients (age 23–94 yrs, mean 67.2 yrs), 96 women and 124 men. We estimated glucose metabolism according to the schedule: an admission plasma glucose level, diurnal glycaemia (in capillary whole blood), concentration of glycosylated haemoglobin (HbA1c). In non-diabetic patients we performed an oral glucose tolerance test. Glycaemia and concentration of HbA1c were determined by the calorimetric method (COBAS, Mira Roche). The patients were divided into four groups: 1. Previously known diabetics. 2. Newly-diagnosed diabetics. 3. Unclassified hyperglycaemia-patients with fasting glucose level ≥ 5,5 mmol/l ≤ 11 mmol/l and postprandial glucose level ≥ 7,8 mmol/l and ≤ 11 mmol/l in diurnal glycae-mia. 4. Euglycemic patients with fasting glucose level below 5,5 mmol/l and postprandial glucose level and below 7,8 mmol/l in diurnal glycaemia.

To the follow-up study (period of double exchange of HbA1c) we included all patients who were classified as hyperglycaemia of unknown cause. We performed the oral glucose tolerance test, and HbA1c concentration was also checked. We used the actual WHO classification.

Results: From 220 patients with stroke, there were 43 (19,5%) previously known diabetics and 20 (9,5%) newly-diagnosed diabetics. 155 patients (70%) were diagnosed as unclassified hyperglycaemia in this phase of stroke. Only 2 patients (1%) stayed euglycemic during the whole hospitalisation period.

The 6 -months follow-up study was performed on 55 patients from the unclassified hyperglycaemia group. Among these patients we recognised 18 patients (33%) with newly-diagnosed diabetes mellitus, 12 patients (22%) with impaired glucose tolerance and 8 patients (14%) with impaired fasting glycaemia. Only 17 persons (31%) appeared to be euglycemic 6 months after stroke. Conclusions: 1. At the onset of ischemic stroke diabetes mellitus was diagnosed in 1/3 of patients – in half of those cases diabetes mellitus was not previously recognised. 2. Impaired glucose tolerance and impaired fasting glycaemia constitute an important but still not well appreciated risk factor of ischemic stroke. 3. Every third case of hyperglycaemia in acute phase of stroke turns out to be stress hyperglycaemia.

**P459**

**Parameters of venous haemodynamics in healthy individuals and report of a patient with cerebral sinus thrombosis.** C. Krogias, S. H. Meves, S. Peters, M. Schöllhammer, T. Postert, Ruhr University Bochum, St. Vinzenz Krankenhaus (Bochum, Paderborn, D)

Background: Recently, the assessment of intracranial venous hemodynamics with transcranial colour-coded duplex sonography (TCCS) has been described. The aim of this study was to provide further data of normal and pathological findings in cerebral venous hemodynamics in order to emphasize the diagnostic value of TCCS in cerebral venous thrombosis.

Methods: 11 healthy volunteers without history of cerebrovascular disease (mean age 31 years) and one 30 years old female patient with right-sided transverse sinus thrombosis were examined with TCCS. Peak-systolic and end-diastolic flow velocity values of the basal veins of Rosenthal (BVR), deep middle cerebral veins (dMCV), vein of Galen (VG), straight sinus (SRS) and transverse sinus (TS) were ascertained. Follow up examinations of the patient with TS thrombosis were performed at months 1, 6 and 12.

Results: Healthy individuals showed peak-systolic flow velocity values of less than 25 cm/sec in all examined cerebral veins. (dMCV = 10,9 ± 2,2 cm/sec; BVR = 12,3 ± 2,5 cm/sec; VG = 12,4 ± 1,5 cm/sec; SRS = 14,4 ± 2,0 cm/sec; TS = 17,4 ± 4,1 cm/sec). Initial examination of the patient with acute right-sided TS thrombosis revealed a missing flow signal in the right TS and a compensatory increased systolic flow velocity value in the left TS (47,6 cm/sec). Follow up examinations at months 1 and 6 showed a normal flow signal of the right TS and a reduction of the increased flow in the left TS without normalization. After 12 months normal values of both TS were measured.

Conclusion: Parameters of healthy individuals are in accordance with published data, confirming the feasibility of TCCS of venous hemodynamics in different neurosonological laboratories. TCCS, an easily available bedside diagnostic tool, provides helpful information in the diagnosis and monitoring of cerebral venous thrombosis.

**P460**

**Preincubation of brain endothelial cells with H202 decreases blood-brain barrier permeability in vitro.** K. Voigt, J. Kraus, P. Oschmann, M. Clauss, B. Engelhardt, University Hospital Giessen, MPI for Physiological and Clinical Research (Giessen, Bad Nauheim, D)

Background: The function of the blood-brain barrier (BBB) can be impaired by generators of free radicals such as H202. Upon hypoxia, H202 is released from primary cultures of rat brain endothelial cells in vitro, and the paracellular permeability of primary cultures of porcine as well as bovine brain capillary endothelial cells is increased. However, ischaemic preconditioning, which involves short term preexposure to free radicals, significantly attenuates ischaemic brain edema formation and BBB disruption in rats in vivo.

Objective: We examined whether preincubation of brain endothelial cells with H202 as a model of ischaemic preconditioning attenuates BBB permeability in vitro.

Methods: The immortalized endothelial cell line bEnd5 from mouse

brain capillaries was grown to confluence in DMEM for two weeks on semipermeable filters precoated with rat tail collagen. Development of functional BBB tightness was verified by measurements of electrical resistance. Culture medium was replaced with Ringer-HEPES solution, and we determined the paracellular permeability of radioactive [14C]-sucrose and [3H]-inulin across the endothelial monolayers after exposure to concentrations of 150, 300, and 500μM H202 for 30 minutes before the permeability assay. In addition, the cells were exposed to 500μM H202 during the assay period. In both cases, permeability was compared to unstimulated controls.

Results: After exposure to H202 for 30 minutes, at all concentrations the bEnd5 cells showed a lower permeability than the controls that had not been preincubated with H202. However, when the endothelial cells were incubated directly with 500μM H202 during the assay, permeability was increased as compared to unstimulated controls.

Conclusion: The results indicate that preincubation of the brain endothelial cell line bEnd5 with H202 decreases paracellular BBB permeability. This seems to be independent of H202 concentration, possibly due to a threshold mechanism. Therefore, short term preexposure of brain endothelial cells to free radicals, such as in ischaemic preconditionng, attenuates BBB permeability in vitro.

P461

**Cerebral blood flow velocity changes after complete recovery aimed surgery in systemic-pulmonary shunt patients.** N. Gungor, U. Can, A. Donmez, D. Sargin, S. Mercan, Baskent University (Ankara, TR)

The impact of systemic-pulmonary shunt and its treatment on cerebral hemodynamics in children has been studied with transcranial Doppler (TCD) in only a few instances and most of them are in patent ductus arteriosus patients. Its clinical consequences are highly variable. In our study, pre- and postoperative TCD in cyanotic and noncyanotic systemic-pulmonary shunt patients were performed in order to find out if recovery aimed surgery makes any change in cerebral blood velocities.

After Ethics Committee approval 12 cyanotic and 22 noncyanotic (7 atrial septal defect, 9 tetralogy of Fallot and 16 ventricular septal defect) children without known neurological disease 6–72 months-old were studied. Four of the cyanotic patients were polycythemic and 8 of them were normocythemic. Patients were evaluated in three groups, 1- non-cyanotic, 2- polycythemic-cyanotic and 3- normocythemic-cyanotic. Peak systolic velocity (PSV), end diastolic velocity (EDV) and mean velocity (MV) and derived parameters such as pulsatility index (PI), resistance (RI) index were recorded with TCD in the preoperative and postoperative periods from the right middle cerebral artery (MCA) of the patients. Synchronized manner hemoglobin, hematocrit, and $O_2$ saturations were also documented. Statistical analyses were performed with Wilcoxon and Mann-Whitney-U tests and $p < 0.005$ was considered as significant.

Preoperative and postoperative cerebral blood flow velocities of 22 non-cyanotic and 8 normocythemic-cyanotic patients were in normal limits. Preoperative PSV and MV but not the other parameters of 4 polycythemic-cyanotic patients were both 26% lower than normal values ($p < 0.002$). Postoperative values turned to normal limits with the augmentation of hemoglobin results.

In conclusion, our study demonstrated that recovery aimed surgery resulted in normalization of the low preoperative PSV and MV values in polycythemic-cyanotic systemic-pulmonary shunt patients but did not make any change in non-cyanotic or normocythemic-cyanotic groups.

P462

**Anticholinergics intake and delirium in acute stroke.** L. Caeiro, M. I. Claro, J. Coelho, J. M. Ferro, R. Albuquerque, M. L. Figueira, Hospital Santa Maria (Lisbon, P)

Background and purpose: The pathogenesis of post stroke delirium is incompletely understood. Delirium was associated with other conditions. The use of medications with anticholinergic (ACH) activity was associated with increase frequency of delirium. In this investigation, we tested the hypothesis that the intake of medications with anticholinergic activity is associated with increased frequency of delirium in acute stroke patients.

Patients and methods: We assessed delirium, using DSM-IV-R criteria and the Delirium Rating Scale, in a sample of consecutive patients with an acute infarct or intracerebral haemorrhage (ICH) ("T4 days). We performed a case-control study: 22 delirious stroke patients (cases) were matched, by gender and age, with 52 non-delirious patients (controls). Cases and controls were compared concerning socio-demographic, predisposing conditions and precipitants of delirium and intake of anticholinergic medications [1) during the days before stroke, 2) during the hospitalisation but before the assessment]. The variables with $p < 0.15$ on bivariate analysis were entered in stepwise logistic regression predicting delirium.

Results: Cases had more frequently ICH, hemispherical strokes, disturbance of alertness, neglect, intake of anticholinergic medications and medical complications than controls. In the final model (Nagelkerke R2 = 0.65) retained ICH (OR = 1.67; 95%CI = 2.7 to 100), anticholinergics before stroke (OR = 1.67; 95%CI = 1 to 333), non-neuroleptics anticholinergics during hospitalisation (OR = 25; 95%CI = 2.17 to 250) and medical complications (OR = 20; 95%CI = 3.4 to 125) were independent predictors of delirium.

Conclusion: Delirious patients presented a higher frequency of intake drug with anticholinergic activity. Drugs with subtle anticholinergic activity play a role in the pathogeneses of delirium in acute stroke and should be avoided in the acute stroke patients.

## Clinical neurophysiology

P463

**Dorsal sural nerve conduction studies in healthy children.** N. Turgut, S. Karasalihoglu, Y. Kücükugurluoglu, K. Balci, G. Ekuklu, U. Utku, F. Tütüncüler, Trakya University (Edirne, TR)

Subject: It is known that in the patients with polyneuropathies, the most distal sensory fibers in the lower extremities are generally first affected. But sensory nerves in the distal part of the feet are not evaluated by sural and superficial peroneal conduction studies. For this reason, nowadays, dorsal sural nerve of the foot is used to diagnose the early stage of polyneuropathy. In this study, we tried to determine the normal dorsal sural nerve conduction values of the childhood population.

Methods: Twenty healthy children (12 girls, 8 boys) were included in the study. Their mean age was 9.3 ± 2.2 years (min: 5.0, med: 9.5, max: 14.0). Peripheral nerve conduction studies of upper and lower extremities were obtained antidhromically. Bilateral sural and dorsal sural nerve conduction studies were also obtained. To test dorsal sural nerve action potentials, surface bar recording electrodes which had active-reference electrode distance of 2 cm were used. Recording electrode was placed over the dorsolateral surface at the midpoint of the fifth metatarsal bone, and just proximal to the fifth toe. The stimulation site was posterior to the lateral malleolus, with the cathode placed 8–10 cm proximal from the recording electrode. Sural nerve conduction studies were obtained by standard antidhromic technique.

Results: Amplitudes, latencies and nerve conduction velocities of the sural and dorsal sural nerves of all the children included in the study were evaluated. Mean sural nerve amplitude was 21.6 ± 9.7 microvolt. Mean dorsal sural nerve amplitude was 9.9 ± 3.5 microvolt. Mean sural nerve conduction velocity was found as 56.4 ± 10.2 m/sn and mean dorsal sural nerve conduction velocity was found as 42.7 ± 5.7 m/sn. Mean sural nerve latency was 2.3 ± 0.7 millisec., mean dorsal sural nerve latency was 2.4 ± 0.4 millisec.

Discussion: In this study, we tried to determine normal nerve conduction study values of dorsal sural nerve in the childhood population. Sural/dorsal sural nerve amplitude ratio was found as 2.1. This ratio was similar to the adult ratios. Our findings suggest that dorsal sural nerve conduction studies may have value to determine neuropathy in the early stages of childhood polyneuropathies.

P464

**An electrodiagnostic study of foot drop cases in Iran (Tabriz).** H. Ayromlou, M. Yazdchi, Z. Agsaiifard, Tabriz University (Tabriz, IR)

Introduction: When there is paralysis of the dorsal extensor muscles of the foot and the toes (tibialis anterior, extensor digitorum longus, and extensor hallucis longus), which are innervated by the deep peroneal nerve, foot drop occurs. Because the tibialis anterior muscle is innervated from the L4-S1 roots, especially the L5 and to a lesser extent L4 root, through the sciatic and ultimately the deep peroneal nerves, a lesion in any of these can cause foot drop. The most common peripheral causes are common peroneal injury and L5 radiculopathy and central causes are parasagittal lesions, spinal cord lesions.

Patients and Methods: In this cross sectional descriptive study 72 patients (55 men & 17 women) with foot drop chief complaint referring for electrodiagnostic ward of Tabriz Imam hospital were chosen and examined clinically and paraclinically.

The standard electrodiagnostic study was performed in all patients.

Results: The age range was from first to 9th decades (most frequent in the second, 6th and 7th decades).

In etiologic study the most frequent cause of foot drop was L5 radiculopathy (40.2%). The other causes were: peroneal mononeuropathy (26.3%), sciatic nerve injury (15.2%), peripheral neuropathy (6.9%), motor neuron disease (5.5%), lumbosacral plexopathy (2.8%) and central nervous system (2.8%). In 21/29 radiculopathies patients the onset of disease was subacute or chronic (72.4%). In 94.7% of peroneal nerve involvements there was acute presentation (18/19).

The most frequent (8/11) cause of sciatic nerve lesion was injection injuries (72.7%) specially with diclofenac Na injection.

In 29 patients with radiculopathy 9 cases had bilateral involvement but in 20 cases it was unilateral.

In 19 peroneal nerve involvement 18 persons had unilateral drop but in 1 patient it was bilateral, also the electrodiagnostic types of injury incuding: 7 demyelinating, 6 axonal and 7 mixed type.

Conclusion:

1. In males foot drop is 3–3.5 times more frequent than in females.
2. The most frequent causes of foot drop in our center are L5 radiculopathies and peroneal nerve injuries.
3. The foot drops due to radiculopathies usually are subacute or chronic.
4. Nearly all patients with peroneal involvement have an acute presentation.
5. The most frequent etiology of sciatic nerve injury is intragluteal injections.

### P465

**Unilateral decreased cortical excitability in a venous angioma of the frontal lobe.** M. Spilioti, I. Ameridou, G. Amoiridis, University Hospital (Heraklion, GR)

Objective: We present a patient with right hand paralysis and sensory loss, considered to be functional. Magnetic stimulation showed increased threshold of the left hemisphere. Neuroimaging studies revealed a venous angioma in the left frontal lobe.

Methods: A 16 year-old girl was admitted to our department because of a second episode of right hand weakness and numbness of acute onset. The first episode occurred 10 days before and lasted for 4 days. Neurological examination on admission revealed paralysis of the wrist and finger extension and flexion, and sensory loss of the hand with a sharp border at the wrist, for all modalities. The deep tendon reflexes were normal. The pattern of weakness and sensory loss was consistent with functional paralysis. Full recovery of the symptoms occurred three days after the patient's admission.

Results: The somatosensory evoked potentials of the median nerve were normal, indicating a functional sensory loss. Magnetic stimulation on admission and after recovery, revealed higher threshold of the left motor cortex for the right arm and leg. CCT and brain MRI were performed and revealed a venous angioma in the left frontal lobe, confirmed by subsequent digital angiography.

Conclusions: An extensive venous angioma in the frontal lobe can be the cause of decreased cortical excitability. The relation of the paralytic episodes to the venous angioma, is quite unclear. Psychogenic factors may have aggravated some minor symptoms.

### P466

**Transcranial magnetic stimulation and functional condition of the blink reflex arch.** A. Remnev, Novosibirsk State Medical Academy (Barnaul, RUS)

We offered a new way of differential diagnostics of the trigeminal nerve (5th cranial nerve) (TN) defeat, facial nerve (7th cranial nerve) (FN) defeat and cerebronuclear ways of facial nerve (CWFN) defeat. This method is that register components of blink reflex (BR) with electrical stimulation (ES) superciliary area and transcranial magnetic stimulation (TMS) in projections a motor cortex of cerebrum. Analyze latency of BR early components (R1) (Russia Patent 1 2125830). TMS is a non-invasive, painless and objective method of diagnostics. All stimulations were performed using a Magstim-200 stimulator (Magstim Company Limited, UK) with 90 mm coil and Sapphire Premiere (Medelec Company Limited, UK).

We examined 85 healthy people (12 children with an age between 10 and 14 years, 38 women with an age between 16 and 46 years, 35 men with an age between 16 and 48 years), 64 patients with Bell's paralysis with an age between 18 and 47 years, 16 patients with central defeat of FN (multiple sclerosis) with an age between 15 and 43 years, 45 patients with various infringements in TN system with an age between 28 and 54 years were surveyed.

The comparison of the characteristics BR, received by ES and TMS allows giving a separate estimation of a functional condition of all three sites of the BR arch: FN, CWFN and TN. So the increase of R1 latency at ES and TMS (differences between healthy people and smitten sides reliable, $P < 0.01$) was registered at the patients with Bell's paralysis (latency accordingly $17.3 \pm 0.4$ ms and $19.4 \pm 0.8$ ms – the data are submitted as $M \pm m$, where M – arithmetic mean; m – standard mistake). At the patients with central defeat of FN – was essentially is increased R1 latency only with TMS ($22.7 \pm 1.2$ ms).

At the patients with TN neuropathy the increase R1 latency was registered only at ES ($19.3 \pm 4.0$ ms).

Thus, the complex applications ES and TMS at the patients with defeats of a trigeminal-facial complex allow objectively distinguish the defeat FN and TN at different levels.

### P467

**Evidence of motor dysfunction in mild cognitive impairment and mild-moderate Alzheimer's disease.** A. Martins da Silva, E. Schiatti, L. Leocani, G. Magnani, M. Natali Sora, R. Mossini, E. Altamura, G. Comi, Scientific Institute Hospital San Raffaele (Milan, I)

Objective: To evaluate motor function in mild cognitive impairment (MCI) and in mild-moderate Alzheimers disease.

Background: Motor dysfunction is considered to occur at a relatively late stage of AD, even though impairment of fine motor skills, such as finger tapping, has been reported at earlier stages. The involvement of motor function in MCI has yet to be clarified.

Design/Methods: Twelve patients with MCI (7 females, mean age $68.6 \pm 10$), 24 patients with AD (16 females, age $70 \pm 9$) mild-moderate (MMSE > 14) and 12 normal control subjects participated in the study. Patients had no motor symptoms or signs at neurological examination. All patients and subjects were right handed according to the Edinburgh scale. Finger tapping on a mouse button was performed 3 times for each side, and the frequency of motor tapping and the right/left ratio were measured using a computerized system.

Results: Compared to control subjects, both patient groups showed significantly slower finger tapping ($p < 0.02$) for both sides (normal subjects: $4.7 \pm 0.8$ right, $3.8 \pm 0.6$ left; AD: $3.1 \pm 1.0$ right, $3.0 \pm 1.0$ left, MCI: $3.0 \pm 0.67$ right, $2.8 \pm 0.5$ left). Moreover, both groups showed a reduced right/left dominance ($p < 0.008$) compared to the normal group (normal: $1.2 \pm 0.07$, AD $1.06 \pm 0.2$, MCI $1.09 \pm 0.12$).

Conclusions: The finding of a reduced frequency of motor tapping in our patients with mild-moderate AD confirms the presence of subclinical motor impairment in relatively early stages of AD. The finding of similar abnormalities in MCI patients suggests that involvement of the motor system may be present even before a diagnosis of AD is made and suggests the need for a larger follow-up study in order to assess whether the presence of motor involvement may have a predictive value for the future development of AD.

### P468

**Nerve conduction studies in chronic venous insufficiency of lower extremities.** E. Gozke, D. Celebi, S. Biber, T. Baltacoglu, M. Cetinkaya, PTT Teaching Hospital (Istanbul, TR)

Objective: In this study, performance of nerve conduction studies (NCS) was designed in order to evaluate nerve damage in cases with chronic venous insufficiency (CVI).

Methods: Findings of NCS performed on 65 legs in 35 cases (18 female, 17 male) with CVI were compared with those of 30 legs of 30 healthy persons (16 female, 14 male).

Results: In cases with CVI, a significant decrease in peroneal nerve compound muscle action potential (CMAP) amplitude and deceleration in tibial nerve motor conduction velocity were detected. Loss of sural nerve sensory nerve action potential (SNAP) was observed to be highly significant in cases with CVI. When intragroup comparisons were performed among patients, as the degree of reflux detected on venous Doppler ultrasonography increased, a decrease in peroneal nerve CMAP amplitude and a significant prolongation in distal motor latency were noticed.

Conclusion: The results indicate that in the diagnosis of nerve damage due to CVI of lower extremities NCS are helpful.

**P469**

**Neurophysiological changes in patients with chronic obstructive pulmonary disease.** H. Ulvi, T. Yoldas, I. Ekici, R. Yigiter, T. Tug, S. Terzi, B. Müngen, Firat University (Elazig, TR)

Purpose: Peripheral neuropathy commonly occurs in patients with chronic obstructive lung disease (COPD). The aim of our study was to investigate the possible effects of COPD on the peripheral nervous system and characteristics of neuropathy with prospective clinical and electrophysiological study, and its correlation with the degree of hypoxemia.

Methods and Results: We enrolled 31 patients, mean age 66.12, with COPD into the study. Arterial oxygen tension ($PaO_2$) $\geq 65$ mmHg was considered as the cut-off value designating tissue hypoxia. According to this cut-off value, the subjects were divided into two groups: Group I, $PaO_2 < 65$ mmHg and Group II, $PaO_2 \geq 65$ mmHg. Patients with other causes of polyneuropathy were excluded from the study. Nerve conduction parameters were studied in all the subjects. Abnormalities of sensory nerve conduction were most common, affecting the sural nerve (29 subjects), median nerve [28], ulnar nerve [26] and motor nerve peroneal (seven), median nerve (two), tibial and ulnar nerve (one). In Group I, severity of neuropathy was correlated with the degree of hypoxemia, but no correlation was observed in Group II.

Conclusion: These findings indicate that peripheral polyneuropathy, particularly sensorial, commonly occurs in association with COPD and that this COPD-related neuropathy is correlated with the degree of hypoxemia. From these data we suggest that electrophysiological studies may be useful in assessing the peripheral neuropathy in patients with COPD.

Key words: chronic obstructive lung disease, peripheric neuropathy, electrophysiological study.

**P470**

**Usefulness of the blink reflex in leprosy patients with neuropathy.** H. Ulvi, R. Yigiter, T. Yoldas, B. Müngen, Firat University (Elazig, TR)

Objectives: Damage of the peripheral nervous system is particularly frequent in leprosy patients. Trigeminal and facial nerves are among the most commonly affected. Electrophysiological studies such as the blink reflex was shown to be an effective method for revealing subclinical involvement of cranial nerves in generalised neuropathies. The aim of our study was to evaluate the efficacy of blink reflex as a method in early diagnosis of cranial nerve involvement in leprosy patients.

Material and methods: We studied 37 leprosy patients (mean age: $38 \pm 17$ years (yrs), range 23 yrs to 62 yrs; 20 female and 17 male) and 35 age-matched healthy subjects (mean age: $34.19 \pm 12.74$ yrs, range 24 yrs to 48 yrs; 20 female and 15 male). Stimulation was made with the cathode over the supraorbital foramen and evoked response recorded from both orbiculares oculi muscles. Blink reflexes were obtained after unilateral electric stimulation of the supraorbital nerve for quantitative analysis of 3 responses, early ipsilateral phasic component (R1), late ipsilateral tonic component (R2i) and late contralateral tonic component (R2c). Nerve conduction parameters were studied in all subjects.

Results: The latencies of both the ipsilateral early phasic component (R1) and bilateral late tonic components (R2i and R2c) in leprosy patients (mean R1 latency in patients, $12.68 \pm 3,43$ on left eye, $13,01 \pm 2,07$ on right eye, vs. controls, $11,28 \pm 2,32$ on left eye, $11,14 \pm 2,18$ on right eye [$p < 0.05$]; mean R2i latency in patients, $37,4 \pm 6,55$ on left eye, $38,25 \pm 4,72$ on right eye, vs. controls, $32,51 \pm 6,65$ on left eye, $30,23 \pm 3,6$ on right eye [$p < 0.05$]; mean R2c latency in patients, $38,01 \pm 9,76$ on left eye, $39,76 \pm 3,48$ on right eye, vs. controls, $32,91 \pm 4,75$ on left eye, $31,67 \pm 5,9$ on right eye [$p < 0.05$]) were significantly prolonged compared with the controls. Out of 37 leprosy patients, 22 (59 %) showed abnormalities R1 latency, 28 (75 %) R2i latency and 31 (83 %) R2c latency. No correlation was observed between prolonged latencies and duration of the disease.

Conclusion: We conclude that blink reflex testing which can be easily and rapidly performed in an EMG laboratory using standard equipment can provide useful, objective information for obtaining early diagnosis and in determination of the degree of cranial nerve lesions.

**P471**

**Central motor conduction after magnetic stimulation in diabetic patients.** G. Kiziltan, M. Erdemir Kiziltan, I. U.Cerrahpasa Medical School (Istanbul, TR)

Aim: Investigate the conduction time in central nervous system, especially in medulla spinalis, in diabetic patients.

Methods: Our study was done with a patient group of 37 people who were at or below the age of 63 and who were diagnosed with either DM Type 1 or DM Type 2 and with a control group of 17 people who were all healthy subjects. In both control and diabetic groups study parameters were motor conduction times between cranial - abductor pollicis brevis, cranial- anterior tibial, C7-abductor pollicis brevis, L4-anterior tibial which were deducted by transmagnetic stimulation of cortical, C7-L4 radix stimulation. Conduction time in medulla spinalis between C7-L4 was evaluated by subtracting the conduction time between cortex-C7 from the conduction time between cortex (CR)-L4. In this study the findings were compared with each other with 'Student's t', 'Mann-Whitney', 'chi-square' and 'ANOVA' tests.

Results: In our study, in diabetics conduction time between C7- L4 in medulla spinalis was found to be significantly longer than the conduction time in healthy subjects. On the contrary conduction times between CR-C7, CR-L4 were found to be not different than the conduction times in healthy subjects. Delay detected in conduction time in medulla spinalis between C7-L4 in diabetic patients was found to be independent of patient's age, duration of diabetes and total neuropathy score. Though the delay in conduction time in medulla spinalis was found to be independent of total neuropathy score, CR-L4, C7-L4 latencies in people with no sural response were longer than the latencies in people with sural nerve response. In addition to these, CR-L4, C7-L4 latencies were longer in subjects with slow fibular nerve conduction velocities than in subjects with normal conduction velocities.

Conclusions: In the guidance of these results, the delay in motor conduction time in medulla spinalis was tried to be explained by the mechanisms causing peripheral neuropathy. When yascular-metabolic mechanism is considered, central nervous system is believed to be less affected by metabolic changes than peripheral nervous system because the permeability of blood-brain barrier is less than the permeability of blood-neuron barrier. For this reason, we thought that, though within the duration of disease peripheric nervous system involvement draws more attention, central motor pathways, especially demyelinization and degeneration of thick axons in distal corticospinal tract which is within the boundaries of medulla spinalis which cause conduction delay, decrease in supraspinal excitatory stimuli extending to medulla spinalis, dysfunction in the potential which evolves from the addition of excitatory stimuli in the anterior horn cells because of temporal dispersion, the carrying of stimuli by small, myelinated fibers in which the conduction is slow and the usage of polysynaptic pathways were all resposible for our findings.

**P472**

**Visual evoked potentials in patients with diabetic neuropathy.** H. Ulvi, T. Yoldas, R. Yigiter, Y. Özkan, B. Müngen, Firat University (Elazig, TR)

Purpose: several studies have shown that diabetes mellitus affects almost all systems and brings about abnormalities in functions of central nervous system (CNS). The aim of our study was to investigate the possible effects of diabetes mellitus on the central optic pathways by means of pattern shift visual evoked potentials (VEP) and its correlation with abnormalities of sensorimotor nerve conduction.

Methods and Results: We studied 31 patients with diabetic neuropathy; the mean age was $56.03 \pm 11.23$ years (yrs) (range 29 yrs to 75 yrs; 18 female and 13 male) and thirtytwo age-matched healthy subjects (control); the mean age was $49.76 \pm 13.11$ years (yrs) (range 27 yrs to 64 yrs; 35 female and 24 male) were also included in the study. Patients with diabetic retinopathy, glaucoma and cataract were excluded from the study. Mean P100 latency and N2-N1 interpeak latency in patients with diabetic neuropathy were significantly longer than those of the normal subjects ($p < 0.05$). No correlation was observed between abnormalities of VEP parameters and abnormalities of sensorimotor nerve conductions. These data indicate that patients with diabetic neuropathy have abnormal CNS function.

Conclusion: We conclude that VEP measurement which can easily be performed in the electromyography laboratory, is a helpful and sensitive method to evaluate involvement of central optic pathways in diabetic neuropathy.

## Dementia/Higher function disorders

P473

**Retroactive interference in verbal tasks under different conditions: an experiment with MCI patients.** N. Beschin, N. Cowan, S. Della Sala, M. Perini, Azienda Ospedaliera, University of Missouri, University of Aberdeen (Gallarate, I; Columbia, USA; Aberdeen, UK)

Introduction: Mild Cognitive Impairment (MCI) is characterized by anterograde amnesia in the absence of overt dementia. Patients with anterograde amnesia typically can recollect an event immediately afterward but forget it within about 1 min. The rate of forgetting and the retrieval deficit are enhanced by proactive interference. Little is known about the role of retroactive interference in amnesia. If forgetting were inevitable, it would allow the possibility that the event has not been encoded into the memory system used for conscious recollection. In contrast, if memory could be retrieved under conditions of reduced retroactive interference, this would indicate that the event has been encoded in the memory system in a weakened form. Patients with MCI are an ideal sample to test these alternative hypotheses.

Material and Methods: A group of patients with MCI and a group of matched healthy controls were presented with stories orally, asking for immediate verbatim recall, and then for delayed recall an hour later. The delay was either filled with other tasks to create a standard amount of interference or followed by period of minimal interference in which the participant reclined in a dark, quiet room.

Results: Average proportion of immediate recall were similar across the two groups. In delay recall, healthy individuals showed 80% savings with the usual interference and 89% savings with minimal interference. The MCI patients showed a marked difference, from 20% savings with the usual interference up to 55% savings with minimal interference. The percent saving was significantly larger for the minimal-interference condition than for the usual-interference condition in both groups.

Conclusions: The results indicate that one of the deficits underlying anterograde amnesia in MCI is increased vulnerability to retroactive interference, implying that either short-term memory has no strict time limit, or some amnesiacs have little or no long-term memory deficit if tested under very low interference conditions. Moreover, since patients with Alzheimer Disease perform at floor in delay retrieval of prose passages, this procedure could add to differential diagnosis between MCI and AD.

P474

**Memory loss in the elderly and its correction.** S. A. Shapovalova, V. N. Grigorieva, Gerontology Center, Medical Academy (Nizhny Novgorod, RUS)

Memory loss in the elderly (60 years and older) is often caused by chronic cerebrovascular insufficiency. The aim of our study was to research possibilities of correction of memory loss by different training programs.

Material and methods: We studied 98 elderly patients with chronic cerebrovascular insufficiency before memory training, after four weeks of in-hospital course of memory training and after 1 year of independent training according to our guidelines. Memory was evaluated according to memory tests by A. R. Luria (immediate and delayed reproduction of a list of words and a short story, of 5 figures and a subject picture). Different training programs were elaborated according to initial memory impairments. Memory training was divided into 3 stages: 1) preparatory stage; 2) learning mnemonics (imagery, image interaction and Method of Loci) and 3) adaptation of memory skills to everyday life.

Results: All patients were divided into two groups. The first group (56 patients) got both medicaments and memory training during their 4-week hospital stay. The second (control) group (42 patients) got medical treatment only. After four weeks (discharge from the hospital) most indicators of visual and speech memory in the first group had improved in 65% of cases of mild memory loss and 79% of moderate memory loss. These effects were long-term: almost half of the patients with mild and moderate memory loss demonstrated memory improvements after 1 year. No improvements were seen in the relevant control groups. Moreover, delayed reproduction of short stories and subject pictures has reliably deteriorated in cases of mild memory loss. As for the patients with severe memory loss, memory training was not effective in both groups.

Conclusion: Differentiated memory training programs help to improve memory performance in elderly patients with chronic cerebrovascular insufficiency in case of mild and moderate memory loss.

P475

**Cognition is physicians' main indicator of treatment efficacy in Alzheimer's disease patients.** P. Johannsen, H. Hampel, S. Hasselbalch, G. Jakab, I. Kloszewska, J. McCarthy, P. Sakka, E. Triau, C. Wouters, S. Qvitzau, Y. Xu, E. Schwam, S. Richardson, R. Schindler, Aarhus University Hospital, Ludwig-Maximilian University, Uzsoki Street Hospital of Budapest Municipality, Medical University of Lodz, N. I. M. T.S Hospital, Neurologie Centrum Leuven, Jeroen Bosch Ziekenhuis, Pfizer Denmark, Pfizer Inc, Eisai Inc (Aarhus, DK; Munich, D; Copenhagen, DK; Budapest, HUN; Lodz, PL; South Yarmouth, USA; Athens, GR; Leuven, B; Den Bosch; Ballerup, DK; New York; Teaneck, USA)

Objective: To examine the domains influencing physicians' judgment of clinical benefit in donepezil-treated Alzheimer's disease (AD) patients, during the pre-randomization phase of the donepezil AWARE (Aricept WAshout and REchallenge) study.

Background: AD patients who do not exhibit improvement are often discontinued from cholinesterase inhibitor therapy. However, although therapeutic benefits may be apparent in several domains, such as cognition and behavior, there is currently no standard clinical approach to determine treatment success in AD patients.

Design: AWARE consists of 3 phases: 1) 24-week, pre-randomization, open-label donepezil treatment phase; 2) 12-week, randomized, double-blind, placebo-controlled phase; 3) 12-week, single-blind donepezil treatment phase. Results from the pre-randomization (first) phase are presented.

Methods: Patients with mild to moderate, possible or probable AD were enrolled in the pre-randomization phase. All patients received donepezil 5 mg/day for 28 days, then 10 mg/day. Clinical benefit was assessed at Weeks 12, 18, and 24. Patients who exhibited decline or no change from baseline on the Mini-Mental State Examination (MMSE) and whose physician was not sufficiently certain of clinical benefit to warrant continued treatment (assessed by formal questionnaire) were rated as showing "no apparent clinical benefit". Physicians indicated which domains (cognition, function [activities of daily living, ADL], behavior, caregiver request, other) most influenced their decision. Patients showing "no apparent clinical benefit" were randomized to receive either donepezil or placebo in the second, double-blind phase of AWARE.

Results: Clinical benefit was classified in 619 of the 817 enrolled patients. The majority (70%) of physicians' decisions were based upon cognition, whereas other domains were less influential (behavior, 14% of decisions; ADL, 10%; caregiver request, 5%; other, 1%). 426 patients showed "observed clinical benefit" (MMSE mean change from baseline, 2.4), and physicians were sufficiently certain of benefit to warrant continued therapy in 400 of these.

Conclusion: Cognition was the most influential domain in the majority of physicians' decisions regarding clinical benefit. There was strong agreement between physician global rating of the patient and the MMSE, supporting the observation that cognition was the main indicator used to assess clinical benefit.

P475a

**Donepezil-treated Alzheimer's disease patients with apparent initial cognitive decline demonstrate significant benefits when therapy is continued: Results from a randomized, placebo-controlled trial.** P. Johannsen, M. Barcikowska, R. Heun, R. Holub, S. Jakobsen, E. Triau, M. Trixler, V. Vagenas, F. Verhey, L. Bergendorff, Y. Xu, N. Kumar, S. Richardson (Aarhus, DK; Warsaw, PL; Bonn, D; Albany, USA; Rudkøbing, DK; Leuven, B; Pecs, HUN; Athens, GR; Maastricht, NL; Ballerup, DK; New York, USA)

Objective: The aim of the donepezil AWARE (Aricept WAshout and REchallenge) study is to determine the nature and extent to which Alzheimer's disease (AD) patients show "no apparent clinical benefit" during 12–24 weeks of initial donepezil therapy benefit from continued treatment.

Background: AD patients who do not exhibit improvement are often discontinued from cholinesterase inhibitor therapy. However, due to the nature of AD, these patients may still experience therapeutic benefits. Since current methods of rating clinical benefit may be biased towards cognition, benefits in other domains might not be identified.

Design: AWARE consists of 3 phases: 1) 24-week, pre-randomization, open-label donepezil treatment phase; 2) 12-week, randomized, double-blind, placebo-controlled phase; 3) 12-week, single-blind donepezil treatment phase.

Methods: Patients with mild to moderate AD received open-label donepezil (10 mg/day) for 12–24 weeks. Clinical benefit was assessed at Weeks 12, 18, and 24; at each time point, patients rated as showing "no apparent clinical benefit" were randomized into the double-blind phase. Patients classified as showing "no apparent clinical benefit" were those who

exhibited decline or no change from baseline on the MMSE and whose physician was not sufficiently certain of clinical benefit to warrant continued treatment (assessed by formal questionnaire). Efficacy assessments included the ADAS-cog, MMSE, and NPI. Results are reported for the double-blind phase as least-squares mean change from baseline for Week 12 intent-to-treat observed cases.

Results: 202 of the 817 enrolled patients entered the double-blind phase and were randomized to continue on donepezil 10 mg/day (n = 99) or switch to placebo (n = 103). Significant differences in favor of the donepezil group compared to placebo were observed (MMSE treatment difference -1.13, P = 0.02; NPI treatment difference 3.16, P = 0.02). Donepezil-treated patients also showed less decline than placebo-treated patients on the ADAS-cog (treatment difference 0.57, P = NS).

Conclusion: Patients initially classified as showing "no apparent clinical benefit" who continued on donepezil for a further 12 weeks demonstrated cognitive and behavioral benefits, compared with those who switched to placebo. Physicians should therefore be cautious when deciding whether to discontinue donepezil treatment, as patients may still experience significant treatment benefits.

**P476**

**Caregiver satisfaction with galantamine treatment for patients with Alzheimer's disease: a large-scale German observational study.** S. Schwalen, H. G. Nehen, Janssen-Cilag GmBH, Elisabeth Hospital (Neuss, Essen, D)

Introduction: Caregivers of people with Alzheimer's disease (AD) suffer a physical and psychological burden. In placebo-controlled trials, galantamine has been shown to reduce caregiver time by about 1 hour per day. This observational study was designed to investigate the burden for caregivers of galantamine-treated AD patients in a naturalistic setting.

Method: Patients with AD who had just started galantamine treatment were enrolled by German neurologists who observed them for 3 months and gave a clinical global impression (CGI) of treatment. Views of non-professional carers were assessed using a validated instrument (Häusliche Pflegeskala) with 28 questions answered on a 4-step categorical scale. The total score ranges from 28 to 112: a lower score represents a higher burden. Carers also reported effects on their own well-being, ability to perform normal activities and to have social contacts and their time burden using 5-step categorical scales. An intent-to-treat analysis was performed using the last observation carried forward method for missing data.

Results: Data were collected from 1260 patients ranging in age from 45 to 100 (mean 75) years. There were more women than men (59%). Most lived with their family (73%), 11% were in nursing homes, 16% lived alone. The majority had moderate AD by ICD-10 criteria (mild 28%, moderate 60%, severe 12%). The median daily dose of galantamine at last visit was 16 mg.

Physicians described the CGI of change as very much or much improved in 41.3% of patients, slightly improved in 35.1%, unchanged in 17.1% and deteriorated in only 6% of patients.

Caregiver burden improved significantly from a moderate level at baseline (mean score 76) to the last visit ($p < 0.001$). Overall, 58% of caregivers rated their burden as improved, 13% as unchanged. On average, caregivers rated their well-being, ability to perform activities of daily life and to have social contacts moderately disturbed at baseline. They also rated their time burden as moderate. All aspects improved significantly by the study end ($p < 0.001$) when caregivers rated them, on average, as not disturbed or mildly disturbed.

Conclusions: The use of galantamine not only improves the lives of patients but also reduces caregiver burden to a meaningful (and statistically significant) extent. This may enable more people to be cared for at home, since caregiver burden is an important factor in determining the time of transition to a nursing home.

**P477**

**Proton magnetic resonance spectroscopy in Alzheimer's disease: differences between 1.5T and 3T.** J. Alvarez-Linera, A. Frank, J. Escribano, A. Sanz, A. Tallón, P. Barreiro, E. Diez-Tejedor, Hospital Ruber Internacional, University Hospital La Paz (Madrid, E)

Background: Diagnosis of probable Alzheimer's disease (AD) is only made when clinical manifestations are already clearly evident. So, it is really important to find tools which lead to an early diagnosis allowing to begin the treatment as soon as possible. Proton magnetic resonance spectroscopy (1H-MRS) is a non-invasive method to investigate changes in brain metabolite composition in different cerebral diseases.

Objective: To evaluate the cerebral white biochemical pattern using proton magnetic resonance spectroscopy (1H-MRS) in aged people and in patients with AD.

Patients and method: Medical and neurological examinations and neuropsychological measures (MMSE, CDR, BDRS, GDS) were used to evaluate physical status and cognitive performance in 22 aged subjects who accepted to participate in the present study. Eleven patients met NINCDS-ADRDA criteria for probable AD and other eleven healthy subjects constituted the control group.

To compare the peak patterns between the two MRS units (1.5T and 3T), a Single Voxel PRESS sequence with TR 2,000 ms and TE 35 ms was used. Volumes of interest (VOI) were selected in the parietal medial cortex. N-acetyl-aspartate (NAA), myoinositol, choline, and creatine resonance signals in an 8-$cm^3$ voxel located in. NAA/creatine, myoinositol/creatine, and choline/creatine ratios were measured, and the mean values were compared.

Results: There are differences between 1.5T and 3T in both groups: AD (average: 0.78 in 1.5T and 0.54 in 3T) and controls (average: 0.6 in 1.5Tand 0.4 in 3T) in mI/Cr ratio, that always is smaller in 3T. There are significant differences between controls and AD with both techniques, although the differences are more pronounced in 1.5T. Although there are differences between 1.5T and 3T in NAA/Cr ratio, they are not significant, and in both they are greater in controls than in AD.

Conclusions: With both techniques it is possible to differentiate between AD and controls, but new specific values for 3T are required.

**P478**

**Long-term changes in the symptoms of dementia in patients with and without cerebrovascular or parkinsonian co-factors treated with donepezil.** J. Kohler, R. Horn, K. Jendroska, M. Riepe, J. Moebius, H. Hampel, University Clinic Charité, University of Ulm, Eisai GmbH, Ludwig Maximilian University (Emmendingen, Bad Honnef, Berlin, Ulm, Frankfurt, Munich, D)

Objectives: Donepezil, a selective reversible acetylcholinesterase inhibitor, is approved for the symptomatic treatment of mild to moderately severe Alzheimer's disease (AD) in over 50 countries. Due to the progressive nature of the disease, as well as the high rate of comorbidity, treatment data from different patient groups should be evaluated to obtain greater insight into the expected response. The treatment response in 3 subgroups was compared after 2 different observation periods: 3 months versus 6 months after initiation of donepezil therapy.

Methods: 2029 Alzheimer patients were enrolled in a multicentre post-marketing surveillance (PMS) study in Germany. 421 patients with concomitant cerebrovascular disease (CVD+), 122 patients with additional parkinsonian symptoms (PS+) and 1188 patients without either of these co-factors (CVD–/PS–) were evaluated in the efficacy analysis. The influence of donepezil on dementia symptoms was categorized using the 7-item Clinical Global Impression scale (CGI). Safety data were also evaluated.

Results: 71.9% of CVD–/PS– patients showed a global improvement after 3 months of donepezil therapy, and 76.7% showed an improvement after 6 months. In the CVD+ group, 69.3% of patients were judged "improved" after 3 months, and 74.9% after 6 months. 70.5% of PS+ patients were judged "improved" after 3 months and 78.2% after 6 months. Interestingly, more patients showed an improvement categorized as "markedly to very much improved" between 3 and 6 months than from baseline to 3 months, especially in the PS+ group. Donepezil was very well tolerated. AEs were reported in only 236 of 2029 patients (11.6%). Extrapyramidal symptoms in the PS+ patients were not exacerbated by donepezil therapy.

Conclusions: Approximately 3 out of 4 patients showed a global improvement after receiving donepezil therapy, irrespective of cerebrovascular and parkinsonian co-factors. In addition to the main treatment effect observed after 3 months, a trend towards further improvement was observed between 3 and 6 months, suggesting an increase of therapeutic effect within the individual patient during this time. Based on these data, an observation period of at least 6 months of donepezil therapy is recommended before judging treatment response.

**P479**

**Health benefits gained with donepezil in patients with moderate to severe Alzheimer's disease translate into cost savings.** H. Feldman, S. Gauthier, J. Hecker, B. Vellas, M. Hux, Y. Xu, E. Schwam, S. Shah, University of British Columbia, McGill Centre for Studies in Aging, Repatriation General Hospital, Toulouse University Alzheimer's Center, Innovus Research Inc, Pfizer Inc (Vancouver, Verdun, CAN; Daw Park, AUS; Toulouse, F; Burlington, CAN; New York, USA)

Background: Donepezil treatment has been shown to be cost effective in mild to moderate Alzheimer's disease (AD). Since AD costs escalate with

disease progression, it is important to study the economic impact of donepezil in more advanced AD.

Objective: This cost-consequence analysis investigated the AD-related costs to society in a double-blind trial of donepezil in patients with moderate to severe AD.

Methods: A total of 289 patients with AD (Mini-Mental State Examination score 5–17) were randomized to receive donepezil (n = 143) or placebo (n = 146) for 24 weeks. AD cost components included patient and caregiver health resource utilization, collected via the Canadian Utilization of Services Tracking (CAUST) questionnaire, and unpaid caregiver time aiding basic and instrumental activities of daily living (ADL). Health resource utilization for 3 months prior to the study was collected so that cost could be used as a covariate in the comparison of treatment costs between groups. Caregiver time, recorded using modified versions of the Physicians' Self Maintenance Scale (PSMS) and Instrumental ADL (IADL) Scale, was valued using the average 1988 Ontario minimum wage.

Results: Patient and caregiver demographics at baseline were similar between treatment groups. Clinical outcome measures of patient global function, cognition, ADL and behavior all showed significant benefits in favor of donepezil compared with placebo. Caregivers of patients receiving donepezil spent less time helping with ADL than caregivers of placebo patients. After adjusting for baseline, mean total societal cost per patient (inclusive of donepezil's unit price), for the 24-week period was Can$9904 (US$6686) for the donepezil group and Can$10,236 (US$6910) for the placebo group. This represented a per-patient cost saving of Can$332 (US$224). Patient health costs were Can$4355 (US$2940) and Can$4321 (US$2917), caregiver health costs were Can$167 (US$113) and Can$136 (US$92), and caregiver time costs were Can$5382 (US$3633) and Can$5779 (US$3901), respectively. Most of the cost saving was due to savings in residential care costs, which were Can$1211 (US$818) for the donepezil group and Can$1806 (US$1219) for the placebo group.

Conclusion: This analysis of resource use in moderate to severe AD patients showed a cost saving of Can$332 (US$224) per patient after 24 weeks of donepezil treatment. This may be due to donepezil's significant clinical benefits on global function, cognition, ADL and behavior.

**P480**

**Tricyclic antidepressants-induced dementia.** L. Curatola, C. Paci, T. Carboni, R. Gobbato, S. Sanguigni, U. O. Neurologia (San Benedetto del Tronto, I)

The principal mental diseases of elderly people are mood disorders and dementia. Recent studies have indicated a risk of learning and memory impairment when patients are treated with antimuscarinic drugs.

In our laboratory we studied 750 patients affected by Dementia for 3 years. 450 were affected by probable Alzheimer's disease (NINCDS-ADRDA), 200 by probable Vascular Dementia (NINDS-AIREN) and 100 by other types of dementia. 20 patients (13 males and 7 females, mean age 78 ± 3) were treated for 20 ± 5 years with tricyclic antidepressants (TCAs). Their caregivers referred that patients presented from 3 ± 1 years cognitive decline, confusion, associated to occasional conditions of delirium.

We utilized ADAS-Cog to evaluate cognitive decline. The severity of dementia was rated by the use of Global Deterioration Scale. Neuropsychological tests showed moderate cognitive impairment in 12 patients (ADAS-Cog mean score 33.8 ± 4.1) and severe cognitive decline in others (ADAS-Cog mean score 48.7 ± 6.3). We started to reduce TCAs gradually within 30 days. After one month patients presented an improvement of cognitive disease. ADAS-Cog mean score was 25.3 ± 2.7 in patients with moderate dementia and 35.3 ± 5.4 in a severe one.

Our patients were affected by tricyclic antidepressants-induced dementia and the withdrawal of these drugs caused a significant improvement. We think that the presence of TCAs in the therapy of demented patients should be carefully investigated.

**P481**

**Hemineglect and right anterior choroidal stroke.** J. L. Renard, D. Lamargue, H. Taillia, T. de Greslan, F. Flocard, D. Bequet, HIA Val-de-Grâce (Paris, F)

Cambier (1983) has demonstrated that the interruption of connections between thalamus and retrorolandic cortex, by a damage of the posterior limb and retrolenticular segment of internal capsule, in relation to an anterior choroidal stroke, could cause a neuropsychological thalamic-like syndrom. We report the neuropsychological study and the imagery of a right anterior choroidal stroke with a left hemineglect.

A 66 year-old, right-handed man had a left proportional hemiplegia and hemianesthesia, a left homonymous hemianopia, a minor hemisphere syndrome with visual hemineglect. Brain MRI showed a right anterior choroidal stroke (posterior limb, retrolenticular segment of internal capsule; hippocampus; a part of internal globus pallidus, pulvinar and right cerebral peduncle). MRangiography found an occlusion of the right internal carotid artery, a circle of Willis on the one hand forwards functional, on the other hand backwards unfunctional. 99m Tc SPECT revealed the deefferented right retrorolandic cortex.

Neuropsychological signs were severe left visual hemineglect with right deviation of eyes and head, constructional apraxia, disturbance of visual memory, motor impersistence and anosognosia. The left hemineglect was obvious during targets crossing, bisection of segments, description of complex pictures, spontaneous or copy drawing, spontaneous, dictated or copy writing, reading of syllables (more than two letters), words or texts with difficulties to change line. This cognitive syndrome was stable six months after.

This patient has presented a total syndrome of right anterior choroidal artery which associates controlateral hemiplegia, hemianesthesia, homonymous hemianopia (Foix, Chavany and al., 1925) and a neuropsychological syndrome with left visual hemineglect, constructional apraxia, alexia due to visuospatial disturbance, motor impersistence, disturbance of visual memory and anosognosia like described by Cambier (1983). This attentional left visual neglect is explained by a disinterest of stimuli coming from the left hemispace and reinforced by a perceptual competition of the two hemispheres with invincible attraction by stimuli coming from the right hemispace. The interruption of right thalamic projections on inferior parietal lobe is the main cause of the attentional system dysfunction. In this particular case, a motivational system dysfunction can be associated in the pathology of this syndrome because of extensive internal temporal injuries.

**P482**

**Reminyl improves cognition and memory within 90 days in Alzheimer patients.** J. Kessler, E. Kalbe, S. Schwalen, Max-Planck Institute, Neurologische Universitätsklinik, Janssen-Cilag GmBH (Cologne, Neuss, D)

Background: Galantamine (Reminyl(R)) is a reversible, competitive, tertiary alkaloid acetylcholinesterase inhibitor which also modulates nicotinic receptors. It has been shown to significantly improve cognition and memory in patients with neurodegenerative disease. In this study we assessed the effects of galantamine to see if it can improve test scores of the DemTect(R), a recently developed cognitive screening instrument, in patients with probable Alzheimer's disease (AD).

Patients and methods: 971 patients with probable AD (mean age 74.6 years, SD 8.41; 434 men and 537 women) were tested with the DemTect(R) before and after 90 days treatment with Reminyl in an open label study. Participants were enrolled by neurologists in private practice in Germany. The DemTect(R), a new screening instrument, consists of 5 subtests: word list learning, number transcoding, digit span reverse, supermarket task, and delayed recall of the word list. It assesses the following cognitive domains: verbal short and long term memory, number processing, language, working memory, cognitive flexibility and speed of information processing. Changes in attention were assessed with a rating scale. Mean DemTect(R) scores before and after therapy were compared using the Wilcoxon (two-tailed) test.

Results: After 90 days treatment with galantamine, the patients with probable AD improved significantly in all 5 subtests of the DemTect(R). The improvements were: word list learning (max. 20) 7.69 to 8.85; number transcoding (max. 4) 1.84 to 2.17; supermarket task (no max.) 11.09 to 12.46; digit span reverse (max. 7) 2.96 to 3.23; delayed recall of the word list (max. 10) 2.48 to 3.33. The overall (raw) score improved from 26.07 (SD ± 9.85) to 30.03 (11.48), the transformed score (max. 18) changed from 7.68 (3.86) to 9.43 (4.49). Improvement in attention was highly correlated with the improvement in the sum score of the DemTect(R) (r = 0.5).

Conclusion: After 90 days galantamine treatment, these patients with mild to moderate probable AD showed clear improvements in several cognitive and memory domains and DemTect(R) was able to document this change over time.

**P483**

**Phospholipase A2 platelet activity in patients with Alzheimer's disease and acute stroke.** E. Krzystanek, G. Opala, H. I. Trzeciak, S. Ochudlo, J. Siuda, A. Gorzkowska, M. Arkuszewski, B. Jasinska-Myga, M. Swiat, Silesian Medical University (Katowice, PL)

Introduction: Phospholipase A2 (E. C.3.1.1.4., PLA2) is a key enzyme responsible for membrane phospholipid turnover. The products of PLA2 action (particularly arachidonic acid) are important factors in signal trans-

duction [2]. There are some findings that PLA2 participates in central nervous system pathology (e. g. multiple sclerosis, epilepsy, Alzheimer's disease) [3]. For many years human platelets have been used as a peripheral model of neurons, because of their membrane features, many receptors and ability to produce amyloidogenic peptides [5]. Thus far there have been no findings about PLA2 activity in acute vascular CNS pathology. Results of the studies in platelets of the patients with Alzheimer's disease and acute vascular process have not been compared.

Purpose of study: Our purpose was to compare PLA2 activity in platelets of patients with Alzheimer's disease and acute ischaemic stroke.

Material and method: Blood samples from 57 patients; 28 patients with Alzheimer's disease (20 females and 8 males, mean age: 71.3 years) and 27 patients (17 females and 10 males, mean age: 73.8 years) with partial anterior circulation infarct were examined. Diagnoses were made using ICD-10 criteria.

Human platelets were sonificated, then PLA2 activity was measured performed according to Jelsema [1] as well as Strosznajder and Strosznajder [4] with slight modifications. As a substrate for PLA2 – catalysed reaction 1-stearoyl-2- [1–14C]arachidonyl-L-phosphatidylinositol (spec.act. 20–50 mCi/mmol) was used. Samples radioactivity was assessed in Beckman LS 6000 IC scintillation counter. PLA2 activity was expressed as nmol of [14C]arachidonate release/min/mg protein. The statistical analysis of results was performed with Student t-test.

Results: PLA2 activity in platelets of the patients with Alzheimer's disease was 1,34 ± 0,45 (nmol/mg/min, mean ± SD), in the group with ischaemic stroke 0,61 ± 0,25.

Conclusion: Comparing Alzheimer's disease group with acute stroke group, activity of platelet PLA2 is higher in patients with Alzheimer's dementia.

#### References


1. Jelsema L C (1987) Light activation of phospholipase A2 in rod outer segments of bovine retina and its modulation by GTP-binding proteins. J Biol Chem 262:163–168
2. Farooqui AA, Horrocks LA (2001) Plasmalogens: workhorse lipids of membranes in normal and injured neurons and glia. Neuroscientist 7:232–245
3. Farooqui AA, Litski ML, Farooqui T, Horrocks LA (1999) Inhibitors of intracellular phospholipase A2 activity: Their neurochemical effects and therapeutical importance for neurological disorders. Brain Res Bull 49:139–153
4. Strosznajder J, Strosznajder RP (1989) Guanine nucleotides and fluoride enhance carbachol mediated arachidonic acid release from phosphatidylinositol. J Lipid Med 1:217–229
5. Pletscher A (1986) Blood platelets as neuronal models: use and limitation. Clin Neuropharmacol 9:344–346


## Epilepsy

**P484**

**Rhythmic epileptiform EEG abnormalities and outcome in acute vascular disease.** F. W. Drislane, M. R. Lopez, A. S. Blum, D. L. Schomer, Harvard Medical School, Brown University (Boston, Providence, USA)

Background: Patients with acute vascular disease (ischemic strokes, hemorrhages, intraoperative hypotension, and anoxia) who have EEGs to look for evidence of ongoing seizures or to assess neurologic function are usually seriously ill. Different EEG patterns such as generalized nonconvulsive status epilepticus (SE), periodic lateralized epileptiform discharges (PLEDs), and other periodic patterns not only determine whether there are ongoing seizures but may also help to predict the clinical course.

Methods: We reviewed EEGs and the clinical course of 74 patients with acute vascular disease and periodic or rhythmic epileptiform EEG patterns, including: generalized electrographic SE, focal SE, PLEDs, and periodic generalized epileptiform discharges. We looked for prognostic factors in the etiology, level of consciousness, and type of epileptiform EEG pattern.

Results: 26 patients had anoxic events, and 26 had ischemic strokes (7 intraoperative). Another 12 had hemorrhages, 6 of these subdural hematomas. Others had acute and severe hypotension or earlier strokes but with new medical precipitants leading to a concern for seizures. The strongest predictive factor was the presence of anoxia – almost all of these patients had generalized electrographic SE on the EEG (3 had focal SE); all were comatose and died. Of the 48 patients without anoxia, those with focal epileptiform discharges were somewhat more likely to survive than those with generalized epileptiform patterns (48 % vs. 27 %; N. S.). Level of consciousness was a better predictor. Patients who were not comatose had a 75 % survival, vs. 4 % for those who were comatose ($p < 0.01$).

Conclusion: Patients with acute vascular disease who have EEGs to determine whether or not they are seizing and to assess their level of neurologic dysfunction are a group with severe illness and high mortality. Anoxic patients with any of these epileptiform EEG patterns rarely survive. Of the others, the different causes of illness (hemorrhage, ischemic stroke, or severe hypotension) were not predictive of outcome. PLEDs and focal SE were common on the EEG of patients with vascular disease, but these 2 patterns did not differ in prognostic importance. The patients with generalized discharges probably had more extensive disease and did not do as well, but the level of consciousness was a better predictor of outcome than was the type of EEG abnormality.

**P485**

**Interictal HMPAO brain SPECT in extratemporal epileptic patients: comparison between basal and activated state.** M. Gudín, B. Catalán, R. Ibáñez, A. Hernández, J. Cano, P. de Luis, A. Soriano, M. del Real, J. Vaamonde, Ntra Sra Alarcos Hospital (Ciudad Real, E)

Background: Interictal brain SPECT is associated to hypoperfusion foci in epileptic patients, extratemporal epileptic patients also have hypoperfusion areas. In previous studies, it was found out that temporal epileptic patients decrease temporal perfusion during Interictal Epileptiform Activity (IEA). In order to evaluate the changes in temporal perfusion of extratemporal epileptic patients during interictal EEG activity we selected a group of patients with extratemporal aura: clonic movements in limbs or atypical signs for temporal epilepsy.

Patients: Five epileptic patients (M/F: 2/3), middle age 35.8, were studied by HMPAO SPECT twice. All of our patients had sensorimotor partial complex partial seizures of probable extratemporal onset. The first study was realized when there was no anomaly in EEG (basal study). In the second study (activated study) the radioligand was injected during a period when the EEG showed at least 10 graphoelements (spikes, polispikes, or sharp waves) per EEG page. Mean seizure frequency was 1 per month. Three of them were treated with lamotrigine (LMT), two with carbamazepine (CBZ). All of them had normal MRI.

Method: The patients were studied by SPECT HMPAO realized with a STARCAM 3.200 equipment, the images were obtained by a high resolution collimator with circular orbit 64 images, 30 sc. per image. Thalamus, temporal cortex, cerebellum were measured by a digital method and the measure was expressed in pixel per area. Thalamus, temporal cortex, cerebellum were measured by a digital method.

Results: Temporal perfusion was increased significantly during activated study compared to the basal one; also there was an increase of thalamic and cerebellar perfusion. Mean cortex perfusion was 25,824 pixel per area in the basal study and 39,064 in the activated one.

Conclusions: Extratemporal epileptic patients show an increase of temporal perfusion while they are having Interictal Epileptiform Activity. These findings are opposed to previous studies in temporal epileptic patients, and may help to differentiate temporal from extratemporal epilepsy.

**P486**

**Methodologic validity of patient's history has an important place in establishing the diagnosis of epileptic attacks.** A. Koçer, E. Gözke, N. Ince, E. Koçer, Dr.Lutfi Kirdar Education Hospital, PTT Education Hospital, Istanbul University, Duzce Medical Faculty (Istanbul, Duzce, TR)

History, physician examination and EEG are required for establishing diagnosis of epilepsy. Accurate history taking depends on physician's recognition of clinical picture of epileptic attacks. Sixty eight patients referring to PTT Training and Research Hospital with complaints of blackout or fainting and with initial diagnoses written down were evaluated pro- and retrospectively. For all of the patients, Diagnoses were classified into 3 groups as ES, NES and epileptic seizures associated with non-epileptic attacks (ES, NES). Initial diagnoses were compared. With definitive diagnoses established subsequent to examinations. In consideration of detailed examinations performed (gold standard) for the differential diagnosis of epilepsy, methodological validity (i. e. sensitivity and specificity) of meticulous history taking was assessed. Initial diagnoses of 68 patients were determined as ES (n = 30; 44.1 %), NES (n = 32; 47 %), and ES/NES (n = 6; 8.8 %). Following review of the cases and completion of the follow-up protocols, established diagnoses were ES (n = 29; 42.6 %), NES (n = 29; 42.6 %) and ES/NES (n = 10; 14.8 %). When the patients were classified as epileptiform cases (ES and ES/NES) and others, the sensitivity and specificity of establishing the diagnosis of epilepsy were 0.641 (64.1 %; 95 % [confidence interval] CI: 0.47–0.79) and 0.621 (52.1 %; 95 % CI: 0.42–0.79) respectively.

Accurate diagnosis could be established for 64.1 % of epileptic and 62.1 % nonepileptic forms by means of history taking. In our study total percentage of accurate diagnosis was found to be 0.632 (63.2%, 95% CI: 0.50–0.74). Under normal policlinic conditions (excluding special epilepsy clinics) the rate of establishing accurate diagnosis based on medical history taken without a regular protocol was found to be 63.2 percent. Since in a domestic source surveillance study, a similar methodological study was not utilized, the values relating to sensitivity and selectivity could not be compared.

**P487**

**No significant association of anti-Gm1 and anti-Gad antibodies with juvenile myoclonic epilepsy.** E. Aykutlu, B. Baykan, C. Gurses, A. Gokyigit, G. Saruhan-Direskeneli, Istanbul University (Istanbul, TR)

Purpose: The presence of anti-ganglioside (GM1) and anti-glutamic acid decarboxylase (GAD) antibodies have been reported in therapy-resistant epilepsy. Our aim was to detect GM1 and GAD autoantibodies in patients with juvenile myoclonic epilepsy (JME) to determine the prevalence of these antibodies and to evaluate their association with therapy-resistance and other various clinical and EEG features of JME.

Methods: The patient's group consisted of 96 consecutive patients with JME diagnosed according to the criteria of the International League Against Epilepsy (ILAE). We studied anti-GM1 and anti-GAD antibodies with enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA), respectively. The patient's group has been evaluated according to the response to therapy, presence of focal clinical and/or EEG signs, evidence of clinical and/or EEG photosensitivity and family history of epilepsy, presence of febrile convulsions, duration of epilepsy and antiepileptic drug treatment.

Results: We found anti-GM1 antibodies in 11 of 96 (11.46%) JME patients and one of 25 (4%) normal controls without a significant difference between the titers. Anti-GAD antibodies were detected in three of 69 patients (4.35%) but in none of the normal controls without a significant difference between the concentrations. Both antibodies did not have any association with the above mentioned clinical and EEG features of JME patients.

Conclusions: The anti-GM1 and anti-GAD antibodies did not correlate with therapy-resistance and other clinical and EEG parameters in JME. The results of our study disclosed that anti-GM1 and anti-GAD antibodies reflected the normal population level in this specific epileptic syndrome.

**P488**

**Epidemiological study of epilepsy among elderly people in Belarus.** H. Navumava, Diagnostical Center (Vitebsk, BLR)

Purpose: In the elderly population seizures are the third most common neurological problem.

Method: We have performed a population-based case ascertainment of all available sources of medical care since May 1986 till December 2001. People aged 50–80 years were analysed. Only cases with active epilepsy (at least one seizure during the last 5 years regardless of treatment) were included. All patients were examined by a psychiatrist or a neurologist.

Results: The incidence rate of epilepsy (registered newly-diagnosed patients) among elderly people in 1986–2001 varied from 16.1 to 40.0 per 100,000 of population. Stroke is the leading cause of new onset epilepsy, which accounts for 37% in women aged 60 or more. However, the leading cause of epilepsy onset in men aged 50–60 is toxic and metabolic disturbances. They may account for up to 39% of all cases. The incidence rate of epilepsy in men was approximately two times higher than in women.

The prevalence rate of epilepsy among elderly people in 1986 was 278.3 and in 2001–302.5 per 100,000 of population. Analysing the average prevalence rate profile of epilepsy according to the age of people, we noticed, that: 1. The prevalence rate of epilepsy was 2.3 times higher in men than in women. 2. At the age of 55–65 the prevalence rate of epilepsy was steadily high.

Anticonvulsants in Belarus are free of charge for the patients suffering from epilepsy. But only 36.25% of the patients receive these medicines regularly (according to our data of selective investigation).

Conclusion: The incidence rates of epilepsy among elderly people in Belarus were similar to those reported by other developed countries.

**P489**

**Juvenile absence epilepsy and focal successful epilepsy surgery.** R. Merino, V. Ivañez, F. Carceller, A. Rodríguez-Albariño, Hospital La Paz (Madrid, E)

Purpose: Co-existence in a patient of idiopathic generalized epilepsy (IGE) and symptomatic focal epilepsy (SFG) is rare. We report a patient with juvenile absence epilepsy and temporal lobe epilepsy. Differential diagnosis between IGE and SFG will help decide whether surgical treatment of focal epilepsy is a viable option.

Methods: The clinical characteristics, MRI and video EEG monitoring diagnostic approach employed in our epilepsy surgical treatment protocol are presented.

Results: We report a 23 year-old woman diagnosed with juvenile absence epilepsy at the age of ten whose seizures have been adequately controlled with valproate. At the age of 14 seizures clinically similar (consisting in loss of contact + automatisms) became intractable. Interictal EEG showed generalized 3.0 Hz frequency spikes waves bursts as well as epileptiform in the right temporal lobe. MRI showed only a thickening of the cortex in the right temporal lobe (T1).

The patient presented two separate epileptic syndromes, IGE and SFG. The latter condition was treated surgically when she was 20. For the last three years, she has received valproate and although her electroclinical anomalies continue, she is seizure-free.

Conclusion: Generalized electroclinical features may be caused by focal brain lesions, but Co-existence in a patient of IGE and SFG intractable so susceptible to surgical treatment can also occur.

In this case, differentiation between the two seizures syndromes allowed surgical treatment of the focal epilepsy and subsequent successful pharmaceutical control of the IGE.

**P490**

**The role of EEG in patients under anti-epileptic treatment.** H. M. Selekler, H. Efendi, S. Komsuoglu, Kocaeli University (Kocaeli, TR)

Introduction: The importance of an epileptiform pattern on an electroencephalogram (EEG) has a known relevance in the initial work-up of persons with suspected epilepsy. But the role of the EEG under anti-epileptic treatment is not clear enough. Diagnostic value of EEG in patients under anti-epileptic treatment and clinical diagnosis of epilepsy were compared in this study.

Material and Method: Patients with mental retardation and any neurological sequel were not included in the study. Subsequently thirty-one female and thirty-five male patients, mean age of $21.53 \pm 14.59$ (range = 5–73 years), who were diagnosed as having epilepsy previously and commenced anti-epileptic treatment by Kocaeli University, Faculty of Medicine, Epilepsy Section were evaluated for the study. Age at onset of seizures, duration of epilepsy, type of seizures, seizure frequency in the past six months, epilepsy medication regimen were documented and then patients' EEGs were recorded.

Results: Abnormality of EEG was found in 41% of patients. Twenty patients had primary generalized tonic-clonic seizures and among them, EEG abnormality was found in only one patient who had generalized intermittent slowing. Centro-temporal spikes were seen in 7 patients and centro-temporal sharp waves seen in 1 patient among 10 children with benign childhood epilepsy. 3 Hz spike and wave complex were observed in 4 of the 8 patients who had absence epilepsy. Generalized polyspikes were determined in 7 of the 9 patients with juvenile myoclonic epilepsy. Among the 13 patients with complex partial seizures, 3 had temporal spike and sharp waves, 1 had temporal slowing, 1 had generalized intermittent slowing and 1 had EEG background slowing. Among 6 patients that had focal motor seizures, frontal spike was seen in only 1 patient. Prevalence of EEG abnormality was significantly higher in patients who had at least one seizure in the past six months compared to the patients who had no seizures in the past six months ($p < 0.05$). There was no statistical significance between pathologic EEG findings and age at onset of seizures, duration of epilepsy, epilepsy medication regimen (monotherapy, double therapy or polytherapy).

Discussion: Abnormality in EEG may not be found in more than half of the patients under anti-epileptic treatment. This is especially true if patients have had no seizures in the past six months. Diagnosis of epilepsy should not rely on only the presence of epileptiform pattern on EEG after the first interview, because epileptiform pattern may reduce the clinical diagnosis of epilepsy by 60% while absence of epileptiform pattern does not exclude the diagnosis.

P491
**Hot-water epilepsy.** A. Yilmaz, E. Ozaydin, M. Kilinc, S. Benli, Baskent University (Ankara, TR)

The terms "reflex" or "sensory" epilepsy are used to describe a seizure precipitated by a sensory stimulus. Hot-water epilepsy (HWE) is an unusual form of reflex epilepsies which are precipitated by photic, auditory, cognitive and somatosensory stimuli. Largest numbers of cases of HWE have been reported from India and Japan. In these series HWE constituted 4.4% of complex partial and generalized tonic clonic seizures. This form of epilepsy seems to be rare in Europe. HWE was found to be more common in males.

We report an 18 year old male who had complex partial seizures with secondary generalisation. His seizures were precipitated during taking bath and washing face with hot or lukewarm water, also by the smell of shampoo. The patient had an aura as if he were in a dream followed by nausea and vomiting, then he became unresponsive; his eyes gazed upward, and he had tonic-clonic contractions that lasted for about one minute without incontinence. He did not declare any spontaneous non-reflex seizure. His seizures began when he was 12 years old. He was born at term without any complication. His psychomotor development was normal. He had a history of meningitis when he was 4 months old, and a single febril convulsion when he was two years old. There was no seizure history in the family. All his neurologic findings and laboratory investigations were normal. His interictal electroencephalography recordings revealed no abnormality. The cranial Magnetic Resonance Imaging showed bilateral mesial temporal sclerosis. Treatment with carbamazepine along with recommendations of lowering bath temperature gave no relief. So his anticonvulsant was replaced with sodium valproate (1000 mg/day). He responded well to the new treatment.

The pathophysiology of epilepsy provoked by hot water is still unknown. Available clinical and experimental data suggest that HWE is precipitated by a combination of both complex tactile and a temperature-dependent stimuli. The most frequently described factors for HWE were pouring hot water over the head, and exposing certain parts of the body such as head, face and neck. In a well documented study on rats, repeated hot water stimuli were shown to have a kindling-like effect, most notably on the amygdala, which produced progressive increases in convulsive responses to stimulation.

Although HWE is generally known to be benign and self-limited, treatment with antiepileptic drugs may sometimes be necessary to control seizure as in our patient.

P492
**Temporal lobe epilepsy in an adult patient presenting with the acute attack of migraine with aura.** D. Aygun, Ondokuz Mayis University (Samsun, TR)

Background: Symptoms associated with migraine can also be seen in other disorders. Similar symptoms may be with epilepsy. We reported a case displaying lateral temporal lobe seizures, unresponsive to previous migraine therapy.

Case Report: A 46-year-old right-handed man presented with new onset aura symptoms described as bright lights or colored lights, scotomas, and figures. Sometimes these symptoms were followed by non-pulsatile bilateral frontal headaches. Severity of headaches was mild. He did not report vomiting, photophobia, and a family history of migraine. He began experiencing these symptoms two months ago and received the diagnosis of migraine. Attacks typically occurred three to four times per month. Treatment with daily 40 mg propanolol for two months did not decrease the severity and frequency of migraine headache attacks. Two interictal electroencephalograms (EEG) showed sharp waves in right and left temporal regions. His magnetic resonance imaging (MRI) and single photon emission tomography (SPECT) were normal. Valproic acid was begun, and his seizures completely stopped.

Conclusion: If a case without family history of migraine experiences atypical migraine aura symptoms with or without headache, in this case, epilepsy should also be considered and EEG should be performed.

## Extrapyramidal disorders

P493
**Involvement of human skeletal muscle in Huntington's disease.** A. Ciammola, J. Sassone, U. Fascio, P. Scalmani, L. Cova, E. Mancinelli, G. Meola, F. Squitieri, V. Silani, IRCCS Istituto Auxologico Italiano, Centro Interdipartimentale di Microscopia Avanzata, Dipartimento di Fisiologia e Biochimica Generali, Istituto Policlinico San Donato, IRCCS Istituto Neuromed (Milan, Isernia, I)

Huntington's disease (HD) is a neurodegenerative disorder characterized by motor, cognitive and psychiatric deficit. HD results from a CAG expansion in the first exon of the IT15 gene, located on chromosome 4p16.3 that encodes a 350 kDa protein called huntingtin (htt). The function of the htt is still unknown. The htt is diffusely expressed in both brain and peripheral tissues (Sharp A. H., 1995). Some evidence suggests that skeletal muscle is involved. Energetic defect with damage of the mitochondrial oxidative metabolism was demonstrated in HD patients (Arenas J., 1998; Lodi R., 2000). Moreover transgenic mouse R6/2 develops severe muscle atrophy and progressive wasting correlate with the formation of ubiquitin and huntingtin-positive intranuclear inclusions (Sathasivam K, 1999). Recently Luthi-Carter et al. (Luthi-Carter R., 2002) demonstrated that R6/2 skeletal muscle is also a target of polyQ-related perturbation in gene expression, showing changes in mRNAs that are deregulated in brain.

We have developed primary adult human skeletal muscle cultures from biopsies of 3 HD patients and 4 age-matched healthy controls. To analyse the effect of the normal and mutant huntingtin, we performed survival assays after different apoptotic stimuli. We found that HD myoblasts are more susceptible to cell death compared to control cells. TUNEL (tdt UTP nick end-labeling) analyses in the same condition confirm the above findings. Then we have demonstrated that increased stress-induced apoptotic death in HD myoblast correlates with greater caspase 3 activation.

The localization in human tissue of normal and mutant htt may be important to understand HD pathogenesis. Many studies suggest that polyglutamine-expanded htt is toxic within the nucleus where it forms aggregates (Sieradzan K. A., 1999). To determine if full-length htt or fragments translocate into the nucleus, we performed immunofluorescence labelling of cultured cells using antibodies that recognize N-terminal or C-terminal htt fragments. Images obtained with confocal microscope revealed that huntingtin is mainly concentrated in the perinuclear region. However immunofluorescence also shows patches of intense labelling in the nuclear compartment of HD and control myoblasts suggesting a physiological function in the nucleus. Then we have confirmed subcellular htt localization by western blot on nuclear and cytoplasmatic fractions.

In conclusion we demonstrated involvement of skeletal muscle in HD. Human muscle cells therefore are a suitable system for the study of the mechanism underling HD. The identification of a different viability in primary human adult skeletal muscle cells may explain muscle atrophy observed in HD and may be useful for monitoring in vivo efficacy of pharmaceutical agents used to prevent HD degeneration in patients.

P494
**Effects of a ballistic unilateral movement on contralateral tremor.** H. Kumru, M. Marti, F. Valldeoriola, J. Valls-Sole, Hospital Clinic (Barcelona, E)

Tremor is a rhythmic mechanical oscillation of at least one functional body region, produced by either alternating or synchronous contractions of reciprocally innervated antagonistic muscles. Tremor is caused by many diseases. It is one of the main features of Parkinson's disease (PD) and Essential tremor (ET), but it is also relatively easily mimicked by patients with no organic central nervous system disorders. The limits between mimicked and pathologically mediated tremors are sometimes difficult to define and document. In the study presented here, we show the effects of a ballistic movement on contralateral tremor in normal volunteers mimicking wrist tremor, in patients with PD, and in patients with psychogenic tremor.

Methods: The study was carried out in 9 healthy subjects, in 10 patients with idiopathic Parkinson's disease (IPD), and in 5 other patients whose tremor was considered psychogenic on the basis of clinical assessment. Subjects were asked to react at the perception of a visual imperative signal by performing a ballistic movement to hit a button located on top of a table with the hand contralateral to the one showing tremor. Accelerometers were attached to the dorsum of both hands. We measured the change induced by the reaction on the frequency, amplitude and regularity of the oscillations recorded in the contralateral wrist joint.

Results: All subjects exhibited regular oscillations according to their rest tremor frequency, ranging from 4.1 to 6.2 Hz. Contralateral ballistic wrist movements induced no change of tremor frequency or amplitude in

IPD patients. However, the same movement induced a significant decrease of tremor amplitude and irregularity in control subjects and in patients with psychogenic tremor. The change occurred always after the onset of the ballistic movement, at a mean interval of 605 ms (SD = 228 ms) in control subjects, and of 548 ms (SD = 264) in patients with psychogenic tremor.

Conclusion: Our study has shown that there is an inhibitory effect of ballistic movements on contralateral voluntarily mediated phasic oscillations, but not in tremor oscillations of IPD patients. The study of that effect is useful to distinguish between psychogenic and IPD tremors.

**P495**

**Efficacy of Botulinum toxin A in the treatment of craniofacial and cervical dystonia over a 14 year period. A retrospective analysis.** F. Simões Ribeiro, M. Rosas, G. Sousa, J. Guimarães, Hospital S. João (Porto, P)

Introduction: Botulinum toxin A (BTX) is the first line treatment of cranio-cervical dystonias - blepharospasm, hemifacial spasms, cervical dystonias and others.

Material and methods: In our outpatient dystonia clinic we made BTX A since 1989. After a 14 year period we did a retrospective analysis of our patients. From January 1989 to January 2003 we treated 246 patients, 181 female and 65 male, aged 16 to 89 (mean 55,65 ± 15,33) with 78 blepharospasm(B); 137 hemifacial spasm(HF) and 31 cervical dystonia(CD).

During this period 69 patients (28 %) discontinued treatment because of: 7 death; 62 unknown cause, primary resistance and secondary resistance.

Efficacy was significant in 225 patients (91.5 %); (85.9 % in B; 95.6 % in HF; 87 % in CD). Thirty four patients (13.8 %), developed side effects, only reported in the first years of treatment. These side effects were minor and transient (mean duration two weeks). There was no treatment discontinuation secondary to adverse effects.

Efficacy remained stable over the years and adverse effects gradually became less frequent, which could reflect better injection technic.

BTX is an effective treatment with transient and minor side effects, mostly related with dosage and experience of the doctors.

This treatment clearly improved the quality of life of our patients.

**P496**

**Abnormal sensorimotor integration in a case of secondary dystonia.** S. Tamburin, S. Marani, P. Manganotti, A. Andreoli, A. Fiaschi, G. Zanette, University of Verona (Verona, I)

Objective: To examine the role of sensory afferences and sensorimotor integration in secondary dystonia.

Case report: A 44 year old man developed writhing movements and a dystonic posture of the right hand associated with slight sensory impairment after cervical whiplash injury. Cervical magnetic resonance imaging revealed a small right posterior C5-C6 lesion of the spinal cord. Surface EMG showed co-contraction of the forearm flexor and extensor muscles. Median nerve somatosensory evoked potentials (SEPs) showed normal conduction times, but spinal and cortical waves were larger in response to stimulation of the affected side. Transcranial magnetic stimulation (TMS) showed the absence of the normal somatotopic distribution of motor inhibition in response to digital stimulation.

Conclusions: We report a patient who developed dystonic symptoms after cervical whiplash injury. Clinical and EMG features favoured the interpretation of the symptoms as dystonia, rather than pseudo-dystonia. Neurophysiological evaluation demonstrated abnormalities of sensory processing and sensorimotor integration, which strongly resemble those found in primary dystonia. This report may confirm the central role of abnormal sensory elaboration in the pathogenesis of symptoms in patients with secondary dystonia. SEPs and TMS appear to be valuable tools to document these abnormalities in dystonic patients.

**P497**

**Cerebral blood velocity (MCA) and cerebrovascular response to apnoea before and after passive orthostasis in patients with idiopathic Parkinson's disease with and without orthostatic hypotension.** U. Sommer, N. Kleiner, M. Suess, F. Suess, G. Gahn, H. Reichmann, T. Ziemssen, University Hospital, Sigma Medizin Technik (Dresden, Thum, D)

Patients with idiopathic Parkinson's disease suffer not only from symptoms involving the motoric system like tremor, rigidity and hypokinesia but also from autonomic failure, especially in later stages of disease. One major problem is orthostatic hypotension, defined as decrease of the systolic blood pressure by more than 20 mmHg when changing from supine to standing position. Often this fall of the blood pressure remains asymptomatic. The aim of this investigation was to perform extensive cardiovascular testing of the autonomic system including the measurement of the cerebral blood velocity and cerebral autoregulation during apnoe-tests.

In this clinical study we examined 22 patients with idiopathic Parkinson's disease in our autonomic laboratory with the VAGUS 2100 (Sigma Medizin-Technik, Thum, Germany). An extensive evaluation of the patients' history was performed, none of the patients complained about orthostatic symptoms. The examination was performed between 8.00am and 2.00pm, exclusion criteria were significant atherosclerosis of extra- and intracerebral vessels as well as peripheral obliterative arterial disease (POAD), diabetes, thyroid dysfunction, myocardial infarction, pacemaker, chronic obstructive pulmonary disease, pheocromocytoma as well as the intake of certain drugs like beta-blockers, anticholinergics, mineral corticoids, diuretics, parasympathomimetics or sympatholytics within 48 hours before the examination. The consumption of alcohol or analgetics, nicotine or caffeine was not allowed 12 hours before the examination. In the first part of the test an extensive autonomic test battery was used for the examination of the patients with continuous measurements of blood pressure, pulse wave, respiration, ECG and Doppler signal of one or both MCA. In the second part three apnoe-tests with a maximum duration of 30 seconds were performed before and during passive orthostasis.

Out of the 22 patients, 11 patients showed asymptomatic orthostatic hypotension (5 female, 6 male; mean age 62 ± 6.4; mean duration of the disease 7.8 years; mean Hoehn and Yahr stage 2.25), whereas the remaining 11 patients (1 female, 10 male; mean age 62 ± 7.4; mean duration of the disease 7.0 years; mean Hoehn and Yahr stage 2) showed no abnormal change in systolic blood pressure. MCA blood velocity was slightly reduced in all patients immediately after passive orthostasis. Our preliminary data show no differences in the cerebrovascular response between the two groups (detailed data will be presented in June).

**P498**

**Progressive supranuclear palsy induced by clebopride.** J. Campdelacreu, H. Kumru, J. Valls-Solé, E. Tolosa, Hospital Clinic (Barcelona, E)

We report a patient who developed parkinsonism with clinical features of progressive supranuclear palsy (PSP) while receiving treatment with the antidopaminergic drug clebopride (CLB). The clinical and neurophysiological abnormalities encountered disappeared after CLB withdrawal.

A 62 year-old woman complained of stuttering and gait instability which was progressive over the previous 5 months. She was being treated with CLB 1.5 mg/day for dyspepsia, a treatment that had been started 10 months before onset of neurological symptoms. On examination she had marked stuttering of speech, masked face, parkinsonism of axial predominance without tremor, postural instability, vertical supranuclear gaze palsy, and pathological frontal reflexes. She was thought to suffer from PSP. The accoustically triggered startle reflex was absent, a finding that supported the diagnosis of PSP.

Because of its known antidopaminergic properties CLB was discontinued. The patient improved gradually and parkinsonism and other PSP-like features disappeared over an 18 month period, with the exception of mild upward gaze limitation. The startle reflex was present at this time.

A video of the patient during CLB treatment and after drug withdrawal will be shown.

Discussion: Our patient had the typical clinical symptoms of PSP. The absence of the accoustically induced startle reflex, a consistent finding in this disorder, supported the clinical diagnosis. The disappearance of both the clinical and the neurophysiological abnormalities upon discontinuation of CLB strongly suggests that the syndrome had been induced by this neuroleptic-like drug. The absence of neurological symptoms and signs 18 months after drug withdrawal supports this interpretation.

Parkinsonism is a well known side effect of antidopaminergic agents. These drugs can also induce a reversible dementia syndrome. To our knowledge, this is the first instance of a PSP-like picture and accoustical startle reflex abnormality that can be attributed to antidopaminergic drug toxicity.

**P499**

**Psychopathological symptoms in patients with cognitive impairment and movement disorders.** A. Gorzkowska, G. Opala, B. Jasinska-Myga, G. Klodowska-Duda, E. Krzystanek, Silesian Medical University (Katowice, PL)

It's known that some neurological problems can influence life satisfaction, physical complaints and effect of treatment. It is hypothesized that non-ac-

ceptable disorders like memory impairment or movement disorders can produce emotional problems and change of behavior.

Our goal of the pilot study was to investigate the kind of psychopathological symptoms among three groups of neurological patients. We examined 32 patients: 11 with Mild Cognitive Impairment (MCI), 13 with Parkinson's disease (PD) and 8 with Other Movement Disorders (OMD). Symptom Check List was used for evaluation of all patients.

In the MCI group value of Somatization scale was 10.1 ± 6.8 and Depression with anxiety scale was 11.0 ± 5.2. In the PD group value of Somatization scale was 11.6 ± 6.6 and Depression with anxiety scale 11.9 ± 6.9. In OMD group value of Somatization 8.0 ± 5.4 and Depression with anxiety scale was 12 ± 5.2.

Comparing MCI and PD groups of patients with healthy people we found the higher scores in two scales: Somatization scale and Depression with anxiety scale. Comparing OMD groups of patients with healthy people we found the higher scores in Depression with anxiety scale.

Somatization and depressive disorders are additional problems in MCI and PD. In the OMD the most important problem is depression. We consider this problem as important for life satisfaction, physical complaints and effect of treatment. Psychological consultation should be recommended in these groups.

**P500**

**Hemihypomimia in Parkinson's disease.** V. C. Zingler, K. Jahn, M. Strupp, T. Brandt, Klinikum Grosshadern, LMU (Munich, D)

Introduction: The clinical symptoms of typical Parkinson's disease are often asymmetric in the early stages of the disease. Bradykinesia also affects the facial muscles, resulting in a mask-like and expressionless face with an unblinking stare. We report on a patient with right-sided, parkinsonian features in whom the lateralization of motor signs also manifested as a hypokinesia of the right-sided facial muscles, i. e., hemihypomimia. To our knowledge, this sign has not been described before.

Case report: A 55-year-old woman had a progressive gait disturbance and difficulty executing skilled movements with her right hand which caused alteration of her signature for at least 4 months. She also complained of pain in her right knee and unsteadiness of her right leg. She had had no history of previous neurological disorders, in particular no stroke. Neurological examination revealed a right-sided cogwheel rigidity and bradykinesia of the upper and lower extremities on the right side. A hypokinesia with reduced and slowed movements was also observed but only of the right-sided facial muscles. Neuropsychiatric evaluation indicated that the patient was depressed and anxious. While cranial magnetic resonance imaging (MRI) was normal, DaTSCAN-SPECT showed an asymmetry of the presynaptic dopamine transporter in the striatal region; there was a significantly lower intensity on the left side.

Conclusions: This is an exceptional case of a right-sided hemiparkinsonism with an obvious unilateral hypokinesia of the face on the same side, i. e., hemihypomimia. Although not known to be a typical feature, the subtle lateralization of hypomimia may remain undetected in patients with Parkinson's disease.

**P501**

**Gabapentin for painful legs and moving toes syndrome.** A. Villarejo, J. Porta-Etessam, A. Camacho, J. Gonzalez de la Aleja, M. Penas, A. Matinez-Salio, Hospital Universitario doce de Octubre (Madrid, E)

Introduction: In 1971, Spillane et al. described a syndrome characterized by pain in the lower limbs associated with spontaneous movements of the toes. The cause of the syndrome remains unclear, and although a number of therapies have been tried, the results are unsatisfactory. We describe a man with painful legs and moving toes syndrome (PLMTS) who was successfully treated with gabapentin (GBP).

Case report: A 66-year-old patient presented with 6-month history of continuous paresthesias and burning pain in both feet. Over the following three months, jerky involuntary movements of all toes of the left foot appeared, and soon spread to the right. He had a past history of pharynx carcinoma treated with radiation therapy and chemotherapy (taxol and cisplatin) five years before the symptoms. On examination, there were intermittent movements of the big toes (frequency of about 1–2 Hz). The movements consisted mainly of flexion and extension, and could be suppressed voluntarily for 30–45 seconds. The neurologic examination revealed absent ankle reflexes and a loss of vibration sense in both feet. Laboratory investigations and MRI of lumbar spine were normal. Motor and sensory nerve conduction studies and an electromyography radiculopathy screen disclosed no abnormalities. Gabapentin 300 mg tid was begun, with improvement of pain and movements within 48 hours. They recurred three months later, and could be controlled doubling the dosage.

Discussion: In most cases of PLMTS a peripheral nerve damage is demonstrated or, as in our case, suspected. GBP, approved as antiepileptic drug, has now wider indications including neuropathic pain and some movement disorders. Although our observation has to be confirmed with other cases, we consider that GBP could be an option in patients with PLMTS.

## General neurology

**P502**

**Misdiagnosis of hysteria in a case of gluten sensitivity and neurological dysfunction.** S. Strazzer, A. Bardoni, D. Brambilla, S. Bernasconi, G. Poggi, E. Castelli, IRCCS "E. Medea" (Bosisio Parini, I)

Several neurological syndromes have been described in association with coeliac disease but it is unclear whether these are directly or indirectly caused by gluten ingestion. To establish an association between neurological disease and gluten sensitivity is not easy because occult sub-clinical coeliac disease occurs commonly without gastrointestinal symptoms. Some authors described a correlation between the duration of symptoms and its severity as well as the presence of neuroradiological findings.

We report on a case of a young woman affected by coeliac disease with a variety of unusual neurological manifestations. For more than 6 years this patient developed a variety of neurological dysfunctions resembling multiple sclerosis, but the negative findings of neurophysiological (EPs) and neuroradiological studies as well as negative cerebrospinal fluid (CSF) resulted in a misdiagnosis of hysteria or Lyme disease as low titer of antibodies to Borrelia burgdorferi was observed in serum, but not in CSF. In the end, she was studied for the presence of coeliac disease owing to minimal intestinal symptoms as well as diarrhea after intake of bread. Despite the absence of antigliadin and a normal duodenal mucosa, the in vitro culture of intestinal mucosa was positive for the presence of IgA. Antibodies to endomysium may be useful for the identification of patients with cryptic coeliac disease. On a gluten-free diet, our patient showed an improved neurological symptomatology with a single episode of emiataxia after erroneous intake of gluten-containing food.

We recommend that patients with neurological dysfunctions of unknown cause be routinely screened for the presence of gluten-sensitivity.

**P503**

**Multidisciplinary study of the prevalence and progression of the mitochondrial diseases. A study of 50 patients.** J. Arpa, A. Cruz-Martínez, Y. Campos, M. Gutiérrez-Molina, F. García-Rio, J. González-Orodea, S. Santiago, C. Pérez-Conde, R. López-Pajares, M. Martín, J. Rubio, P. del Hoyo, A. Arpa-Fernández, M. Sarriá, M. Blanco, V. Mejías, S. Escalante, S. Monteagudo, J. Gracia, R. Merino, J. Nos, F. Vivancos, F. Palomo, T. Lacasa, C. Morales-Bastos, J. Arenas, Hospital Universitario "La Paz", Hospital "La Luz", Hospital "12 de Octubre", Hospital Severo Ochoa (Madrid, E)

In the present series of 50 patients we describe various clinical phenotypes of mitochondrial disease (MD) studied over the past 10 years, those which are most frequent in adults. Patients were classified into two groups: 1) with an exclusively myopathic clinical picture (mitochondrial myopathy = MM), 35 (70%); and 2) with mitochondrial encephalomyopathy (MEM), 15 (30%). The prevalence of MD in Madrid Health Area 5 is of 5.7/100,000 in the population over 14 years of age. Clinical signs of peripheral neuropathy (PN) are significantly more frequent in MEM than in MM ($P < 0.002$). The electrophysiological assessment reveals the significantly more frequent presence of myopathy in the MM group than in MEM ($P < 0.03$) and, vice versa, PN in the MEM group with respect to the MM group ($P < 0.02$). We have observed 2 patients with a subclinical axonal motor neuropathy not previously described in MD. Jitter was found to be altered in 10/18 cases studied. Another frequent finding is an increased slope of the metabolic response (dV'O2/W), reflecting some degree of work inefficiency. Currently available therapy in MD is generally disappointing, though it appears to be partially helpful in certain cases. MEM patients had accelerated risk of death compared with MM patients ($P < 0.01$).

**P504**

**Ischaemic cell death is necroapoptotic.** M. Kilinc, Y. Özdemir-Gürsoy, A. Can, T. Dalkara, Baskent University, Hacettepe University, Ankara University (Ankara, TR)

It is well known that both necrotic and apoptotic cell death processes are activated after focal cerebral ischemia and reperfusion injury. However up

to now, no study has been performed demonstrating that these two pathways are activated in the same cell, and at the same time.

Immunohistochemical studies have shown that activity of caspase-3, an enzyme which acts both as an initiator and executor of the apoptotic process, increases after cerebral ischemia. Cathepsin-B, one of the lysosomal proteases, is a marker of necrotic cell death. Cathepsin-B is also found to be overexpressed and activated within ischemic neurons after onset of focal cerebral ischemia. When the enzyme is activated, this can be detected first by the appearance of dense granular cathepsin-B immunoreactivity within the cells, and later by diffuse staining in the extracellular space consistent with degranulation of the lysosomes. Colocalization of caspase-3 activation along with cathepsin-B may give an answer to the question whether these two pathways are activated in the same cells, and at the same time after focal cerebral ischemia.

To evaluate this, we used a transient middle cerebral occlusion model in adult male Swiss Albino mice (5 animals in each group). Ischemia was induced by an 8–0 nylon monofilament coated with a resin-silicon hardener mixture. The filament was introduced to the left common carotid artery and then advanced into the middle cerebral artery until an approximately 80 % drop in the cerebral blood flow was detected by laser Doppler, and left in this position for 1 hour. For reperfusion, the filament was withdrawn slowly. Mice were killed 0–5 minutes, 1, 3, 6, 12 and 24 hours after reperfusion. Blood pressure and blood gases were monitored and maintained in the normal range throughout the experiments. After perfusion with saline followed by a formalin solution, the brains were extracted and formaline fixed. Brain slices obtained by cryosectioning were then stained by double immunofluorescence histochemistry by an antibody to caspase-3p20 and later by an antibody to cathepsin-B. The antibody to caspase-3p20 detects the active form of caspase-3 while the cathepsin antibody detects mature and precursor forms of cathepsin-B.

We observed that caspase-3p20 immunoreactivity became prominent in neuronal perikarya within the middle cerebral artery territory at the time of reperfusion and 1–24 hours later, in a decreasing manner as time of reperfusion increases. We detected cathepsin-B activity by the appearance of dense granular cathepsin-B immunoreactivity within the cells, more prominent after 3 hours of reperfusion; and diffuse staining in the extra cellular space after 12 and 24 hours, consistent with degranulation of the lysosomes.

These data demonstrating the increase in both caspase-3 and cathepsin B activity after transient focal cerebral ischemia suggest that ischemic cell death has a mixed (necroapoptotic) phenotype.

## P505

**Amelioration of ataxia in cortical cerebellar atrophy with the GABAergic drug gabapentin.** J. Gazulla, J. M. Errea, I. Benavente, C. Tordesillas, C. Ríos, J. A. Salvador, Hospital San Jorge, Hospital de Barbastro, Departamento de Documentación Científica. (Huesca, Barbastro, Zaragoza, E)

Cortical cerebellar atrophy (CCA) has been found to be associated with an almost selective loss of Purkinje cells in the anterior vermis of the cerebellum. Its neurochemical correlate is a loss of gamma-aminobutyric acid (GABA)in deep cerebellar nuclei and cerebrospinal fluid. GABAergic drugs have not been used to treat this disorder up to date, but the drug gabapentin was found capable of improving cerebellar signs in a case of atrophy of the cerebellar cortex caused by deficiency of hexosaminidase A.As this disease is associated with damage to GABAergic circuits in the central nervous system, it was considered that it could constitute an adequate therapeutic model for other diseases with a similar neurochemical substrate.

The objective of this work has been to find out the efficacy of the GABAergic drug gabapentin in the treatment of the cerebellar signs caused by CCA, either after single doses of the drug or after continued administration during four weeks.

Methods: Ten patients with CCA received gabapentin in single doses of 400 mg, in three occasions on alternate days; thereafter, daily administration of 900 to 1600 mg of gabapentin was continued during at least four weeks. An ataxia scale based on the International Cooperative Ataxia Rating Scale, that included items to evaluate intermalleolar distance, walking capacities, knee-tibia test, finger-to-nose test and a total ataxia scale, was employed to evaluate the cerebellar signs at baseline and after administration of the drug. Informed consent was obtained from every patient; the study was approved by Hospital San Jorge ethics committee.

Results: A statistically significant improvement of the ataxia scores was found after single doses of 400 mg of gabapentin and after the administration of 900 to 1600 mg of the drug during four weeks, as compared to the results obtained at baseline. Friedmann test and Wilcoxon's paired t test were used in the workout of the statistical study. An evident clinical improvement was observed in every case.

Conclusions: Gabapentin has been demonstrated to be capable of improving the cerebellar signs in cases of CCA, after single doses and after continued administration of this drug during four weeks. GABAergic enhancement or substitution could play an important therapeutic role in the treatment of diseases of the cerebellar cortex associated with a deficit of GABA, of degenerative or other causes.

## P506

**Posterior leukencephalopathy due to cyclosporine neurotoxicity in patients after allogeneic stem cell transplantation.** P. Günther, W. Hermann, D. Schneider, A. Wagner, University of Leipzig (Leipzig, D)

Background: Cyclosporine A is widely used as therapeutic agent for immunosuppressive treatment after stem cell transplantation. Although several neurotoxic side effects are known the pathogenesis of the involvement of central nervous system remains not fully understood. A reversible posterior leukencephalopathy with altered consciousness, seizures, headache, hypertension and visual disturbances is rare but the most serious complication.

Case report: We report four patients who developed coma and seizures under immunosuppressive therapy with cyclosporine A. All patients underwent allogeneic stem cell transplantation because of malignant hematological disease two to five months before symptoms occurred. In all patients no severe infections, metabolic nor other toxic factors were found. MRI showed white matter abnormalities indicating posterior leukencephalopathy. Cyclosporine A blood levels were monitored and symptoms resolved with dose reduction or withdrawal of the drug in three patients. One patient died because of a relapse of the hematological disease.

Conclusions: Immunosuppressive treatment after allogeneic stem cell transplantation with cyclosporine A can provoke severe neurological complications. Since posterior leukencephalopathy due to cyclosporine A neurotoxicity is a reversible syndrome it should be always considered in differential diagnosis in case of coma and seizures.

## P507

**Effects of gabapentin on posture in the young adult.** V. Wiener, M. Manto, Hôpital Saint-Pierre, FNRS Hôpital Erasme (Brussels, B)

Gabapentin (GBP) is a widely used anti-epileptic drug. GBP is also recognized for its properties against neuropathic pain. Its mechanism of action is undetermined. One of the side effects reported in studies including patients treated with GBP is ataxia/loss of equilibrium. These studies have considered GBP as an adjunct therapy for the neurological disorder. We analysed the effects of GBP alone on static parameters of posture in 10 young adults (mean age 30 ± 7 years, 2 women). GBP was given orally up to 900 mg/day over 5 days. The following parameters of posture were studied: lateral displacement (Delta X, mm), antero-posterior displacement (Delta Y, mm), total travelled distance (TTW, mm) of the center of pressure (Footscan, RS Scan, Belgium). Four conditions were considered: feet apart eyes open (FAEO), feet apart eyes closed (FAEC), feet joined eyes open (FJEO), feet joined eyes closed (FJEC). Statistical significance was set at 0.05. The tolerance was good. In the condition FAEO, Delta X, delta Y and TTW were respectively 4.6 ± 1.9 mm, 7.9 ± 4.2 mm, 432.0 ± 271.0 mm before GBP, and were 5.8 ± 2.3, 8.8 ± 3.6, 535.9 ± 300.9 with GBP. In the condition FAEC, Delta X, delta Y and TTW were respectively 5.3 ± 2.0 mm, 8.8 ± 2.9 mm, 429.9 ± 315.1 mm before GBP, and were 7.2 ± 4.8, 11.1 ± 7.3, 493.1 ± 257.4 with GBP. No statistical difference was found ($p > 0.05$). The same observation was made for the feet joined conditions. Parameters of posture were clearly unaffected by a dose of 900 mg of GBP.

Our study argues against a postural disturbance induced by a monotherapy of GBP in the young adult.

## P508

**Changes in pupil's reaction to light in myasthenia gravis.** D. Fotiou, G Rizos, I. Kourtis, C Tsalamas, A. Goulas, F. Fotioy, Ahepa University Hospital, Lab of Fluid Mechanics, AUTH (Thessalonica, GR)

Background: Controversial reports have been made for the association between myasthenia gravis (MG) and central CNS functions.There are also reports about pupil and eye movements in MG. Pupillometry is a useful method for testing the pupil's reaction.

Objective: To investigate the pupils reactions to light in MG patients before the treatment with anticholinergic drugs (pyridostigmine) and in normal controls.

Materials and methods: A new system for recording the pupil's reaction to light was developed in the Laboratory of Clinical Neurophysiology in

collaboration with the Department of Fluid Mechanics of the Aristotle University of Thessaloniki.

This system is computer controlled and fully automated. It includes an infrared digital video camera capable of recording 260 frames/second. The high frame rate allowed for a very accurate measurement of the reaction time as well as of the instantaneous velocity and acceleration of the pupil and other interesting parameters under a variety of external stimuli. Full statistical analysis of the results is performed on line using ensemble averaging of all the important parameters. We study 10 patients (7 female, 3 male) main age 35 ± 12 with the diagnosis of MG (group A). All of the patients were in class II-III according to clinical classification of myasthenia gravis foundation, had positive acetylcholine receptor antibody titres and were positive in repeated stimulation test (Desmet). The MG patients were re-examined with pupillometry one month after the treatment with pyridostigmine. As control group (group B) we examined 10 healthy persons statistically comparable. All of the patients were drug free for at least one week and they have not any obvious ANS dysfunction.

Results: There was a statistical significant difference in parameters in pupils reaction to light between the two groups mainly in the maximal acceleration to constriction ($p < 0001$), in reduced amplitude ($p < 0005$) and in maximal velocity of pupillary constriction. Abnormal values were observed and after the treatment with anticholinesterasic drugs between the groups but there was improvement in the parameters before and after treatment.

Conclusion: These results suggest that there may be a possible involvement of central cholinergic transmission in MG, involvement of the iris sphincter is common in MG and that pupillometry is an easy method for diagnosis of MG and checking the response to treatment.

**P509**

**Polymerase chain reaction in diagnosis of tuberculous myelitis: report of two cases.** C. Bensa, L. Landrau, S. Chanalet, C. Lebrun, M. Chatel, CHU Nice (Nice, F)

Introduction: Myelitis is a common pathology but 25 % of cases remain of undetermined aetiology.

Case n°1: A woman, 61 years old, first seen in March 2002, without any prior pathological event, presented, since January 2002, lower limb paresthesiae, walking impairment, Lhermitte's sign and sphincters dysfunction. Examination revealed proprioceptive ataxia and tetrapyramidal syndrome. Her clinical status progressively worsened. Spinal cord MRI showed several cervical and dorsal T2 hypersignals. Cerebrospinal fluid (CSF) was normal on 3 successive lumbar punctions. Serologies, auto-antibodies, anti-neuronal antibodies screenings were negative; no inflammatory syndrome was present at that time. Cerebral MRI, ophthalmologic exam, bone marrow biopsy, salivary gland biopsy were normal. Steroid treatment was started but clinical signs worsened. Then, a biological inflammatory syndrome appeared. Thoracic scanner showed in the upper right lobe a micro-nodular image congruent with tuberculosis sequelae. CSF Mycobacterium Tuberculosis PCR (MT-PCR) was positive. CSF culture remained negative. A antituberculosis quadri-antibiotherapy was started; the patient improved and has recovered normal walking.

Case n°2: This patient, 45 years old, HIV positive with severe lymphopenia and chronic C hepatitis presented walking difficulties in November 2002. Neurological examination found a tetrapyramidal syndrome with proprioceptive ataxia. Spinal cord MRI revealed T2 hypersignals and T1 gadolinium enhancements. All serologies, JC virus PCR, HTLV screenings were negative and CSF chemistry remained normal. Mycobacterium tuberculosis culture was negative but MT-PCR was positive on two successive samplings. A quadri-antibiotherapy was prescribed in December 2002 but poorly tolerated. No clinical nor MRI improvement has been seen till February 2003.

Discussion: Tuberculous myelitis is the most frequent aetiology of non-traumatic paraplegia in developing countries; tuberculosis prevalence has been recently increasing in occidental countries. Tuberculous myelitis diagnosis is difficult as cerebrospinal fluid remains normal in half the cases (Vukusic et al. 1998).

Conclusions: In suspected tuberculous myelitis, two investigations are highly relevant: MRI which may show suggestive lesions and CSF specific MT-PCR: its specificity is 97 % and its sensibility ranges from 32 % to 80 % whereas BK culture from CSF amounts only up to 17 %.

**P510**

**Unremitting venous congestion.** K. M. Gormley, M. A. James, D. A. Hilton, N. J. Gutowski, Royal Devon and Exeter Hospital, Peninsula Medical School (Exeter, UK)

A 59 year old man complained of minor visual blurring and went to his optician. There was no significant past history. He had papilloedema. He had normal imaging and a normal cerebrospinal fluid (CSF), except for a raised pressure of 38 cm of water, therefore benign intracranial hypertension was diagnosed. Three months later he developed partial seizures with secondary generalisation. A magnetic resonance venogram (MRV) showed both superior sagittal sinus and right lateral sinus filling defects. He was commenced on warfarin. By the following month he had deteriorated developing extrapyramidal features and cognitive impairment. Further magnetic resonance imaging (MRI) showed extensive bilateral cerebral white matter changes, the MRV revealed occluded transverse sinuses and a discontinuous sagittal sinus. CSF pressure was elevated with a lymphocytosis. Subsequent extensive investigations were normal. His condition was steroid-responsive. Off steroids he declined again and a right frontal brain biopsy including leptomeninges was non-specific. He continued to have more seizures requiring sedation on intensive care. Further MRIs showed new areas of non-enhancing focal signal abnormality in the temporal and cerebellar regions. He continued to deteriorate and died. Post mortem revealed extensive white matter degeneration with focal infarction secondary to acute-on-chronic venous congestion. This is an unusual presentation of unremitting venous congestion.

**P511**

**Modified Bielschowsky silver impregnation combined with hematoxylin counterstaining as a potential tool for the simultaneous study of inflammation and axonal injury in the central nervous system.** N. Grigoriadis, A. Lourbopoulos, D. Karussis, C. Symeonidou, I. Yatziv, E. Shohami, T. Ben-Hur, I. Milonas, Aristotle University of Thessalonica, Hadassah Hebrew University (Thessalonica, GR; Jerusalem, IL)

There is increasing evidence regarding the close relationship between inflammation and neuronal damage in several neurodegenerative disorders of the central nervous system. Among them, Multiple Sclerosis and brain injury have long been considered to contain both inflammatory and degenerative elements in their pathology. Hematoxylin – eosin and the Bielschowsky's silver staining are among the very basic tools for an initial evaluation of inflammation and axonal injury, respectively. Both stains are performed in separate, sometimes serial, sections of the same tissue. A combined performance of both methods in the same section could highly contribute to a direct visualization of both processes simultaneously. However, the major problem is the non-specific background when any of the several existing protocols is followed for Bielschowsky's staining. It is therefore impossible to identify the inflammatory infiltrates under these conditions, since hematoxylin cannot stain the cells due to the silver unselective impregnation. As it has recently been reported, a standardised low environmental temperature enables a significant reducing of background. We propose a further modification of the Bielschowsky's staining protocol, where an accurate manipulation of pH during the incubation periods (12.05–12.25) and washes (11.55 ± 0.05) leads to a total reduction of the background and allows the counterstaining of the section with hematoxylin. According to this modification, axons and infiltrates were successfully stained simultaneously in the same CNS section in the experimental allergic encephalomyelitis and traumatic brain injury models. Axonal injury could easily be identified and correlated to the existing inflammation in both models. We therefore propose the combination of both stains as a basic cost effective and time safe tool for the study of inflammation and neurodegeneration in the CNS.

**P512**

**Encephalopathy due to severe hyponatremia with cortical and basal ganglia lesions. A case report with MRI and SPECT studies.** A. Nouh, D. Milhaud, A. Barbaud, C. Delpirou-Nouh, B. Viaud-Rivallin, C. Heroum, CHU Montpellier (Montpellier, F)

Objective: To demonstrate that osmotic encephalopathy could be the result of severe hyponatremia, even in the case of gradual and progressive correction of the natremia.

Subject: We report the case of a female patient, 60 year old, who was first admitted to the Infectious Disease Department after the rapid installation of high Fever, Confusion, Headache, Vomiting and Diarrhea. Blood chemistry showed natremia at 130 mmol/l. Blood smear was positive for Plasmodium Falciparium.Treatment was initiated with Intra-Venous Qui-

nine, and three liters per day of 5% Dextrose. Over the next 48 hours, the patient became, progressively, stuporous, drowsy, and then developped seizures and further deterioration into unresponsive reaction with Glasgow coma scale at 6. The patient was transferred into our unit.

She was intubated with mechanical ventilation. Blood chemistry showed natremia at 110 mmol/l, kalemia at 2.8 mmol/l, plasmatic osmolality was at 229 mmol/l. We began hydric restriction for gradual natremia correction with 1 liter of saline 0.9% perfusion.

Natremia increased to 115 mmo/l at the sixth hour, 123 mmol/l at the fifteenth hour, and 132 mmol/l at the twenty eighth hour.

The extubation was only possible on day 15. Two MRI (T1, T2, T1 with Gadolinum injection, Flair, Diffusion) were performed, on days 13 and 35; they revealed widespread linear and punctuate hyper-signals with slight gadolinium enhancement, in the cortico-subcortical regions and in the basal ganglia (BG), especially the Putamina. There was no myelinolysis centro-pontine. The SPECT showed an extensive cortical hypo-perfusion and the same in the BG.

Clinical course was marked by a vigil coma stat, with spastic quadriplegia, mute and permanent trismus, no response to orders was obtained. Patient could open her eyes without purpose.

Conclusion: Brain lesions with severe clinical stat were due to metabolic encephalopathy with bilateral cortical and BG lesions after severe hyponatremia related to perfusion of high amount of Dextrose, though, the correction was gradual and progressive. MRI findings are very similar to lesions observed after cerebral anoxia. It seems that brain lesions occurred in accord with the severity and the duration of the hyponatremia. The rapid correction can probably be incriminated as some authors mentioned it in the litterature (Calakos, et al. (2000) Neurology 55(7):1048), otherwise, this was not observed with our patient.

P513

**Asymmetrical cortical and subcortical involvement on MRI in acute hepatic encephalopathy.** V. García-Gil, M. Varela, B. Gómez-Ansón, A. Escorsell, M.-J. Martí, Hospital Clinic (Barcelona, E)

Introduction: Hepatic encephalopathy (HE) secondary to acute liver damage is not uncommon. Perhaps due to high rate of mortality and difficulties in managing these patients, there are very few reports concerning neuroimage on acute HE. We describe a patient with severe acute alcoholic hepatitis who presented seizures, visual hallucinations, delusions and asymmetrical cortical and subcortical involvement on MRI, in keeping with cerebral oedema and cortical laminar necrosis.

Case report: A 40-year-old enolic non cirrhotic woman was admitted to emergency after three days of progressive abdominal swelling and black depositions without haemodynamic consequences. Physical examination revealed jaundice, clear ascitis, melaenas, drowsiness and no malnutrition signs. Liver function was severely impaired (prothrombin activity 35%), and increased ammonia levels were present. In the emergency room, the patient developed progressive stupor and coma followed by generalized seizures with respiratory compromise and the patient had to be mechanically ventilated.

Cranial CT at this point showed cortical swelling in the right temporal region, but was otherwise normal. EEG excluded epileptic status. CSF sample cultures and PCR were negative for herpes virus. Toxic screening including opiates and cocaine was also negative.

When sedation was stopped the patient remained with impaired consciousness and there were partial seizures affecting the right limbs. A repeat EEG showed diffuse slowing and right temporal PLED (periodic lateralized epileptiform discharges) and sodium valproate was started. MRI showed cortical swelling and increased signal on T2-weighted images in the right cerebral haemisphere and in the left fronto-temporal region. There was some mass effect on the right lateral and 3rd ventricle. Increased T2-signal was also present in both thalami, head of the right caudate, and in the periventricular white matter. There was no contrast enhancement.

Fifteen days after admission, the patient's level of consciousness improved, although there were visual hallucinations, delusions, myoclonus and an abnormal memory. Follow-up MRIs showed some improvement, but persistent signal change and cortical swelling in a bilateral, asymmetrical fashion.

Discussion and conclusion: There is limited evidence of cortical involvement seen on MRI in patients with acute HE. Recently reported a case of acute HE with diffuse, symmetrical cortical involvement, similar to that of hypoxic brain damage, evolving to cortical laminar necrosis.

In our patient, MRI showed asymmetrical cortical and subcortical lesions, seen as swelling, and signal change. The asymmetrical pattern of involvement seen in our case has not been described yet in acute HE, and its histological correlation and significance remain unknown.

P514

**Seizure disorder associated with normal neuroimaging studies in Wegener's granulomatosis.** V. Mastorodimos, M. Spilioti, M. Mamoulaki, H. Kritikos, D. Boumpas, A. Plaitakis, University Hospital of Crete (Heraklion, GR)

Objective: Wegener's granulomatosis (WG) presenting with seizures is quite rare. To our knowledge all the reported cases of WG had abnormal neuroimaging findings. We describe a patient presented with seizures who later fulfilled the American College of Rheumatology (ACR) criteria for WG.

Case summary: A 41 year old male was admitted to the Neurology Department because of new onset generalised tonic-clonic seizure. He had a similar episode one month prior to his admission. His past medical history was essentially negative.

Physical examination, including fundoscopy and neurologic examination, was normal. Meningeal signs were absent. Laboratory investigation revealed WBC 11.200, Hct 37.5, ESR 44 mm/hr, CRP 5.16 mg/dl. Serum biochemistry was normal. Mantoux skin reaction was negative. The chest x-ray on admission revealed hilar enlargement with basilar fibrotic lesions bilaterally. High Recolution Computerised Tomography (HRCT) of the lung showed small ground glass opacities. The bronchoscopy with cytology revealed no abnormalities. Lung biopsy revealed no granulomas or vasculitis. The CT, Magnetic Resonance Imaging (MRI) and MR angiography of the brain were normal. Lymphocytic pleocytosis (52 cells/mm$^3$) was found in the cerebrospinal fluid (CSF). CSF glucose was normal whereas CSF protein was slightly elevated. Multiple CSF cultures for infectious microorganisms were negative. The serum antineutrophil cytoplasmic autoantibodies with cytoplasmic pattern (c-ANCA) titer was positive. The patient was treated initially with steroids for possible WG despite absence of renal and sinus involvement. Five months later the patient developed right pleural effusion and microscopic hematuria. A renal biopsy was performed that confirmed the diagnosis of Wegener's granulomatosis.

Conclusions: Seizure disorder with normal neuroimaging findings can be a presentation of Wegener's granulomatosis. In this patient, CSF analysis revealed aseptic meningitis suggestive that the seizure disorder was due to small vessel vasculitis. Seizures as a lone neurologic manifestation in young adults should raise suspicion of Wegener's granulomatosis.

P515

**TIA, migrainous TIA and sleep apnea.** M. Beaudry, A. Brassard, M. Hudon, C. H. Sagamie, UQAC (Chicoutimi, CAN)

Background: Sleep apnea syndrome (SAS) is considered as a putative risk factor for stroke. It has been found that up to 70% of stroke survivors present an apnea-hypopnea index (AHI) greater then 10 per hour. Similar figures have been found in transient ischemic attacks (TIA) patients. Because of a similar frequency in TIA it is postulated that SAS is present before stroke and not the contrary.

Methods: We recruited 22 TIA patients, 8 patients with seemingly migrainous accompaniments and 15 controls (spouse of TIA cases). All were subjected to neuropsychological tests, a nocturnal polysomnogram and standard questionnaire.

Results: All 3 groups were well matched on age (62.77 ± 9.6, 60 ± 7.6, 64.5 ± 7.5 respectively).Controls were found to be heavier on Body Mass Index vs patients (28.4, 28.75 and 30.5). It was found that migrainous TIA had a lower AHI compared to TIA or controls (10.29, 3.47 and 9.8). This finding was significant. No patients with migrainous TIA had an AHI superior to 10 whereas 45% of TIA had that finding (43% of controls had AHI superior to 10).

Conclusion: Our results confirm other findings on the high frequency of OSAS in TIA population. It was observed that no patient in the migrainous TIA group had an AHI superior to 10. This suggests different physiopathological mechanisms. Although vasospasm exists in migraine it does not seem to be related to the hemodynamic instability associated with sleep apnea.

## Genetics

P516

**Sporadic and familial Parkinson's disease are quite similar in Crete, Greece.** C. Spanaki, A. Plaitakis, University of Crete (Heraklion, GR)

A prospective study was performed on all patients with parkinsonism seen consecutively at the University Hospital of Crete between 1997 and

2002 according to a predesigned protocol, in order to evaluate the role of genetic factors in the pathogenesis of Parkinson's disease. A detailed history, which included a questionnaire of 40 inquiries, was obtained from each patient with symptoms and signs suggestive of PD. All patients underwent thorough physical and neurologic examinations. PD was diagnosed according to established criteria. The final diagnosis was confirmed by two neurologists with expertise in extrapyramidal syndromes. All patients were examined for the presence of motor fluctuations, dyskinesias, painful dystonias, psychosis and dementia. Secondary forms of PD were excluded.

Two hundred forty seven patients were diagnosed as suffering from definite PD. Sixty-two of them (25%) had one or more similarly affected relative(s) (familial PD), 163 patients (66%) had no other affected relatives (sporadic PD) and 22 patients had relatives who exhibited just tremor and could not be classified into any of the above categories.

Clinical and epidemiologic characteristics did not differ significantly between familial and sporadic PD cases. Age at disease onset was 63.9 ± 1.4 years for familial and 64.2 ± 0.8 years for sporadic Parkinson's disease patients. Disease duration was 8.5 ± 0.8 years and 7.1 ± 0.5 respectively. Males and females, smokers and non smokers, urban and rural residents were equally distributed among the two groups. Clinical characteristics, such as type of onset, predominant symptom, presence of dyskinesias, fluctuations, dementia, psychosis, as well as dopa dosing and response to treatment were similar between the two groups.

Conclusion: Familial Parkinson's disease on Crete is clinically indistinguishable from the sporadic form of the disease. Detection of the molecular defect(s) responsible for these cases could have implications for elucidating the etiology of primary (idiopathic) Parkinsonism.

**P517**

**Pedigree analysis of late onset, L-dopa responsive, Parkinson's disease in Crete suggests a complex inheritance pattern.** C. Spanaki, A. Plaitakis, University of Crete (Heraklion, GR)

Parkinson's disease is etiologically heterogeneous with rare autosomal dominant and recessive forms of the disorder being linked to single gene defects or to specific chromosomal loci. However, the etiology of the common, late-onset, sporadic disease remains unclear. We performed a prospective study to explore genetic aspects of Parkinson's disease in natives of Crete. All patients with movement disorder, seen consecutively between 1997 and 2002, were thoroughly investigated. Detailed genealogic information spanning 4–5 generations was obtained for each patient on whom Parkinson's disease was diagnosed and the neurological status of their relatives was ascertained. The diagnosis of Parkinson's disease was confirmed in 247 index cases, the overwhelming majority of which had the typical late-onset, L-dopa-responsive form of the disorder. Two hundred and forty seven controls were drawn at random from the local population and were matched to Parkinson's disease patients.

The estimated relative risk for Parkinson's disease was 2.99 (95% CI 1.88–5.25, $p < 0.001$) for a first-degree relative (> 50 years) of patients with late-onset Parkinson's disease. About 25% (62/247) of index cases had one or more relatives affected by Parkinson's disease, but analysis of pedigree data revealed no clear Mendelian inheritance. Eleven pedigrees were identified in which Parkinson's disease originated from both paternal and maternal sides (bilineal transmission). A high proportion of the bilineal offspring older than 60 years (31 out of 47 or 66%), were affected by typical, late-onset Parkinson's disease. In contrast, in the succeeding unilineal generation only 6.5% (6/92, $p < 0.001$) of siblings older than 60 years had Parkinson's disease. Also in the preceding generation (that of the parents of the bilineal cases), 17.5% (14/80) of siblings older than 60 years were affected by Parkinson's disease.

Conclusion: When Parkinson's disease is transmitted through both parents, a high proportion of the bilineal siblings are affected by the disease. A marked dilution in the risk for developing Parkinson's disease was observed in the succeeding unilineal generation. These data suggest that digenic or other type of polygenic inheritance may underlie the disorder.

**P518**

**Comparison of clinical and electrophysiological alterations in patients with spinocerebellar ataxias type 1 and type 2.** M. Rakowicz, E. Zdzienicka, M. Niewiadomska, A. Sulek, D. Hoffman-Zacharska, R. Poniatowska, E. Pilkowska, C. Glazowski, J. Zaremba, U. Zalewska, Institute of Psychiatry and Neurology (Warsaw, PL)

Background: Spinocerebellar ataxias type 1 (SCA1) and type 2 (SCA2) belong to the group of neurodegenerative disorders of autosomal dominant inheritance caused by the expansion of trinucleotyde CAG. Trunk and limbs ataxia, dysarthria, gaze palsy, sensory and motor axonal neuropathy, pyramidal signs, and impairment of cognitive functions were the dominant signs in both disorders. In most SCA cases a clinical examination does not reveal any lesion of peripheral neuron, but electrophysiological studies can demonstrate this kind of subclinical damage.

Objective: Clinical and electrophysiological comparison of the functional state of somatosensory pathways, the lesion of peripheral nerves, and the degree of the cerebellar and cervical spinal cord atrophy in magnetic resonance imaging (MRI) for the verification of SCA1 and SCA2 phenotypes in patients molecularly confirmed.

Methods: 20 patients with SCA1 and 14 cases with SCA2 were neurologically examined using the International Co-operative Ataxia Rating Scale. Somatosensory evoked potentials followed median (Mn-SEPs) and tibial (Tn-SEPs) nerves stimulation and neurography of axillary, median, ulnar, femoral, peroneal and sural nerves were performed. Central, peripheral sensory conduction times, peripheral conduction velocity and amplitudes of evoked potentials at different levels were analysed. MRI images were performed to measure the brain's atrophy, cerebellum and cervical spinal cord.

Results: The age of patients in SCA1 (mean 42.3 ± 9.8) and SCA2 (mean 39.9 ± 13.4), and the duration of the disease 7.3 ± 4.2 and 11.4 ± 12.2, respectively, were not statistically different. The CAG repeats in both cases were in the range: in SCA1 from 45 to 62, in SCA2 from 37 to 52 and correlated inversely with the age of disease onset: $r = -0.89$, and $r = -0.56$ respectively. Neurological evaluation disclosed dominant pyramidal syndrome in the SCA1 group. For the SCA2 patients the decreased muscle tone and reflexes were more characteristic. Ataxia Rating Scale was similar in both groups of patients and ranged from 1 to 53 and from 4 to 69 respectively. In SCA1 patients, the spinal conduction time was significantly prolonged in 80% of Mn-SEPs and in 73% cases of Tn-SEPs, while in SCA2 patients it was 70% and 62% respectively. The neurography revealed motor and sensory neuropathy in all examined patients: axonal dying-back in 57% of SCA1 and 71% of SCA2 cases accompanied by demyelination in 43% and 29% patients respectively. The lesion of sensory fibres was most pronounced in SCA2 group, especially in peroneal nerves. MRI showed more evident cerebellar and brain stem atrophy in SCA2 patients, while the flattening of cervical spinal cord in 55% of SCA1 patients was observed.

Conclusions: The results provide further evidence for a similar type of lesion of the motor and sensory peripheral fibres, and of somatosensory afferents, despite different features of phenotypes in those two forms of SCA.

**P519**

**Alpha1 antichymotrypsin gene polymorphism and clinical outcome after traumatic brain injury.** E. Dardiotis, K. Aggelakis, K. Paterakis, A. Karantanas, P. Kolia, A. Komnos, A. Papadimitiou, G. Hadjigeorgiou, University of Larissa (Larissa, GR)

Unfavorable clinical outcome after traumatic brain injury (TBI) has been associated with the presence of hemorrhagic diffuse axonal injury (DAI). Although MRI has been shown to be much more sensitive for DAI detection compared to CT-scan, and due to expense and less availability, its application in TBI patients has been limited for TBI patients whose clinical status is in discordance with the CT findings.

Alpha1 antichymotrypsin (ACT) is located on chromosome 14 and is a member of the serine protease inhibitor superfamily. ACT is an inhibitor of the neutrophil cathepsin G, which via the cathepsin G platelet receptor promotes platelet aggregation. A polymorphism in the signal peptide sequence (-17Ala/Thr; allele A and allele T) of the ACT gene has been found to confer a significant risk for hemorrhagic stroke in normotensive subjects.

Using standard molecular methods we determined the ACT genotypes and alleles and tested their ability to predict the type of DAI (hemorrhagic or non-hemorrhagic) and the clinical outcome after six months in 72 survivors (13 women and 59 men; mean age = 31.9 ± 12.8 years) of TBI. Six-month favourable outcome defined in advance by means of the Glasgow Outcome Scale as good recovery or with moderate disability whilst severe disability or vegetative state referred as unfavourable outcome. Brain MRI imaging was performed in all patients under a single criterion, namely discrepancy between normal or minor brain CT findings and the Glasgow Coma Scale estimation. The possible association of the ACT polymorphism with the presence of hemorrhagic DAI and clinical outcome was tested using the Fisher's exact test.

Results: Thirty-five patients (35/51, 68.6%) with at least one T allele had unfavourable clinical outcome compared to 8 (8/21, 38.1%) of patients without the T allele ($p = 0.02$). Patients with hemorrhagic DAI also showed an increased frequency of T allele carriers (19/22, 86.4%) compared to pa-

tients with non-hemorrhagic DAI (32/50, 64 %) but this difference did not reach statistical significance ($p = 0.05$).

Conclusions: We have shown that ACT genotype influences the six-month clinical outcome in survivors after TBI. Our results suggest that this influence is probably due to the tendency of T allele carriers for hemorrhagic DAI.

P520

**An mtDNA C5586T transition in a patient with progressive external ophthalmoplegia.** A. Kladi, M. Panas, N. Kalfakis, K. Spengos, D. Vassilopoulos, University of Athens (Athens, GR)

Introduction: Progressive external ophthalmoplegia (PEO) is a common clinical manifestation of mitochondrial disease. The disorder is due to various large mitochondrial DNA (mtDNA) deletions or point mutations of tRNA genes.

Case report: A 64-year-old woman reported a progressive bilateral eyelid ptosis over the last 8 years. She also reported occasional diplopia. The neurological examination was normal, apart from bilateral ptosis and reduced lateral eye movements. Her family history was negative. The routine laboratory investigations were normal. Only the resting serum lactate was mildly elevated. Electromyography and Magnetic Resonance Imaging of the brain were normal.

Methods and Result: Genomic DNA was isolated from blood cells using the salting out lysis procedure. Southern analysis of mtDNA revealed no single or multiple deletions or duplications. In a search for point mutations, sequencing revealed a homoplasmic C to T transition at 5586 nucleotide in a non-coding region of mtDNA. A PCR assay digested with Dde I restriction endonuclease was designed to confirm the presence of the transition.

Discussion: Our finding was detected in a non-coding region, where no other DNA variations have previously been reported. Variations found in other non-coding regions are known to influence the susceptibility for mutations in distal genes. Our finding was not a polymorphism, since it was not found in 200 healthy controls and the search for the usual PEO mutations in our patient was negative. Therefore, it is possible that our finding is a mutation related to the pathogenesis of the disease.

P521

**Spinocerebellar ataxia and hypergonadotropic hypogonadism associated with familial sensorineural hearing loss.** S. Papapetropoulos, N. A. Georgopoulos, E. Chroni, E. S. Papadeas, P. A. Dimopoulos, V. Kyriazopoulou, M. B. Davis, L. Eunson, G. Kourounis, V. A. Tzigounis, Medical School of Patras University, Institute of Neurology (Patras, GR; London, UK)

The association of cerebellar ataxia and hypergonadotropic hypogonadism is a rare genetic disorder with a recessive mode of inheritance.

We report a case of a woman with early onset spino-cerebellar ataxia, primary amenorrhea due to hypergonadotropic hypogonadism, and a late onset sensorineural hearing loss. Additional family members from the father side are affected with late onset hearing loss, suggesting a recessive mode of inheritance.

Physical examination revealed height 160 cm, and weight 62kg. Her skull was normocephalic with relative mid-face hypoplasia and an upper cleft lip, giving the impression of prognathism. Her breast development was Tanner Stage III for the right breast and Tanner stage IV for the left breast and her pubic hair development was Tanner stage V. External genitalia were those of a normal female, without clitoris enlargement. She presented no clinical signs of Turner syndrome and she was clinically euthyroid.

On neurological examination, she was intellectually intact with a normal I. Q. Her gait was broad based, she was unable to tandem walk and had a positive Romberg's sign. There were signs of cerebellum dysfunction including a mildly slurred speech, bilateral horizontal gaze evoked nystagmus, dysmetria of limb movements, clumsiness of finger and toe tapping, dysdiadochokinesia and mild action tremor of the hands. Muscle strength and tone were normal. The deep tendon reflexes were all either reduced or absent. Vibration sense was moderately impaired. The neurophysiological evaluation showed that the sensory evoked potentials of all tested nerves were absent.

Pure tone audiometry demonstrated a sensorineural hearing loss in bass frequency.

On Ultrasound, her uterus was hypoplastic with no evidence of endometrium and relatively hypoplastic ovaries without any evidence of growing oocytes in the ovarian stroma.

MR scan showed mild vermian and cerebellar hypoplasia.

Her karyotype was 46XX. DNA analysis for expansions at the SCA 1, 2, 3, 6, 7 and FRDA loci were negative.

P522

**Fabry disease: beneficial long-term effects of agalsidase alfa confirmed in FOS – the Fabry Outcome Survey.** A. Mehta, M. Beck, F. Dehout, A. Garcia de Lorenzo, A. Linhart, G. Sunder-Plassmann, R. Ricci, U. Widmer, Royal Free Hospital, University of Mainz, CHU de Charleroi, Formacion Medica Continuada Hospital Universitario, Charles University, University of Vienna, UCSC, University of Zurich (London, UK; Mainz, D; Charleroi, B; Madrid, E; Prague, CZ; Vienna, A; Rome, I; Zurich, CH)

Deficient activity of the lysosomal enzyme alpha-galactosidase A in patients with Fabry disease results in the accumulation of neutral glycosphingolipids, primarily globotriaosylceramide (Gb3), in various organs and tissues, including the central nervous system and neurons of the dorsal root ganglia and the autonomic nervous system. The progressive deposition of Gb3 leads to a wide variety of symptoms including acroparaesthesia, pain crises, hypohydrosis, intestinal dysmobility and personality changes, as well as renal failure, cardiovascular disease, cerebrovascular accidents and dermatological lesions (angiokeratoma). The wide range and variable nature of the signs and symptoms of Fabry disease frequently leads to misdiagnosis and inappropriate treatment. This is particularly important as enzyme replacement therapy (ERT) has recently been successfully introduced to treat the disease. FOS – the Fabry Outcome Survey – is a European outcomes database for patients who are receiving, or candidates for, ERT with Replagal™ (agalsidase alfa; TKT-5S, Danderyd, Sweden). Currently, 336 patients (182 males and 154 females) are included in FOS, making it the world's largest studied cohort of patients with Fabry disease. Of these, 217 are receiving treatment with agalsidase alfa, with 58 % having been treated for more than 1 year. At entry into FOS, males were 35.3 ± 12.6 and females 40.7 ± 16.9 years of age; 35 patients were under 18 years of age (18 boys and 17 girls). The majority of patients in FOS have manifestations of Fabry disease in at least six organ systems, with neuropathy, gastrointestinal symptoms and impaired renal function being major causes of morbidity.

Analysis of FOS data supports the results from previous clinical trials in relatively small numbers of patients. These have shown that ERT with agalsidase alfa has positive effects on the renal, neurological and cardiac manifestations of the disease, as well as improving quality of life. In FOS, 12 months of agalsidase alfa treatment not only stabilized renal function but, in patients with mild renal damage, significantly ($p < 0.001$) improved the glomerular filtration rate. Likewise, agalsidase alfa treatment significantly ($p < 0.05$) improved eight out of eleven measures of pain-related quality of life, as assessed using the Brief Pain Inventory.

This first analysis of efficacy data from FOS suggests that ERT with agalsidase alfa produces clinically important benefits in patients with Fabry disease.

P523

**Interleukin-1A [–889] genetic polymorphism increases the risk of multiple system atrophy.** O. Combarros, J. Infante, J. Berciano, J. Llorca, University Hospital Marques de Valdecilla, School of Medicine (Santander, E)

Background: The pathogenesis of multiple system atrophy (MSA) involves a significant local inflammatory response within the brain as evidenced by the presence of activated microglial cells that produce proinflammatory cytokines such as interleukin-1A (IL-1A). A polymorphism (allele 1 or low secretor of IL-1A, and allele 2 or high secretor of IL-1A) in the regulatory region [–889] of the IL-1A gene is related to the amount of cytokine produced.

Objective: Our purpose was to investigate whether IL-1A allele 2 (a high secretor of IL-1A) is associated with increased risk for MSA.

Methods: The study included 30 patients (47 % females; mean age 57.0 ± 12.1 years; range 36–74 years) fulfilling the criteria for MSA, and 110 control subjects (50 % females; mean age 58.1 ± 10.9 years; range 35–71 years) without clinical evidence of neurologic disease or familial history of neurodegenerative disease.

Results: The IL-1A allele 2 was overrepresented in MSA compared with controls, and a gene dose effect for this allele was observed: the risk of developing MSA with two copies of the IL-1A allele 2 (odds ratio = 5.1) was approximately triple that of one copy of the IL-1A allele 2 (odds ratio = 1.6) ($p$ for trend = 0.018).

Conclusions: This is the first report of a cytokine gene association with increased risk of MSA, presumably by increasing the amount of cytokine-mediated inflammation in the brain. Our results suggest that patients with MSA display a heritable proinflammatory phenotype associated to overrepresentation of IL-1A allele 2, that may contribute to the development of MSA.

P524
**GAA expansion size and age at onset of Friedreich's ataxia.** O. Combarros, I. Mateo, J. Berciano, J. Llorca, V. Volpini, J. Corral, University Hospital Marques de Valdecilla, School of Medicine, Molecular Genetics Centre (Santander, Barcelona, E)

Background: The size of the smaller GAA expansion (GAA1 allele) within intron 1 of the FRDA gene located on chromosome 9q13 is the major determining factor for the variability in age at onset of Friedreich's ataxia (FA), whereas the size of the larger GAA expansion (GAA2 allele) has been a poor predictor of age at onset.

Objective: We have assessed the predictive value of GAA1 and GAA2 sizes in regard to age at onset of FA.

Methods: We selected a group of 40 patients homozygous for GAA expansion on FRDA gene. Relationships between GAA expansion sizes and age at onset of FA were studied using both the Pearson correlation coefficient (r) and multiple linear regression.

Results: We split the data based upon GAA2 alleles of more or less 800 repeats, which is the largest size of the GAA1 allele in our series, and GAA1 size accounted for 43% of the variation in age at onset ($r = -0.66$), and smaller (less than 800 repeats) GAA2 alleles accounted for 42% of the variation in age at onset ($r = -0.65$).

Conclusions: For the first time ever our analysis showed that GAA2 alleles of less than 800 repeats are as good predictors of age at onset as GAA1 alleles. FA mutation is supposed to cause a loss of function, and smaller GAA expansions do not totally abolish processing of the frataxin. Our data suggest that beyond a threshold of 800 repeats, the residual expression of frataxin is too low to influence the age at onset of FA.

## Neuro-immunology

P525
**Interleukin-1 beta (IL-1B) and interleukin-1 receptor antagonist (IL-1RA) genes polymorphisms and clinical course of ischaemic stroke.** G. Gromadzka, I. Sarzynska-Dlugosz, A. Ciesielska, A. Czlonkowska, Institute of Psychiatry and Neurology (Warsaw, PL)

Background: Proinflammatory cytokines are thought as mainly responsible for the onset of postischemic inflammatory cascade. One of the proinflammatory cytokines that has been identified as an important mediator of inflammation induced by IS is IL-1. IL-1 levels exhibit stable inter-individual differences which are associated with polymorphisms in nucleotide sequences of the genes for IL-1alpha, IL-1B and IL-1RA which are found in a cluster on chromosome 2. Of those, our interest has focused on two: C-511T polymorphism in the IL-1B gene and VNTR (variable number tandem repeats) polymorphism within the fourth exon of the IL-1RA gene (IL-1RN). Allele-2 of the IL-1RN polymorphism is associated with increased production of IL-1RA; the less frequent allele T of IL-1B gene polymorphism is associated with a high-secretor phenotype.

Aim of study: to assess whether molecular variations at the IL-1B and IL-1RN loci influence the clinical course of IS.

Methods: Genotyping was performed in 301 patients with IS. IL-1RN VNTR polymorphism was typed by polymerase chain reaction (PCR) method; genotyping of the C-511T polymorphism in the IL-1B gene was performed by PCR-restriction fragment length polymorphism (PCR RFLP) method.

Results: Genotypes frequency for IL-1B: C/C: 48.65%, C/T: 40.22%, T/T: 11.13%; IL-1RN: 1/1: 53.82%, 1/2: 33.22%, 2/2: 7.31%, 1/3: 4.98%, 2/3: 0.67%. The frequency of allele T of IL-1B gene: 40.86%; the frequency of allele 2 of the IL-1RN: 40,86%. Any significant differences were noticed between carrying of subsequent genotypes and stroke types, size and number of lesions. The carrying of genotype A/A of the IL-1B gene was associated with more frequent occlusion in carotid artery. The carrying of allele 2 of IL-1B gene was associated with significantly higher 30-days mortality rate, as compared with "non carrying" of this allele ($p = 0.05$). The homozygotic carrying of allele 2 of IL-1RN was associated with significantly better neurological outcome as measured using the Scandinavian Stroke Scale (SSS) at entry and at discharge ($p = 0.03$; $p = 0.008$; respectively), activity daily living (ADL) measured using Barthel Stroke Scale at 7th day after CI ($p = 0.04$), and lower degree of independency measured with Rankin Scale ($p = 0.04$) as compared to the carrying of the rest of IL-1RN genotypes.

Conclusion: Results of our study suggest that genetic variation at the IL-1B and IL-1RN loci in Polish stroke patients population is a genetic factor that influences the clinical course of the disease.

P526
**Autoimmune reaction causes less degeneration of neurons injured with 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP) intoxication in mice.** I. Kurkowska-Jastrzebska, E. Balkowiec-Iskra, I. Joniec, T. Litwin, A. Przybylkowski, A. Ciesielska, A. Czlonkowski, A. Czlonkowska, Institute of Psychiatry and Neurology (Warsaw, PL)

Many recent studies showed the evidence that inflammatory reaction may have a protective effect in neurodegenerative processes. An important part of inflammatory response after neuronal injury are autoreactive T lymphocytes and macrophages infiltrating affected area. Autoreactive T cells are present in physiologic state and are activated during any brain injury. They are able to diminish degeneration and stimulate regeneration of neurons injured by other factors.

In the present study we investigated an influence of autoimmune reaction induced by administration of myelin protein, MOG 35–55, on toxic neurodegeneration of dopaminergic cells of the substantia nigra (SN) produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MOG administration to animal causes an induction of autoimmune response directed towards myelin protein, that starts autoimmune encephalomyelitis (EAE) with typical clinical signs.

C57Bl6 mice, young (2 months) and old (8–10 months old), received MOG in CFA, 6 days later followed by MPTP 40 mg/kg – toxin which selectively injures dopaminergic neurons in the SN. Dopamine content in striatum (HPLC), TH content in striatum (immunoblotting) as well as the number of TH + cells (immunochemisty) in the SN was estimated on the 3rd and 7th day after intoxication. The glial reaction (GFAP, CR3) and lymphocytes influx to the SN were also evaluated.

MPTP as given alone caused depletion of dopamine content (by 60%), TH content in striatum (by 20%) and of the cells number in the SN (by 55%) as compared to control. Mice receiving MOG in CFA before MPTP intoxication showed less neuronal impairment than mice receiving only MPTP. Dopamine content was higher by 19%, TH content in striatum as well as cell number in the SN was bigger by 15% as compared to MPTP group. No differences between young and old mice were marked. Glial reaction was smaller in mice receiving MOG in CFA than in mice receiving only MPTP as measured by microglial CR3 receptor expression in the SN and striatum and astrocytic GFAP expression in striatum. Lymphocytic infiltration in the SN and striatum was less in MOG treated mice but consisted of less CD8 + cells than in MPTP only treated mice.

Our study showed that autoimmune reaction induced prior to toxic impairment decreased dopaminergic neurons damage caused by MPTP. It indicates that autoimmune process may exert neuroprotective action in toxic degeneration in the central nervous system.

P527
**A nitric oxide releasing derivative of flurbiprofen inhibits experimental autoimmune encephalomyelitis in C57BL/6 mice.** A. Kurne, A. Bergami, L. Gasparini, R. Furlan, E. Ongini, G. Martino, Hacettepe University, San Raffaele Scientific Institute, Nicox Research Institute (Ankara, TR; Milan, Bresso, I)

Nitric oxide (NO)-releasing derivatives of non-steroidal anti-inflammatory drugs (NSAID) represent a new class of anti-inflammatory compounds which are more efficacious in blocking ongoing inflammation compared to conventional NSAID. We tested their therapeutic efficacy in experimental autoimmune encephalomyelitis (EAE), the elective model of multiple sclerosis. NO-releasing derivative of flurbiprofen (HCT 1026) was administered per os to C57BL/6 mice immunized with 50 microgram of MOG 35–55 in CFA and 500 nanogram of pertussis toxin. Mice eating a normal diet or with flurbiprofen only were used as controls. Administration of HCT-1026 beginning on the day of immunization significantly delayed the EAE onset ($27.8 \pm 2.9$ days postimmunization vs. in flurbiprofen treated mice vs. $20.3 \pm 3.7$ days and $11.0 \pm 0.5$ days postimmunization in controls; $p < 0.01$ logrank test) and reduced the severity of symptoms (mean maximum score $0.9 \pm 0.3$ vs. $1.6 \pm 0.2$ in flurbiprofen treated mice vs. $2.4 \pm 0.2$ in control mice; $p = 0.0033$ and $p = 0.0191$ Mann-Whitney, respectively). In 2 out of 7 mice (28%) HCT 1026 prevented the development of the disease. Therapeutic efficacy of HCT 1026 was not associated to decreased T cell proliferation ability or decreased IFN gamma release in response to in vitro MOG 35–55 re-stimulation. However MOG 35–55 specific T cells from HCT 1026 treated mice released increased levels of IL-10 as compared to T cells from sham-treated control mice.

We conclude that oral administration of the NO-releasing derivative of flurbiprofen HCT 1026 is able to inhibit EAE development in MOG 35–55-immunized C57BL/6 mice without overt side effects.

P528
**Splenocytes from donor mice ingesting IFN-alpha transfer protection against EAE in SCID recipients.** S. Brod, University of Texas (Houston, USA)

T cells from IFN fed, but not mock fed, immunized donors protect against active EAE in immuno-competent recipients (Brod et al. 2001). The mechanism of ingested IFN can be dissected by adoptive transfer of protective cells from IFN fed donors into B6 scid recipients.

C57BL/6 6–8 week old females were actively immunized s. c. with 200 mcg MOG peptide 35–55 and 800 mcg MT in IFA on day 0/7 and followed for disease. Non-immunized donor B6 mice ingested 100 IU of murine IFN or mock murine IFN daily for 14 days before spleen cells (SC) were harvested. SC from the MOG immunized donor mice were restimulated in vitro with 30 mcg/ml MOG peptide 35–55. A second set of splenocytes from the mock or IFN fed non-immunized donor mice were activated in vitro with 2.5 mcg/ml Con A. 100 million (M) MOG-restimulated cells were passively transferred i. p. into 8–10 week old female B6 scid recipients. Recipients also received EITHER 80 M Con A-activated SC from mock fed donors (control) OR 80 M Con A-activated SC from IFN fed donors (active treatment) and followed for EAE.

The recipients of MOG peptide AND Con A-activated mock fed SC (control) all showed clinical EAE progressing over the 2 weeks to peak scores of 2, 2.5, and 3.0. The recipients of MOG peptide AND Con A-activated IFN-a fed SC (active treatment) showed much less severe EAE.

We determined beta actin, IL-2, and IFN-g mRNA transcripts from control and active treatment spinal cords using Q-PCR. The mean measured cytokine of interest transcript levels were normalized to the beta actin control (normalized mean = COI mean/beta actin x 100) and expressed as % beta actin molecules. There were clear differences in the levels of pro-inflammatory IL-2 and IFN-g transcript (Th1-like cytokines) in the CNS in the scid recipients of IFN fed cells compared to the scid recipients of mock fed cells. There are detectable levels of IL-2 (normalized ratio = 0.19, 0.47) and IFN-g (normalized ratio = 0.13, 0.29. 0.35) transcripts in the mock recipients respectively but no detectable (below the threshold of detection = 20 transcripts) IL-2 and IFN-g transcripts in the scid recipients of IFN fed donors, differences that correlated with the clinical outcome. Differences in IL-4 and IL-10 will be measured. The use of whole spinal cord homogenates from EAE reconstituted scid recipients can provide useful data on important cytokines critical for EAE pathogenesis.

P529
**The influence of age and gender on the TNF-a mRNA expression in murine model of Parkinson's disease.** A. Ciesielska, I. Joniec, A. Przybylkowski, G. Gromadzka, A. Czlonkowska, A. Czlonkowski, Institute of Psychiatry and Neurology, Medical Academy (Warsaw, PL)

The incidence of Parkinson disease (PD) changes with gender and age. The reason for the gender difference in this disease is still unknown but a higher prevalence of PD in men suggests a link between gonadal hormone, such as estrogens, levels and PD. The involvement of immune mechanisms in the etiopathogenesis of PD is documented. The neuroinflammation is regulated by numerous signal molecules, including cytokines. TNF is one of the key proinflammatory cytokines implicated in immune response observed in PD. Estrogens have been shown to play a major role in inflammatory processes. One mechanism by which estrogens could modulate the immune reaction is regulation of the cytokines expression.

Age is an important risk factor for PD. Advanced age is associated with increased expression levels of various proinflammatory cytokines in that may enhance the brain's susceptibility to neurodegeneration.

We investigated the influence of age and gender on TNF gene expression in a murine model of PD induced by 1-methyl-4-phenyl-1,2,3,6 tetrahydropiridine (MPTP). The level of TNF mRNA was measured by RT-PCR in the striatum of male and female C57BL/6 mice (3 and 12 months old) after 6h; 1, 3, 7, 14, 21 days post MPTP intoxication.

Administration of MPTP caused a marked increase of TNF-a mRNA in striatum of young and aged male and female mice. In young and aged male mice the TNF mRNA showed similar expression pattern. TNF mRNA was rapidly increased already at 6h after MPTP injection and peaked at 1-day time point. The second increase of TNF mRNA was detected at the 7th day post intoxication. We observed that the increase of TNF mRNA was more prominent in the aged than in the young male. The pattern of expression of TNF mRNA in young and aged female mice differed from that observed in male mice. In female mice the significant increase of TNF mRNA was 24h after intoxication. The elevation was observed at 3-day time point. The expression of TNF mRNA had diminished at 7 day but not yet recovered to control level. The TNF mRNA was higher in aged female than in young, although this difference was less, as compared with that observed in male mice.

The data indicate that there is an age- and gender-dependent difference in the TNF gene expression profiles in striatum by MPTP injection. These observations may help in better understanding the age and gender difference which exist in Parkinson disease.

P530
**Interferon beta-1b modulates serum levels of soluble VCAM-1 in primary progressive multiple sclerosis patients.** D. Bahner, A. Bitsch, C. Klucke, E. Elitok, T. Bogumil, A. Dressel, T. Polak, H. Tumani, F. Weber, S. Poser, B. Kitze, Klinikum Fulda, Ruppiner Klinikum, Georg-August University, University of Ulm, Specialized Therapeutics, University of Greifswald, Klinikum Kassel, MPI for Psychiatry (Fulda, Neuruppin, Göttingen, Ulm, D; Montville, USA; Greifswald, Kassel, Munich, D)

The formation of CNS lesions in multiple sclerosis (MS) involves the migration of monocytes and lymphocytes from the blood into the parenchyma and is associated with an opening of the blood-brain-barrier (BBB). Adhesion molecules like sE-selectin and soluble vascular cell adhesion molecule-1 (sVCAM-1) mediate the complex process of immune cells trafficking across the BBB. Interferon beta-1b (IFNB-1b) upregulates sVCAM-1 levels in serum of patients with relapsing remitting MS (RRMS), proposing that the affection of T-cell/endothelial cell interaction was one possible mechanism of IFNB-1b action. This particular effect still has to be proven in primary progressive MS (PPMS). In the current study, serum concentrations of sVCAM-1 and sE-selectin were analyzed longitudinally in 18 PPMS patients before, during and after 12 months of treatment with IFNB-1b. SVCAM-1 but not sE-selectin levels of untreated patients were significantly elevated compared to age- and sex-matched controls. During drug therapy there was a significant early and sustained increase of sVCAM-1 (overall $p < 0.0001$). Levels remained elevated in most patients until three months after withdrawal of therapy. Concomitant infections were associated with higher sVCAM-1 levels. Neutralizing antibodies to IFNB-1b developed in 10 patients and were associated with lower sVCAM-1 levels. In contrast sE-selectin levels exhibited some fluctuations during therapy, but none was statistically significant. In conclusion, IFNB-1b modulates the adhesion cascade in patients with PPMS similar to its effects in RRMS, thus pointing to a possible role in treatment for this group of patients. To prove effectiveness in treatment of PPMS larger clinical, placebo-controlled trials are warranted.

## Motor neuron disease

P531
**Atypical symptoms and signs in patients with amyotrophic lateral sclerosis.** Z. Stevic, A. Vujic, R. Trikic, S. Pavlovic, S. Apostolski, Institute of Neurology (Belgrade, YU)

Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal progressive disorder characterized by muscle wasting and weakness. According to El Escorial diagnostic criteria for ALS (World Federation of Neurology, 1994), significant sensory or sphincter abnormalities exclude the diagnosis of ALS. The aim of this study was to investigate the frequency of atypical symptoms and signs in ALS patients.

Methods: The study population included 360 ALS patients, diagnosed in the period from 1994 to 2001 at the Institute of Neurology, Belgrade, Yugoslavia. Nineteen cases with familial ALS(FALS) were also included, 17 with mutation on Cu/Zn superoxide dismutase (SOD1) gene Leu144Phe and two patients with mutation of SOD1 gene Ala145Gly. The patients from both groups fulfilled the criteria of probable or definite ALS according to El Escorial criteria. In some patients sensory evoked potentials and cystometrograms were performed.

Results: 66 (18.3%) out of 360 ALS patients (51 males and 15 females, mean duration of the disease 19 months) had various symptoms and signs, atypical for ALS. The spinal onset of disease was more often than bulbar onset, 63 (94.5%) vs. 3 (4.5%). Sensory symptoms such as tingling, paraesthesiae, hyperaesthesiae and pain were present in 41 (11.4%) patients. Sensory examinations showed that 26 (39.4%) ALS patients had dysaesthesiae and hypalgesia in the hands and in the feet. In 14(21.2%) patients vibratory sensation was reduced in the feet. Five FALS patients with mutation Leu144Phe had paraesthesiae and reduced vibratory sensation in the feet. None of the patients with mutation Ala145Gly had any sensory symptoms or signs. In the remaining 30 (45.4%) ALS patients no objective sensory signs were found. Electrophysiological examinations of patients with atyp-

ical symptoms and signs revealed moderate distal symmetrical sensory polyneuropathy in 18 (27.3 %) patients. Among them 4 were FALS patients with the same mutation of SOD1 gene. Urinary abnormalities were present in 23 (34.8 %) patients, among whom 11 (47.8 %) had FALS with mutation on SOD1 Leu144Phe.

Conclusion: The sensory symptoms and signs, as well as urinary abnormalities were not infrequent in the observed group of our ALS patients. Those signs were more commonly seen in patients with spinal than the bulbar onset of ALS. The presence of atypical symptoms and signs in patients with ALS may indicate importance of SOD1 gene mutation screening.

P532

**Mitochondrial protein expression in a cell-culture model of CuZn superoxide dismutase-related familial amyotrophic lateral sclerosis: a proteomic study.** C. Wood-Allum, S. Allen, P. Shaw, Sheffield University Medical School (Sheffield, UK)

Introduction: Amyotrophic lateral sclerosis (ALS) is an adult-onset, incurable neurodegenerative disease causing progressive limb and bulbar muscle weakness. Some 10 % of ALS is familial, of which 20 % is the result of mutations to CuZn superoxide dismutase (SOD1). Recently, increasing evidence suggests that mitochondrial dysfunction may be important in ALS pathogenesis.

Aims: In order to clarify the molecular basis of the mitochondrial dysfunction previously observed in our cell-culture model of SOD1-related familial ALS, this study aims to examine changes in mitochondrial protein expression and post-translational modification due to expression of mutant human SOD1.

Methods: 2-D gel electrophoresis of mitochondrially-enriched preparations of NSC34 cells stably transfected with normal human SOD1, G93A mutant human SOD1 or empty vector alone, was used to identify protein spots whose expression in cells expressing mutant human SOD1 was different to that in control cells (non-parametric, paired Wilcoxon t-test). MALDI-TOF mass spectroscopy was performed on the spots of interest and database searching used to generate candidate protein identities.

Results: Three proteins are up-regulated in cells expressing G93A mutant human SOD1 compared to those expressing normal SOD1 and 2 are down-regulated. To date, 3 of these have been identified. One down-regulated protein forms part of the mitochondrial anti-oxidant defence system. Western blotting has been used to confirm both the identity of this protein and its down-regulation in NSC34 cells expressing mutant human SOD1. The functional significance of these changes is under investigation.

P533

**Pathological crying as the initial presenting symptom in amyotrophic lateral sclerosis.** A. Czaplinski, A. Steck, P. Andersen, S. Hartmann, M. Weber, University of Basel, University of Umea, Cantonal Hospital (Basel, CH; Umea, S; St.Gallen, CH)

Inappropriate and uncontrollable bouts of laughing and crying belong to the major symptoms in amyotrophic lateral sclerosis (ALS). Pathological laughter and crying occurs in 19–49 % of ALS patients and may be present in patients with and without pseudobulbar palsy. However, studies investigating the natural history of the disease indicate that pseudobulbar symptoms with loss of control of emotion occur generally in later stages of the disease.

A 73-year old, previously healthy nun was candle crafting when she experienced an acute, unprovoked, uncontrolled crying spell lasting 45 minutes. She lacked prior depression or prior crying spells. During this episode she rested her head on the table. When lifting her head she noticed difficulties speaking and weakness in her right arm. Over the next few days she additionally developed mild dysphagia and weakness progressed to her right leg. At the time of the first neurological consultation she was alert, and her mental status was normal. Neurological examination revealed moderate dysphagia and dysarthria, weakness of her right arm and leg in a pyramidal pattern but no wasting or fasciculations. Plantar responses were flexor bilaterally. Deep tendon reflexes were brisk, more pronounced on the right. Sensory examination was normal. A vascular event was considered the most likely diagnosis. However, CT scan, MRI, including diffusion weighted imaging (DWI) excluded a focal cerebral lesion. EEG, ultrasound studies and routine laboratory tests showed no abnormalities. One week later the patient was re-examined by the neurologist. By then she had developed shortness of breath. A few fasciculations were noted in her right arm and leg. Muscle tone in her right arm was slightly reduced, but increased in her lower limbs. Deep tendon jerks remained brisk with down going plantar responses. Needle electromyography (EMG) showed signs of both acute and chronic denervation and fasciculation potentials in all four limbs. Motor and sensory conduction velocities were normal. The diagnosis of probable ALS according to the revised El-Escorial criteria was made. IgM anti-GM1 antibodies were absent. There was no mutation in the SOD1 gene. During the next weeks, motor deficits, respiratory insufficiency and bulbar signs progressed. Four months after symptom onset the patient died of respiratory failure. To our knowledge, there are no prior reports of crying spells resulting from pseudobulbar palsy and heralding or signifying ALS.

P534

**Anti-annexin V antibodies (aANX V) in the cerebrospinal fluid and serum of patients with amyotrophic lateral sclerosis.** J. Ilzecka, Z. Stelmasiak, Medical University (Lublin, PL)

Introduction: Annexins belong to a family of calcium-dependent phospholipid-binding proteins. Their function is still unclear. Autoantibodies generated against annexins were detected in inflammatory and autoimmune diseases. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, in which inflammatory and autoimmune mechanisms may play a role.

Objectives: The aim of this study was to measure the anti-annexin V antibodies (aANX V) in the cerebrospinal fluid (CSF) and serum from ALS patients and control group people.

Methods: The study involved 25 (13 males/12 females) ALS patients with an average age of 58, and 20 (10 males/10 females) age-matched controls. The ALS patients were diagnosed according to the El Escorial criteria. The average duration of ALS was 16 [3–60] months. The aANX V were measured by the enzyme-linked immunosorbent assay.

Results: The aANX V were positive in the CSF of 16 % (4/25), and in the serum of 8 % (2/25) patients with ALS. All aANX V were detected in patients with bulbar onset of ALS and in patients with short duration (up to 8 months) of the disease. The aANX V were not detected in the CSF and serum from controls.

Conclusions: Results showed that aANX V may be involved in neurodegeneration in bulbar type of ALS onset and in the early phase of the disease. The presence of aANX V may be associated with inflammatory or autoimmune reaction which is observed in ALS.

P535

**Incidence of presumptive tardive dyskinesia in elderly patients treated with olanzapine or conventional antipsychotics.** B. Kinon, V. Stauffer, C. Kaiser, D. Hay, S. Kollack-Walker, Eli Lilly and Company (Indianapolis, USA)

Background: Incidence rates of presumptive tardive dyskinesia (TD) were compared in acutely psychotic or agitated elderly patients treated with olanzapine (OLZ) or conventional antipsychotic (CNV) drug therapy.

Methods: Patients without TD were randomized to OLZ (2.5–20 mg/day; $n = 150$) or CNV (dosed per label; $n = 143$) therapy, and underwent a 6-week drug tapering/drug initiation period, followed by reassessment of TD. Patients remaining without TD after six weeks were treated with OLZ or CNV for up to 1 year. Primary analysis was time-to-TD incidence, defined as rating on the Abnormal Involuntary Movement Scale (AIMS) of either: A) moderate severity ($\geq 3$) in 1 body region or mild severity ($\geq 2$) in 2 or more body regions, or B) moderate severity ($\geq 3$) in 1 body region.

Results: Patients in CNV group were at a greater risk for presumptive TD than patients in OLZ group (criteria A or B, $p < 0.05$). Incidence of presumptive TD that persisted for at least 1 month was lower and differed between treatments only for criterion B (moderately severe symptoms; $p < 0.05$).

Conclusions: In elderly patients who are at a greater risk for developing TD, these data revealed a lower risk of developing dyskinetic symptoms in patients treated with olanzapine versus conventional antipsychotics.

P536

**Cerebrospinal fluid cyclic guanosine 5' monophosphate level in patients with amyotrophic lateral sclerosis.** J. Ilzecka, Z. Stelmasiak, Department of Neurology, Medical University (Lublin, PL)

Introduction: The role of cyclic guanosine 5' monophosphate (cGMP) in neurodegeneration of motor neurons in amyotrophic lateral sclerosis (ALS) is still controversial. It was proposed that cGMP can prevent motor neuron death mediated by glutamate excitotoxicity and oxygen free radicals.

Objectives: The aim of the study was to measure cGMP levels in the cerebrospinal fluid (CSF) from ALS patients and control group people, and to investigate whether there is a relationship between CSF cGMP levels and clinical parameters of the disease.

Methods: The study involved 30 (18 males/12 females) ALS patients with an average age of 55, and 20 (10 males/10 females) age-matched controls. The average duration of ALS was 18 [3–48] months. The ALS patients were divided into the groups according to the clinical state of patients (mild/severe), type of ALS onset (bulbar/limb), and duration of the disease (short – up to 12 months/long – over 12 months). The cGMP was measured by the enzyme-linked immunosorbent assay. The Mann-Whitney U test was used to examine the differences between the groups. The correlation analysis was performed using Spearman's correlation coefficient.

Results: The CSF cGMP level was significantly lower in ALS patients (mean 2.08 SD 1.45; median 1.64 (0.48–6.45) pmol/ml, compared with controls (mean 3.90 SD 2.58; median 3.23 (0.99–9.74) pmol/ml ($p < 0.05$). The clinical state of ALS patients, type of ALS onset, and duration of the disease did not influence CSF cGMP levels ($p > 0.05$). There was also no significant correlation between CSF cGMP levels and clinical state of patients, and duration of the disease ($p > 0.05$).

Conclusions: Because extracellular cGMP may have a neuroprotective activity, a decrease in cGMP level, observed in this study, could lead to accelerated motor neuron death. Thus, it is possible that elevation of cGMP content may be useful therapy for ALS. Results showed that CSF cGMP can not be a marker of ALS activity.

## Multiple sclerosis

**P537**

**A multicentre, randomised, double-blind, placebo-controlled, phase III study of subcutaneous Rebif in the treatment of secondary progressive multiple sclerosis – 6-year results.** M. Freedman, J. Abdalla, F. Forrestal, Ottawa General Hospital, Serono International S. A. on behalf of the SPECTRIMS Investigators

Objective: To investigate the efficacy and safety of IFN-beta 1a (Rebif®) in a 3 year extension to the original SPECTRIMS study in secondary progressive multiple sclerosis (SPMS).

Background: In the double-blind, placebo-controlled phase of SPECTRIMS, 618 patients were randomised to one of three treatments (IFN beta-1a 22mcg tiw, 44mcg tiw, or placebo tiw) for 36 months; no statistically significant benefit of IFN on disability was seen (for 44 mcg tiw, HR 0.83 (0.65–1.07) and for 22 mcg tiw, HR 0.88 (0.69–1.12), each compared to placebo), although highly significant treatment effects were found for relapse rate and MRI measures. Treated patients with pre-study relapses were also less likely to progress than placebo patients.

Design/Methods: The placebo-controlled phase was followed by three consecutive one-year dose-blinded extensions after re-randomisation of the placebo group at month 36 to receive either IFN beta-1a 22mcg or 44mcg tiw; patients on active treatment continued on their original medication. The primary efficacy endpoint for this extension was 'time to 3-month confirmed EDSS progression' (ITT; IFN beta-1a 44mcg tiw versus placebo as the primary comparison, adjusted for centre).

Results: 396/618 (64 %) patients completed up to Month 72 on study. No statistically significant differences were noted for either dose groups (6 years active treatment) compared to the placebo group (3 years active treatment) on the primary endpoint (44mcg tiw: HR = 0.87; 95 % CI 0.69, 1.10, $p = 0.247$; 22mcg tiw: HR = 0.87; 95 %CI 0.70, 1.10, $p = 0.241$). The treatment by gender interaction remained significant at six years ($p = 0.006$).The female subgroup treated with IFN had a statistically significant benefit in both dose groups compared to placebo (44mcg tiw: HR = 0.64; 95 % CI 0.48, 0.87, $p = 0.004$; 22mcg tiw: HR = 0.67; 95 %CI 0.50, 0.91, $p = 0.009$) at 6 years. A non-significant trend was observed in the relapsing pre-study subgroup in favour of treatment (HR = 0.8095 %CI 0.60, 1.08, $p = 0.148$). Relapse rates were significantly less with IFN than placebo/IFN groups (rate ratio = 0.71, 95 % CI 0.57,0.88; $p = 0.002$ for both doses compared to placebo). Drug therapy was well tolerated, and no new safety concerns were noted during this extension. Treatment discontinuation as a result of an adverse event over the 6-year period was 14.4 % in the placebo/44mcg tiw group, 12.7 % in the 44/44 mcg tiw group, 8.6 % in the 22/22 mcg tiw group, and 7.9 % in the placebo/22 mcg tiw group. MRI and antigenicity data are pending.

Conclusions: The results of this dose-blinded extension confirm the findings of the original 3-year double blind study. IFN beta-1a (Rebif®), particularly at the dose of 44mcg tiw, provides modest benefit on disability in SPMS that is more apparent in women and in patients who experience relapses at baseline. The significant benefit on relapses seen at 3 years is also maintained. This study represents the longest controlled follow-up of IFN treated patients with SPMS.

**P538**

**Intraventricular transplantation of neurospheres attenuates acute experimental allergic encephalomyelitis.** T. Ben-Hur, O. Einstein, D. Grigoriadis, R. Mizrachi-Kol, E. Reinhartz, D. Karussis, O. Abramsky, Hadassah-Hebrew University Hospital, University of Thessalonica (Jerusalem, IL; Thessalonica, GR)

Objectives: To study the clinical and pathological value of neural precursor cell transplantation in an animal model of Multiple sclerosis

Background: Multiple sclerosis (MS) is an immune-mediated demyelinating disease without known treatments to enhance myelin regeneration. Transplanted neural precursor cells (NPCs) can remyelinate efficiently acutely demyelinated focal lesions. However, the clinical-therapeutic value of cell transplantation in experimental autoimmune encephalomyelitis (EAE), an animal disease model of MS, is unknown. We therefore examined the effects of NPCs transplantation on the clinical and pathological course of acute EAE in Lewis rats.

Methods: Newborn Lewis rat striatal NPCs were expanded in spheres as nestin +, PSA-NCAM +, NG2[-] cells, which could differentiate in vitro into astrocytes, oligodendrocytes and neurons. BrdU-tagged spheres were transplanted into the lateral ventricles of female Lewis rats, which were induced with EAE at the same day of transplantation. Severity of EAE was determined using standard clinical scores. The fate of transplanted cells was examined by double immunofluorescent-stainings for BrdU and GalC or NG2 or GFAP. Perivascular inflammatory infiltrates were counted on H&E stained brain sections and expression of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function antigen-1 (LFA-1) was determined by computerized image analysis of immunohistochemical stainings. Lymphocyte proliferation was determined by 3H-thymidine incorporation and cell death by TUNEL stains.

Results: Transplanted NPCs migrated into inflamed white matter tracts and differentiated into oligodendroglial and astroglial lineage cells. NPC spheres transplantation attenuated the clinical severity of EAE, as determined by maximal clinical severity and by cumulative burden of disease. Sphere-transplanted rats also exhibited an attenuated inflammatory process in the CNS, as determined by a reduced number of perivascular infiltrates and decreased expression of ICAM-1LFA-1. NPC spheres also inhibited basal proliferation and proliferative responses to Concavalin-A (ConA) and to myelin oligodendrocyte glycoprotein (MOG) peptide of EAE rat-derived lymphocytes in-vitro.

Conclusions: Intraventricular transplantation of NPC spheres may attenuate the clinical course of EAE, probably via an immunomodulatory mechanism that inhibits inflammatory responses in vivo and in vitro.

**P539**

**Could mononuclear cell adenosine deaminase activity be considered as a surrogate marker for multiple sclerosis?** L. Feuillet, R. Guieu, E. Fenouillet, I. Malikova, H. Rochat, A. Ali Cherif, J. Pelletier, CHU Timone (Marseille, F)

Objective: To determine potential value of activity of mononuclear cell adenosine deaminase (MCADA) as a surrogate marker of inflammation in Multiple Sclerosis (MS).

Background: CD 26 is a multifonctional type II cell surface glycoprotein widely expressed in T and B cells and which has a dipeptidyl peptidase activity. Furthermore, in human, CD 26 binds to mononuclear cell adenosine deaminase (MCADA). MCADA is an enzyme that deaminates adenosine (Ado) into inosine, thus protecting lymphocytes against toxic concentration of Ado. MCADA is also implicated in T cell activation via non-covalent binding to the T cell antigen CD26. It was previously shown that CD26 + lymphocytes population is increased in patients with MS. Because MCADA and CD26 are coexpressed on T cells, we evaluated MCADA activity in a group of patients with MS and the influence of corticosteroids on MCADA activity.

Design/Methods: Twelve successive patients were screened for MCADA activity when they experienced neurological signs suggestive of MS relapse. We restricted our study to the 10 patients whose relapse onset was ≤ 1 month. 7 patients were fulfilling definite MS criteria according to Mc Donald (all relapsing remitting forms) while 3 did not meet yet temporal dissemination criterion. Therefore, their cerebrospinal fluid analysis showed inflammatory pattern whereas initial cerebral MRI was fulfilling Barkhof criteria in two cases. These 3 patients were considered as having clinical isolated syndromes of an inflammatory demyelinating process. Among these 10 patients (sex ratio M/F:1/4), age ranged from 21 to 43 years

old (mean 32.4 ± 7.7), mean duration of the disease was 17 ± 21months (0.3–68 months), mean EDSS was 2.5 [2–4], mean relapse rate was 2.7 ± 2.4 [1–9]. The last relapse occurred within the last month in all cases, from 7 to 30 days (15.6 ± 10.2 days). Only one patient received interferon beta for less than 1 month. Blood samples for MCADA were studied before IV corticosteroid treatment (methylprednisolone 1 g/day) and the day after the third perfusion. Ten healthy volunteers matched for age were also analysed for MCADA activity.

Results: Mean MCADA activity in the control group was 1.49 ± 0.1. In the patient group, MCADA activity was significantly increased 2.33 ± 0.8 (ANOVA $p < 0.05$). After the third day of methylprednisolone, MCADA had decreased from baseline, with mean value 1.46 ± 0.4 (wilcoxon $p < 0.05$). These results obtained after treatment did not differ with safety control values.

Conclusions: MCADA activity may be a useful surrogate marker of MS relapse since its value is increased during relapse and decreased after corticosteroid treatment, going down to safety controls values. Further studies are needed to confirm these results.

**P540**

**Testing a high-dose protocol (500 mcg every-other-day, subcutaneously) for interferon beta-1b treatment of MS. Clinical and laboratory tolerability evaluated in a multicentre study.** M. Clerico, A. Protti, E. Pucci, L. Manneschi, I. Pesci, P. Barbero, A. Pipieri, M. A. Cucci, A. Ricci, E. Verdun, G. Giuliani, E. Montanari, L. Durelli, University of Turin, Niguarda Hospital, Civil Hospital (Turin, Milan, Macerata, Fidenza, I)

Objective: To evaluate the clinical and laboratory tolerability of interferon (IFN) beta-1b administered using a high-dose protocol (500 mcg every-other-day [EOD]).

Background: About 30% of relapsing-remitting (RR) MS patients receiving treatment with standard dose IFN beta-1b (250 mcg, EOD) display only a partial response to treatment with persistent clinical or MRI activity. A multicentre trial underway in Italy to test the possibility of treating these partial responders with an increased dose of IFN beta-1b (375 mcg EOD; the OPTIMS trial) provided good preliminary safety data, but also showed that some patients still do not respond well to this dose. In an IFN beta-1b dose-finding study (Greenstein et al, 1987) patients treated with 500 mcg experienced no more relapses during follow-up, although that dose, administered without a dose escalation protocol, was not well tolerated.

Design and methods: Multicentre study (4 Italian MS centers) in outpatients suffering from clinically definite RR or relapsing-progressive MS, who are either partial responders after at least 1 year of chronic treatment with 250 or 375 mcg IFN beta-1b or who have very aggressive RR MS (> 2 relapses/year; progression of 1 point/year in the EDSS score; or with persisting active lesions in two consecutive MRI scans, 2–6 months apart). The patients will be treated with IFN beta-1b 500 mcg, EOD, for 1 year. This therapeutic dose will be reached after an escalating titration. Clinical and laboratory side effects will be carefully monitored.

Results: Twelve patients have been recruited: 4 with no prior treatment; 5 previously receiving IFN beta-1b 250 mcg, EOD; and 3 previously receiving IFN beta-1b 375 mcg, EOD. IFN beta-1b 500 mcg has been well tolerated, with the usual side effect profile observed for IFN beta treatment in MS. No new or unexpected side effects were observed. Clinical side effects (fever, flu-like symptoms, fatigue, local skin reactions) were mild, with the exception of one case of moderate depression. Laboratory side effects (increased liver enzymes, anemia) were mild, with the exception of one case of moderate leukopenia associated with a mild subclinical hypothyroidism, which occurred during the first months of treatment, decreasing thereafter.

Conclusion: IFN beta-1b 500 mcg, EOD was well tolerated and further testing of long-term tolerability and clinical and MRI efficacy in selected patients is warranted.

**P541**

**Does fatigue in multiple sclerosis have a "sleepiness" component? A pilot study.** E. Szabadi, C. S. Constantinescu, R. Langley, G. Niepel, C. M. Bradshaw, University of Nottingham (Nottingham, UK)

Patients suffering from multiple sclerosis (MS) complain of fatigue and many show excessive daytime sleepiness [1]. Recently, it has been reported that the wakefulness promoting agent modafinil is effective in alleviating fatigue and reducing sleepiness in MS [1]. The Pupillographic Sleepiness Test (PST) provides an objective quantitative index of sleepiness by measuring pupillary fluctuations in darkness (pupillary fatigue waves) [2]. In a pilot experiment we examined whether a single dose of modafinil is effective in reducing pupillary fatigue waves in MS patients suffering from fatigue.

Five patients with MS (3 females, 2 males; 4 relapsing/remitting, 1 primary progressive), with prominent complaints of fatigue which failed to respond to treatment with amantadine were recruited. Measurements included the fatigue assessment instrument (FAI) and severity score (FSS), the Epworth Sleepiness Scale (ESS), visual analogue self-ratings of alertness, anxiety and contentedness, and physiological tests, including the PST (pupil diameter, total power of fluctuations obtained from a Fast Fourrier Transformation, pupillary unrest index [PUI]) and the critical flicker fusion frequency (CFFF) test. Patients were given either modafinil 200 mg or placebo in two sessions one week apart according to a double-blind crossover design.

The ESS score was 13.8 ± 1.5 (mean ± s. e.mean), and the FSS score was 5.704 ± 0.68, indicating severe fatigue and sleepiness. Modafinil reduced ratings of sleepiness (ESS), increased alertness, and contentedness, and decreased anxiety, compared to placebo ($p < 0.05$). Modafinil caused an increase (0.49 ± 0.37 mm) in pupil diameter, and reduced the power of pupil fluctuations and PUI. Three patients continued on maintenance treatment with modafinil 200–400 mg/day: all showed improvement in the FSS score.

In summary, measures of sleepiness co-existed with measures of fatigue in the patients studied, suggesting a contribution of sleepiness to fatigue in MS. Modafinil was effective in increasing measures of alertness in these patients, including pupillary fatigue waves as assessed by PST. The PST may be a useful tool for objective assessment of the sleepiness component of fatigue and the response to anti-fatigue drugs.

**P542**

**Temporal expression of plasma membrane calcium ATPases in the spinal cord during the clinical course of experimental autoimmune encephalomyelitis.** A. Nicot, M. Kurnellas, S. Elkabes, New Jersey Medical School (Newark, USA)

Increasing evidence indicates that axonal/neuronal pathology correlates with neurological decline during both multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), an animal model reproducing some characteristics of MS. Yet, the molecular mechanisms underlying neuronal deficits remain undefined. We previously reported that mRNA and protein levels of PMCA2, an essential pump expressed exclusively in neurons and involved in $Ca2+$ extrusion, are dramatically decreased during acute, severe EAE in the Lewis rat, a disease characterized by progressive ascending paralysis without remission. Expression of PMCA2 was decreased at onset of symptoms and remained low thereafter (Nicot et al, 2003, Brain, 126, 398). Our present investigations indicate that the changes in PMCA2 levels are selective and specific to this isoform, as the expression of PMCA1, 3 and 4 is not modified during the course of the disease as revealed by RT-PCR. Moreover, in agreement with our findings in the Lewis rat, PMCA2 expression is decreased at disease onset and thereafter, in a model of chronic EAE in C57BL/6 mice.

To determine the pattern of PMCA2 expression during recovery, we used an acute, remitting EAE model and further analyzed PMCA2 mRNA and protein levels in the spinal cord by real-time PCR and Western blot. We found that PMCA2 expression is decreased two-fold at disease onset but returns to control levels during remission.

These results, taken together, indicate that specific changes in PMCA2 expression correlate with disease course. Our findings also suggest that the reduction in PMCA2 levels is an early event common to different EAE paradigms and is reversible during remission. Thus, early alterations in PMCA2 levels and the consequent calcium dyshomeostasis may lead to neuronal damage, a hypothesis under current investigation in primary cultures.

**P543**

**Clinical and immunological study of multiple sclerosis patients treated with repeated pulse doses of methylprednisolone.** M. Krassa, M. Paschalidou, D. Parissis, M. Danielides, I. Milonas, Ahepa University Hospital (Thessalonica, GR)

Background: It is well established that pulsed therapy with high-dose methylprednisolone is effective in patients with multiple sclerosis (MS) in acute relapse. The effect of repeated course of intravenous (i.v) methylprednisolone on the clinical course of the disease is less clear, however some promising results have emerged from magnetic resonance imaging (MRI) studies.

Aim: The goal of this study was to investigate a) the potential clinical

benefit of high-dose i. v. methylprednisolone in MS patients and b) the effect of this therapy on immunological markers relevant to MS.

Patients-Methods: 20 patients (13 females, 7 males, age range = 17–62 years, mean age = 39.5) suffering from definite MS were subjected to the following courses of treatment: a) 11 patients (mean EDSS = 3.6) were treated with steroids 1 gr methylprednisolone daily for the first 5 days followed by 1 gr for 3 consecutive days every 4 months b) 9 patients (mean EDSS = 4.42) were used as a control group treated with steroids 1 gr methylprednisolone daily for 5 days, only during the relapsing period. The following immunological parameters were determined in the serum using standard methods: CD4+, CD8+, CD4+/CD8+ ratio and the cytokines IL-2, IL-6, TNF-a. These parameters were determined 6 times during the 13 months observational period, together with clinical evaluation according to a standardized protocol.

Results: In both groups of patients a significant decrease in the mean relapse rate (compared to the 13 months pre-trial rate) was noticed. The first group of patients showed a reduction in the mean EDSS by approximately 0.5 grades, where EDSS did not change in the second group. CD4/CD8 ratio was also reduced in both groups, but not on a significant level.

Finally, the mean percentage measurements of elevated IL-2, IL-6 and TNF-a which took place during the 13 months period did not significantly differ between the two groups.

Conclusion: Treatment with repeated pulses of i. v. corticosteroids may have a positive influence on the short-term outcome of MS patients. This effect does not appear, according to our results, to be related to any marked alterations in the cytokine profile. Larger studies are needed to evaluate the long-term efficacy of the treatment on disease progression and its safety.

**P544**

**A longitudinal study on the evaluation of neopterin as a marker of disease activity in multiple sclerosis.** F. Bagnato, F. Deisenhammer, R. Zivadinov, A. Tancredi, C. Gneiss, V. Durastanti, M. Zorzon, D. Fuchs, E. Millefiorini, University La Sapienza, University of Innsbruck, Cattinara Hospital, University of Padua (Rome, Padua, Trieste I; Innsbruck, A)

Neopterin is a low-mass compound produced by interferon (IFN) gamma activated macrophages and it is recognized as a marker of T1-cells activation. Neopterin's levels have found to be higher in patients with several autoimmune, neoplastic and infectious conditions than in healthy individuals (HI). In addition, neopterin is used as a pharmacodynamic marker of IFN beta activity in patients with multiple sclerosis (MS) or other disease that requires type I IFN for treatment as well. The aim of the present study was to test the role of neopterin as a biological marker of disease activity in MS patients during a short-time course of the natural history of the disease.

Thirty RR-MS patients (10 males and 20 females, [mean ± SD] age 34.4 ± 8.5 years, disability score at the Expanded Disability Status Scale (EDSS) 1.4 ± 0.6, MS duration 4.9 ± 4.8 years) were monthly clinically assessed and imaged over a three-month period. The relapses' number and the EDSS score served as clinical measures, whilst the lesion load (LL), on T2 and T1 pre/post-contrast images and the brain parenchyma fraction (BPF) were used as Magnetic Resonance Images (MRI) metrics. Samples' sera were collected at each time point concomitantly to the MRI and clinical assessment for the evaluation of neopterin, by the means of an enzyme-linked immunosorbant assay. Sera from 20 HI were drawn and used as controls. A student T-test was used to analyze differences between quantitative variables. Time effect on MRI and biological (i. e. neopterin) metrics was assessed by the means of the Random-Effect Models.

Eight (26.7%) patients experienced a clinical relapse out of whom, 3 (10%) required steroids treatments. Baseline T2, T1 and T1-post contrast LL were 11.7 ± 18.9, 3.1 ± 8.1, 0.2 ± 0.4, respectively and did not change over time. A moderate albeit significant ($p = 0.01$) decrease of BPF was observed. Baseline levels of neopterin were similar between patients (4.7 ± 1 nmol/l) and HI (5.6 ± 6.1 nmol/l) with a significant increase of neopterin values over the three-month time period ($p = 0.04$). Such an increase was modestly correlated ($r = -0.13$) with the decrease in the BPF but the association was not significant.

These preliminary results suggest that levels of neopterin may fluctuate over time in patients of MS. Further analyses are ongoing in order to precisely quantify neopterin as a biological marker of disease activity in MS by the means of both clinical and MRI metrics in this cohort of patients.

**P545**

**Bone strength in multiple sclerosis.** A. Achiron, Y. Barak, I. Siev-Ner, S. Edelstein, Z. Rotstein, Sheba Medical Center, Abarbanel Mental Health Center, Weizman Institute (Tel-Hashomer, Bat-Yam, Rehovot, IL)

Multiple sclerosis (MS) patients are at increased risk for osteoporosis due to reduced mobility, decreased exposure to sunlight and recurrent steroid treatment. We assessed bone strength in 256 MS patients (171 females, 75 males) through quantitative ultrasound measurement of cortical bone. Tibial Speed of Sound (SOS, m/sec) was measured at midpoint of the tibial shaft using a Soundscan 2000 (Myriad Ultrasound Systems, Rehovot, Israel) and results were compared to age and gender matched population norms. T score distribution in male MS patients was similar to normal population. In contrast, for female MS patients T score distribution was significantly different from population norms, reflected by increased SOS in 30.4% (T score intervals 1–2 and > 2 above normal values; $p = 0.001$), compared with 7.4% in controls.

These findings held true for both patients younger and older than 45 years of age. Increased neurological disability and specifically motor involvement were more frequent in patients with increased SOS ($p < 0.05$). Bone strength was preserved in MS patients. In a subgroup of female patients increased SOS was conceivably related to spasticity.

**P546**

**A refined disease score reveals that the induction of experimental allergic encephalomyelitis in Lewis rats can be augmented by pertussis toxin but not aluminiumhydroxide.** S. Busse, M. Klabunde, A. Dressel, University of Greifswald (Greifswald, D)

Experimental allergic encephalomyelitis (EAE) is an autoimmune central nervous system (CNS) disease which can be induced by immunization with CNS antigens. However, disease induction is variable and several scoring systems are applied. The first classification of EAE was introduced by Swanborg who divided the symptoms into three stages. After using Pertussis toxin (PT) as an additional adjuvant rodents were affected more severely and the score had to be expanded.

We aimed to establish an optimised protocol to induce EAE and to distinguish more subtle differences in diseases severity, therefore we established a new scoring system with eleven categories. The disease severity in animals treated with myelin basic protein (MBP) dissolved in complete Freund's Adjuvant (CFA) was compared to animals in which PT and/or Aluminiumhydroxide ($Al(OH)_3$) was added to the regimen. Statistical significance was determined with the students T test.

Ongoing disease was assessed by scoring weakness of tail, hind leg and foreleg in three grades each, using the loss of weight as an additional parameter. A few days after the animals gained their maximum weight the disease broke out. At onset (stage 1) the rats were lethargic. The illness caused weakness starting with the most distal part of the tail (stage 2), then additional the middle (step 3) and later the whole tail (stage 4). Afterwards the hind legs (stages 5–7) and forelegs (stages 8–10) were affected and graduated in weak, middle and strong paresis. Stage 11 was defined as death due to EAE.

The mean loss of weight during ongoing disease ranged from 7.1% to 15.9% among the groups. Female rats [42] were affected to a greater extent than male rats [15] with regard to loss of weight ($p = 0.3135$) and severity of disease ($p = 0.0016$). The additional application of $Al(OH)_3$ did not change the disease course. In contrast, rats that were treated with MBP/CFA or MBP/CFA/$Al(OH)_3$ were affected to a lesser extent than animals in which PT was added to the adjuvant. Also, the onset of disease occurred earlier with the addition of PT.

We established a refined 11 point scoring system to distinguish subtle differences in EAE disease severity. Applying this scale we found that female rats are more severely affected than male rats (mean score 6.64 vs. 4.7). The additional use of $Al(OH)_3$ in the adjuvant had no influence on the course or severity of EAE. The application of PT, however, led to an earlier onset and an increase in disease severity ($p = 0.0016$).

**P547**

**Assessment of the feasibility and capability of nine clinical algorithms to measure variation in multiple sclerosis patient care (ASAP) – the basic methodology of a benchmark pilot.** D. Poehlau, G. Japp, P. Reuther, A. Simonow, K. Piwernetz, C. Leopold, W. Hipp, D. Vermeij, Kamillus Klinik, Asklepios, ANR, Quanup, Medinet, Serono (Asbach, Falkenstein, Neuenahr, Herborn, Grünwald, Oberhaching, Unterschleissheim, D; Draguignan, F)

Background: Multiple Sclerosis (MS) is the most common non traumatic neurological disease of young and middle aged people. In middle Europe the prevalence is about 100–160 patients in 100,000 inhabitants.

Life expectancy of MS patients is getting normal, efficacious disease modifying drugs (DMD) and other symptomatic treatment strategies are available. However, applicable definitions of quality of patient care in terms of achievable benefits, avoidable risks and avoidable costs are still missing.

The concept "Total Quality Management in MS Patient Care" (TQMS) addresses these challenges and implies benchmarking to identify centres of excellence by the comparison of predefined quality indicators.

Recently the Task Force Essentials of the European Charcot Foundation published clinical algorithms for nine critical domains to measure interventions and outcomes in MS patient care intended for the development of quality indicators.

Objectives: The final objective of ASAP is to assess the capability of these nine clinical algorithms. Preliminary steps are the establishment of a client-server network with the participating MS centres, the creation of a central database for performance and outcome monitoring and the evaluation of the functionality of this type of networking for participating centres.

Methods: Process and outcome indicators using basic neurological data (history, examinations, diagnosis, treatment) and the UNDS (United Kingdom Neurological Disability Scale) were developed. Data were documented in the software PROMISE (Practice-Oriented Multiple Sclerosis Information System to Ensure MS Patient Care according to Quality Standards).

Organisation and coaching of participating centres was done by QUANUP e. V. (society for quality development in neurology and psychiatry), an initiative of German neurologists, psychiatrists and their professional associations.

Results: 19 performance and 39 outcome indicators were derived from the clinical algorithms and integrated in an XML- (Extended Markup Language) based client-server system. Data entered in PROMISE are uploaded via Internet into the central server database that is able to process an unlimited number of data due to the use of WebObjects. Aggregated, anonymized data are evaluated and within a few minutes graphs in jpeg format are created. These graphs are automatically resent as E-Mail: attachments and can directly be viewed on-line.

Benchmark evaluations of 16 participants having access to data of about 1300 MS patients revealed great variations in process and outcome indicators.

**P548**

**Year-person post marketing survey for multiple sclerosis centres: clinical and MRI data.** G. Iuliano, R. Napoletano, M. Tenuta, A. Esposito, Ospedali Riuniti di Salerno (Salerno, I)

Background: Italian district Multiple Sclerosis Centers treat with interferons (IFN) patients (pts) with 1 or more relapse/year for 2 years, and EDSS up to 5.5, and/or secondary progression up to EDSS 6.5 (with IFN 1b). In this paper we update our simple year/person registry to December 2002.

Methods: Years/person is the standard method to evaluate little groups, with different observation periods, often with previous therapies, sometimes switching from drug to drug. Only patients with complete history are eligible, to avoid different relapse risks. They are divided in: 0-without therapy; 1-IFN1b; 2-IFN1a (i. m.1/w); 3-azathioprine (AZA); 4- IFN1a (22 or 44 mg s. c.3/w). Patients start or switch therapy when having 1 relapse/year for two years. Secondary progressive SM are treated with IFN 1b.

Results: From 55 patients (age onset 18–49, mean 30.42) we obtained 504 years (without therapy = 78, before therapy = 238, IFN1b = 34, IFN1a 1/w = 53, IFN1a 3/w = 38, AZA = 49). Group 0 has lower relapse rate (RR) (0.333) and is excluded from further evaluation. Relapse rate is similar before treatment in groups 1–2–3–4 (mean 0.916, SD 0.807), and MRI parameters are not different, so a single control group was made. During therapy, relapse rate for IFN1b is 0.618 (SD 0.817), for IFN1a1/w 0.585 (0.795); for IFN Ia3/W 0.762 (0.983); for AZA 0.469 (0.739). Kruskal-Wallis test is significant ($p = 0.000501$); Neuman-Keuls comparisons ($p < 0.05$) show AZA rate significantly lower vs all the other groups; interferon rates are significant vs years before treatment. EDSS is not significant except for higher scores for IFNIb, due to secondary progressive patients in this group; they lose significance if these patients are excluded. Stratification till the fifth year does not show differences in RR; a reduction of EDSS scores is evident for IF1b, though significance is not reached if secondary progressive patients (10yrs) are excluded. As to MRI new lesions, the odds ratio was 0.16 (0.04–058) for IFN1b, for IFN1a1/w it was 0.48 (0.10–0.68); for IFN1a3/w 0.07 (0.01–0.28). They are significantly different vs no therapy and among them. Data about enhanced lesions are not significant. Besides 1 allergic reaction to AZA, the most common side effect was protracted fever after injection; there were no major events.

Conclusions: Longitudinal data are maybe the best contribution of epidemiological monitoring on disease modifying drugs in Italian district SM centers, besides practical managing of the drugs.

**P549**

**Middle latency auditory evoked potentials in multiple sclerosis.** H. Efendi, B. Kara, S. Komsuoglu, Kocaeli University (Kocaeli, TR)

Multiple sclerosis (MS) is a disease characterised by multiple areas of demyelination in the central nervous system. In multiple sclerosis the involvement of central nervous system, auditory pathways is usually explored by means of brain-stem auditory evoked potentials (BAEPs) which however investigate just their brainstem tract, thus, middle latency auditory evoked potentials (MLAEPs) have been proposed to increase the detectability of lesions along the whole auditory pathway. MLAEPs are composed of several components that can be detected from 10 to 50 msec after stimulus onset. In this study middle latency auditory evoked potentials (MLAEPs) were studied in definite MS patients in addition to brain-stem auditory evoked potentials (BAEPs).

Monaural BAEPs and MLAEPs were studied in 40 definite MS patients (29 women, 11 men, and mean age 34.2, range 21–59). These 40 MS patients cross-matched with an age and sex distribution similar healthy people as a control group (29 women, 11 men, mean age 34.0, range 20–56). Stimulations, recording and analysis of MLAEPs performed by Nihon Kohden Neuropack systems. During the recording, the subjects were awake, eyes closed, and they comfortably sat in a dimly lit room. Monaural responses were recorded from Cz-ipsilateral ear. For each ear, series of 2000 responses were averaged and then superimposed to test waveform consistency. MLAEPs were abnormal in (30/40) of MS patients, BAEP responses were abnormal only in (13/40) patients. 18/40 patients had abnormal MLAEPs with normal BAEPs whereas the opposite was detectable only in one patient. All of the MLAEPs parameters (No, Po, Na, Pa, Nb, Pb) in the MS group statistically differed from those obtained in the control group. We also examined the correlations between MLAEPs parameters and the EDSS scores, the number of attacks and duration of the illness. Weak correlations were found between EDSS scores and right Na latency, duration of illness and right Pa latency, the number of attacks and bilateral Na latency. There was no significant correlation between BAEPs parameters (I-III, III-V, I-V inter peak latency) and the EDSS scores, the number of attacks, duration of illness.

In conclusion, our data demonstrated that MLAEPs give additional information on both the occurrence and the extent of auditory impairment. We concluded that MLAEPs could be useful in combination with other multimodal evoked potentials in MS patients.

**P550**

**Psychiatric disorders as a primary manifestation of multiple sclerosis.** V. Barba, C. Mavrogenidou, E. Vardaki, A. Anastasopoulou, T. Stifougias, N. Matikas, Evagelismos General Hospital (Athens, GR)

Background: Although psychiatric manifestations are relatively common among patients with Multiple Sclerosis (MS), there are few studies of MS presenting as a pure psychiatric disorder.

Goals: The possible correlation between psychopathological abnormalities and the central nervous system lesions and the therapeutic approach of those patients are discussed in relation to literature.

Methods: We studied 8 patients (3 males and 5 females) 23 to 49 years old, with psychiatric disorders (Bipolar disorder, Schizophrenia, Atypical psychosis) as a primary manifestation of MS. All patients except one were on medication – either neuroleptic or antidepressant or mood stabilizer – prior to diagnosis of MS and none of them had a family history of psychiatric illness. Review of their medical records documented minor neurological symptoms that were either overlooked or dismissed as atypical.

The brain and spinal cord magnetic resonance imaging (MRI) and the presence of oligoclonal bands in the cerebrospinal fluid confirmed the diagnosis of MS.

The administration of corticosteroids to those patients did not cause any exacerbation of the psychiatric disorder, whereas 2 of 4 patients treated with interferon deteriorated.

Conclusions: We cannot establish a clear relationship between CNS lesions and psychiatric disorders in those patients. The use of corticosteroids should be considered in their treatment but interferon should be used with caution.

P551
**T-cell activation in glatiramer-acetate treated multiple sclerosis patients: analysis of expression of CD69 marker.** O. Zapletalova, P. Hradilek, A. Lochmanova, University Hospital, Institute of Immunology (Ostrava, CZ)

Objective: To compare the immunological response profile in 24 glatiramer acetate (GA) treated multiple sclerosis (MS) patients with clinical course of the disease and 10 healthy controls.

Backgrounds: Auto-reactive T lymphocytes are considered to play a crucial role in chronic inflammation in the central nervous system (CNS) of MS patients. Several lines suggest an important role of interferon gamma (IFN-gamma) in the pathogenesis of MS. It has been recently reported that IFN-gamma mediates a sustained elevated intracellular Ca2 + influx and significantly augments proliferation response to sub-optimal doses of phytohemagglutinin (PHA) in T cells of active MS patients as compared with healthy controls. Immune modulation therapy like IFN-Beta and GA are known to significantly decrease proliferation of pre-activated T cells. It is thought that GA-reactive regulatory T cells are activated in the CNS by cross-reacting myelin antigens presented by local antigen presenting cells. It is at present unknown whether and how strongly the immunological response to GA correlates with the clinical course of the disease.

Materials and Methods: The effects of GA therapy on in vitro proliferative response of lymphocytes derived from MS patients receiving this therapy were analysed. T-cell activation by expression of early surface antigen expression on lymphocytes from 24 MS patients at various periods of therapy and 10 controls was monitored. Peripheral blood CD69 positive T-cells subsets were analysed after 3, 6, 12, 15, 18 and 24 months after therapy initiation and compared with healthy controls.The clinical course of MS was analysed. Proliferative response was measured in PHA stimulated whole blood of MS patients and controls. The following antigens were determined: CD4FITC/CD69PE/CD3PerCP,CD8FITC/CD69PE/CD3PerCP.

Results: The proliferative activity measured by the expression of activation marker CD69 on CD4 + T lymphocytes showed similar trend in almost all patients: significant and transient decrease at 3 and 6 months followed by slight increase at 12 and 15 months. After 24 months of treatment the average level is comparable with healthy controls.The expression of CD69 on CD8 + T lymphocytes showed decrease at 3–18 months, comparable level with healthy controls was reached at 24 months.The low expression of CD69 on CD8 + T lymphocytes could be connected with low number of CD8 + T cells in MS patients. In correlation with clinical course (MS relapse) we found in 2 cases extremely high level of CD3/69 in the beginning of GA treatment.The other patient presented extremely high level of CD4/69 during MS relapse at month 18 of the therapy followed by significant decrement with clinical stabilisation of the illness.

Conclusion: The predictive value of the expression of early activation marker CD69 is limited. In our small study we found that the reactivity of this marker corresponds with clinical course of some of our MS patients. Further following and analysis is necessary.

P552
**Change of Interferon-beta preparations and the influence on the course of multiple sclerosis in the daily practice.** J. Haas, Jewish Hospital Berlin (Berlin, D)

There is an ongoing discussion concerning the efficacy of the different dosages of the INF beta preparations and the mode of application respectively. To prove the relevance of switching from INF beta 1a sc or 1b sc to INF beta 1a im and vice versa we analysed our data base. Since1996 we documented in a standardized way the data of all MS patients seen in our outpatient clinic (data base MUSIS). Out of 1815 MS patients 315 were treated with INF beta 1a im. We identified 71(16 m 55f) patients who had switched, five between three INF beta preparations. From INF beta 1a im to INF beta 1a sc 27 and to INF beta 1b 11, from INF beta 1b sc to INF beta 1a im 32 and from INF beta 1a sc to INF beta 1a im 6. The first analysis revealed no influence of age and gender concerning the risk for switching. The main reason for switching from INF beta 1b or 1a sc to INF beta 1a im were skin reactions or handling. The main reason for switching from INF beta 1a im to INF beta 1a sc or 1b sc was ongoing activity of the disease. The majority of the patients (59 %) who switched from one INF beta preparation to another received more than two different immunological therapies. 20 had one more agent, 6 had two additional agents, 10 had three additional agents and 5 had four additional agents. The high number of therapeutical approaches revealed inefficacy, high risk for side effects or non compliance. 25 (35 %) of the 71 patients had need for an escalating therapy (mitoxantrone or cyclophosphamide) based on the criteria of the consensus of the German speaking MS experts. The necessity for an escalating therapy was to be observed in 15 of 38 patients who had switched from INF beta 1a im ti INF beta 1a sc or INF beta 1b and in 10 of 33 who had switched vice versa.

The analysis of our data has so far revealed no risk for switching from INF beta 1a im to INF beta 1b or 1a sc concerning treatment failure. A further analysis will be done concerning number of exacerbations, progression and side effects.

P553
**Multiple sclerosis: psychological disorders related to diagnosis.** S. Pires-Barata, I. Henriques, Hospital do Espirito Santo (Évora, P)

Introduction and purpose: Different neuropsychiatric disturbances and psychological reactions were described in multiple sclerosis (MS) patients. Depression, anxiety and helplessness feelings are often observed in clinical practice. We looked for the prevalence of such syndromes and symptoms in our patients with MS.

Materials and Methods: We performed a 30 minutes interview to 12 MS patients (7 women), at least 6 months after the diagnosis. Age range was 24 to 50 years. We used the Symptom CheckList-90 scale (SCL-90) as a screening tool to look for psychopathology signs. This scale evaluates the emotional adjustment to the disease, using items such as somatization, obsessive/compulsive, interpersonal relations, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism, eating disorders, sleep disorders, thoughts of death and guilty feelings. All patients were classified according to the EDSS scale. Descriptive analysis was used.

Results: Thoughts of death were observed in 10 patients and 9 referred guilty feelings about the disease. All 12 patients showed obsessive compulsive symptoms (scores varied from 1.2 to 2.6 out of 4).All patients presented sleeping disorders and 9 out of 10 showed loss of appetite. Only one patient showed maximum score for depression. Psychotic symptoms were present in all patients (scores varied from 0.3 to 2.6 out of 4) and paranoid ideation was present in 11 out of 12 (scores varied from 0.3 to 2.7 out of 4).

Discussion: Obsessive/compulsive behaviours, depressive symptomatology, loss of appetite and sleep disorders were observed in our MS patients. The presence of obsessive compulsive symptoms might be related with feelings of control loss in the disease course. Guilty feelings may be related with self-blame. Since disease progression requires a very demanding physical and psychological adjustment a multidisciplinary team is needed to follow MS patients.

## Peripheral neuropathy

P554
**The molecular diagnosis of pure neural leprosy.** R. Scola, F. Cunha, M. Werneck, L. Werneck, Hospital de Clinicas Universidade Federal do Paran (Curitiba, Fortaleza, BR)

Objective: Study the diagnostic yield of polymerase chain reaction (PCR) in pure neural leprosy (PNL) and correlation with clinical forms.

Background: Leprosy is a disease of nerves, skin and other tissues. The clinical diagnosis may be defined with at least two of three principal signs: hypopigmentation and/or skin infiltration, nerve thickening and/or sensitive abnormalities with the presence of Mycobacterium leprae (ML) in the skin or nerve. Leprosy represents in developing countries a severe problem of public health. The pure neural leprosy (PNL), condition with one or more nerves are affected without skin lesion, with a prevalence of 3.9 % to 8.2 % per 10,000 patients. In these cases, even with accurate investigation, the diagnosis is difficult. Recently the use of polymerase chain reaction (PCR) for ML DNA identification in nerve biopsies has been an alternative for the differential diagnosis of PNL.

Design/Methods: Fifty-eight patients with clinical suspicion of PNL were studied from two reference centers (Curitiba and Fortaleza, Brazil). All cases were submitted to a specific protocol including dermatological and neurological examination, routine blood tests, search for ML in skin and nasal mucous, electromyography, nerve biopsy and PCR of the nerve biopsy. The nerve biopsies were divided in two fragments: one for PCR and another for frozen studies (haematoxilin-eosin, Gomori trichrome and search for ML with Fite-Faraco stain). From the sample for PCR, DNA was extracted and the specific sequence ML DNA was amplified with primers ML 1 and ML 2, according to Woods Cole (1989). All tests had at the same time a positive and a negative control ML.

Results: From 58 patients, 41 were males and females. The disease duration ranged from 2 months to 8 years (mean of 1.9). The age ranged from 15 to 77 years (mean 42.1). The patients were classified according to Ridley and Jopling (1966) in Borderline-Tuberculoid (BT) 40 cases and Tuber-

culoid polar (TT) 18 cases. The neuropathy pattern was multiple motor-sensitive in 36 cases, multiple sensitive in 7 cases, motor-sensitive mononeuritis in 11 cases and sensitive mononeuritis in 4 cases. The main nerves involved were ulnar, common peroneal, posterior tibial, superficial peroneal and sural. The ML in nerve biopsy was positive in 20 cases (34.4%) of BT and none of TT. Of these, 5 cases had normal PCR. The OCR was positive in the nerves of 29 patients (50%). Of these, the ML was not found in 14 cases (24.12%), which 12 cases (20.7%) were BT and 2 cases (3.4%) of TT. The ML in biopsy and PCR were negative in 24 cases (41.4%)

Conclusion: PCR is a useful diagnostic method in pure neural leprosy and allows to confirm the diagnosis of pure neural leprosy in cases with negative AFB in the biopsy. This allows us to suggest first to search AFB in the biopsy. If negative, proceed to PCR to detect sequences of the ML in nerve specimen.

P555

**Sensory chronic inflammatory demyelinating polyneuropathy: a study of 28 cases with nerve biopsy.** A. Ferreira, P. Lozeron, C. Lacroix, D. Adams, G. Said, CHU Bicêtre (Le Kremlin-Bicêtre, F)

A pure sensory clinical presentation is reported in 5% of chronic inflammatory demyelinating polyneuropathy (CIDP). Data about clinical presentation, clinical course and electrophysiological findings are scarce. We studied 28 idiopathic CIDP patients without weakness at first clinical examination and with segmental demyelination/remyelination on nerve biopsy performed in our Department.

Eighteen patients were male. The mean age at onset was 53.9 years (range 33–79). The mean duration before the diagnosis was 3.4 years. Initial symptoms were paresthesia (n = 13), numbness (n = 10), pain (n = 10) or ataxia (n = 4) and were localized more frequently in lower limbs, distally and unilaterally. The first examination showed sensory disturbances over both lower limbs in 27 cases, all four limbs in 14 and over the trunk in 4. The sensory changes were symmetric in 23 cases and distal in 21. Large fiber sensory and thermoalgic disturbances were present in 27 and 24 cases respectively. Tendon reflexes were preserved in 7 cases. One patient had trigeminal nerve involvement and another developed transient diplopia. Two patients had a postural tremor. The mean CSF protein level was 0.74 g/l (range 0.22–2.5). CSF analysis was normal in 12 cases. On electrophysiologic study only one case fulfilled the criteria of the Ad Hoc Subcommittee of the American Academy of Neurology (AAN). On nerve biopsy all the patients had segmental demyelination/remyelination, 19 had axonal loss, 6 hypomyelination, 4 inflammation and 2 onion bulb formations. Three patients developed motor deficit on average 4.7 years after the onset (range 1–7). One of them fulfilled and another was near the criteria of the AAN. The other patients did not develop weakness during a mean follow-up of 6 years (range 0.5–26). The course was progressive for 26 patients, secondary progressive for one and relapsing for another one. Disability, mainly due to ataxia, was present at first examination in 10 patients and at last examination in 14 patients. No specific treatment was administered for 7 patients. An improvement was noted for 5/15 patients treated with oral prednisone, 3/10 patients with intravenous immunoglobulins, 0/3 patients with plasmatic exchanges and 0/4 patients with immunosuppressive treatment.

Diagnosis of sensory CIDP may be difficult because of non specific clinical features, normal CSF findings and lack of evidence of demyelination on electrophysiologic study. Thus nerve microscopic study is essential for diagnosis of sensory neuropathies of unknown origin. The prognosis is also uncertain: strength can remain normal during a long time. Motor nerve demyelination evidenced on electrophysiologic study seems to be a risk factor for development of weakness.

P556

**Course and prognosis of neuropathy associated with IgG or IgA monoclonal gammopathy.** M. Hézode, D. Adams, C. Théodore, C. Lacroix, G. Saïd, Centre Hospitalier Universitaire de Bicêtre, Institut Gustave Roussy (Le Kremlin-Bicêtre, Villejuif, F)

Background: We have recently shown that peripheral neuropathies associated with IgG or IgA monoclonal gammopathy (IgGA-MG) represent an heterogeneous group. Their management is still difficult and their long-term outcome poorly documented.

Objectives: Aim of the study was to evaluate the course and the prognosis of neuropathy associated with IgGA-MG.

Methods: Among the 110 consecutive patients with neuromuscular disease associated with IgGA-MG investigated over the past 24 years in our department, we obtained the follow-up in 94, in files, by phone or letter. All patients had a combined neurological or hematological management. We evaluated neuropathy, hemopathy, possible systemic manifestations and studied the survival.

Patients: Mean age was 61 y.; MG was IgG for 75 pts, IgA for 19. Patients had CIDP (n = 36), multiplex neuritis (n = 14) secondary to granulomatosis (3), vasculitis (5) and neurolymphomatosis (1), distal symmetrical polyneuropathy (DSP) (n = 35), ALS (n = 2), motor neuropathy (n = 3). Neuropathy was severe (bedridden) in 10 pts, moderate (walking with aid) in 37 and mild in 47. 57 pts had a MGUS, 25 a myeloma (including 6 solitary plasmacytoma), 5 an amyloidosis, 15 a POEMS, 1 a lymphoma.

Results: Most patients had a regular follow-up (mean duration: 5 years).

68 patients (72%) were treated for a progressive or disabling neuropathy (n = 35) or malignant MG (n = 33) using: radiation for plasmacytoma, chemotherapy for malignant MG, amyloidosis and progressive neuropathy with MGUS, corticosteroids for vasculitis or granulomatosis, IgIV, plasma exchanges in CIDP with MGUS.

Neuropathy improved in 32/68 pts (47%), including those with solitary plasmacytoma and vasculitis, remained stable in 15 pts (22%). It worsened in 21 pts (31%) including amyloidosis (n = 4/5), POEMS (n = 6), ALS (n = 4) and idiopathic axonal neuropathy (n = 7). In this latter group, amyloid deposits were found after a second look of nerve biopsy in 2 of them, initially managed as MGUS or myeloma.

Among 24 patients without treatment, 21 remained stable (of which 15 DSP) but 3 worsened including one with POEMS and one who declared a stomach cancer.

7/57 MGUS (12%) switched to myeloma (n = 6) or amyloidosis (n = 1). All cases of solitary plasmacytoma improved after treatment, without any malignant transformation; in one pt the plasmacytoma was recurrent (mean follow-up: 10 yrs). Three patients developed a POEMS syndrome before 3 years. Among the 41 pts having a lambda light chain only 24% remained MGUS on last follow-up.

Thirty pts (32%) died from: POEMS syndrome (n = 8), amyloidosis (n = 6), myeloma (n = 3), vasculitis (n = 1) and of other or unknown origin (n = 11). Survival was better in patients with MGUS than with amyloidosis, POEMS or myeloma.

Conclusion: Specific treatment is efficient in most patients (70%) with peripheral neuropathy associated with IgGA-MG. Prognosis and survival are still poor among patients with amyloidosis and some patients with POEMS.

P557

**Charcot-Marie-Tooth Type 2 neuropathy with mutations in the ganglioside-induced differentiation-associated protein 1.** M. J. Chumillas, F. Mayordomo, A. Cuesta, P. Alonso, J. J. Vilchez, Hospital Universitari La Fe (Valencia, E)

Introduction: Ganglioside-induced differentiation-associated protein 1 (GDAP1) gene related neuropathy has been described associated to both demylienating or axonal CMT phenotypes.

Objective: To describe the clinical, electrophysiological and pathological features of a series CMT II patients showing mutations in the GADP1 gene

Patients and methods: Screening for mutations in the GDAP1 gene was performed in patients with autosomal recessive or sporadic CMT2 phenotype. All patients had been investigated with a standard clinical protocol, conventional EMG and nerve conduction studies and sural nerve biopsy in the index case.

Results: GADP1 mutations were found in 10 patients belonging to 4 families and in one sporadic case. The disease had an early-onset, usually in the first or second year of life with distal motor and pansensorial deficit beginning in legs and rapidly extending to hands and arms; muscle atrophy was very pronounced. Severe disability with loss of walking capacity appeared in the second to fourth decade. Some cases manifested hoarseness due to vocal cord palsy after the second decade. Motor nerve conduction velocities were above 40 m/sec. but often were unobtainable due to distal muscle atrophy; in these cases latencies to proximal muscles were preserved. The main pathological feature in sural nerve biopsies was a depletion of myelinated fibers particularly those of larger diameter with signs of axonal atrophy. In addition there were demyelinating features like thinly myelinated fibers and proliferation of Schwann cell forming onion bulbs usually around regenerative clusters.

Conclusion: GADP1 related neuropathy is the most frequent cause of the severe CMT2 autosomal recessive forms. Distal nerve motor conduction testing may often be misleading due to severe distal muscle atrophy; in these cases latencies to proximal muscles can be useful. Axonal degeneration is the main pathological feature but signs of Schwann cell dysfunction are also present.

P558
**Clinical and magnetic resonance imaging findings in patients with brachial plexopathy after radiation for cancer.** M. C. Petit-Lacour, A. Signate, G. Said, D. Adams, CHU de Bicêtre (Le Kremlin Bicêtre, F)

Background: Diagnosis of underlying mechanism in brachial plexopathy developing in patients previously treated by radiotherapy for cancer is still difficult.

Objectives: To report the MRI findings in patients with suspected tumoral or radiation-induced brachial plexopathy.

Methods: In this retrospective study, we reviewed the files of patients who underwent MRI of brachial plexus between 1997–2002. We compared clinical and MRI findings in patients considered as tumoral plexopathy, radiation-induced or unkown origin. Imaging protocol was as follows: with a shoulder coil, three planes on T1 weighted images, coronal T2 and coronal T1 after gadolinium and fat suppression.

Patients: Fifteen patients were studied in this monocentric study. Mean age was 57.6 years (extremes: 36–75), 14 females, 1 male. They had been treated for breast cancer (n = 14) or lymphoma (n = 1). They were divided in three groups: in group I (n = 7), patients were suspected of tumoral relapse because of a local palpable mass or skin metastasis, in group II (n = 6), patients had no palpable mass and had complete remission of the cancer, in group III patients had no palpable mass but had an evolutive cancer (metastasis, increased tumoral biological marker).

Results: In group I, MRI showed a focal mass encasing the brachial plexus (BP)(4/7) or a fusiform thickening of the BP (3/7). The lesion was in hyperintense signals on T2 weighted images (3/7) and in low signal on T2 (4/7). The lesion was always enhanced after gadolinium. Modification of the symptoms after chemotherapy confirmed the final diagnosis of tumor recurrence in 5 patients; 5 patients died. In group II, MRI showed a diffuse thickening of the BP in high signal T2 (5/6), enhanced in one case, and a normal BP in 1 case. Two patients had also a focal mass, suspected of regional recurrence but not close to the BP. In this group there was a follow-up of 6 to 14 years (mean 12 years) to confim the diagnosis of radiation inducéd plexopathy. In group III, MRI showed a pattern similar as group II in one case and a mass suspected of recurrence explaining the plexopathy in 1 case.

Conclusion: The distinct MRI pattern found in radiation- and tumor-induced brachial plexopathy may be useful to identify tumoral recurrence in previously radiated patients and complete clinical evaluation in the assessment and direction therapy.

P559
**Autoantibodies in chronic idiopathic sensory neuropathy.** M. Sgandurra, R. Nemni, L. Sanvito, G. Santuccio, E. Calabrese, N. Canal, Don C. Gnocchi Foundation (Milan, I)

Introduction: Sensory Neuropathies (SN) are relatively uncommon and are constituted by a heterogeneous group with different etiologies. In the last decades some acquired SN have been associated with specific serum autoantibodies and in particular anti-Hu antibodies (Ab) have been found in paraneoplastic SN, Ab to MAG, sulfatide (S) chondroitin sulfate C (ChS) and disyalogangliosides (DG) have been found in predominantly SN associated or not with monoclonal gammopathy.

Objective: The aim of this study is to detect the prevalence of autoAb to neural antigens (NAg) in chronic idiopathic sensory neuropathy (CISN).

Patients (Pts) and methods: We studied the serum from 35 pts (27 men and 8 women) ranging from 33 to 80 years of age who had CISN by clinical criteria. Known causes of SN were excluded. In all pts the disease had a slowly progressive course for more than a year; the duration of symptoms ranged from 13 to 118 months. Electrophysiological studies were carried out in all pts and showed the predominant involvement of sensory nerve fibres and the absence of sensory nerve action potential in most of the clinically affected limbs.

Binding of Ab to sections of rat central nervous system (CNS) and dorsal root ganglia (DRG) neurons was examined by indirect immunocytochemistry (ICC). Sera were also tested by western immunoblot (WB) of human whole CNS, DRG and skeletal muscle homogenates prepared from autopsies and by ELISA for Ab to MAG, S, ChS and DG.

Results: By ICC, sera from 13 pts immunostained cytoplasm (7/13) or cell membrane (6/13) of neurons. By WB, sera from 15/35 pts reacted with proteins of different molecular weight (45–50 kD, 62–64 kD, 200 kD). By ELISA, 18 of 35 pts had high titre serum Ab to neuropathy-related NAg: 15 to S (42.8%), 6 to DG (17.1%), 4 to ChS (11.4%) and 2 to MAG (5.7%). Altogether reactivity to NAg was detectable in 32 of 35 pts, in 17 reactivity was directed against more than one antigen.

Discussion: Our results indicate that Ab to NAg are detectable in a high percentage (91.4) of pts with CISN. In only 3 pts with CISN, Ab to NAg were not detectable. Our study indicates that either a primary or secondary derangement of the immune system is almost ever-present in CISN even if its relevance for the disease has still to be defined.

P560
**Familial amyloidotic polyneuropathy in Crete, Greece.** C. Spanaki, M. Tzagournissakis, A. Plaitakis, University of Crete (Heraklion, GR)

FAP is an autosomal dominant hereditary disease characterized by polyneuropathy and autonomic dysfunction. Genetically it has been related to more than 50 transthyretin (TTR) gene mutations. Different mutations are associated with similar clinical presentation although the phenotype of the same mutation may vary. Here we report clinical and molecular data, as well as treatment outcome of all Cretan patients with Familial Amyloidotic Polyneuropathy (FAP) seen at the University Hospital of Crete. All patients underwent thorough clinical and laboratory investigations including rectal and/or nerve biopsy. Molecular analysis was performed in all patients. Exon 2 of the TTR gene was amplified from genomic DNA of patients and normal controls by PCR using an appropriate set of primers. The PCR products were digested with the restriction enzyme NsiI and an agarose gel electrophoresis followed. Eight patients (4 men and 4 women), members of 5 unrelated families were studied. All patients had positive family history for polyneuropathy. Extended pedigrees spanning 5 generations were constructed. Three of the patients belonged to one family, two to another and the remaining three were members of 3 separate families. The age of disease onset ranged between 27 and 43 yrs (median = 30,7 yrs). The patients presented paresthesias, progressive weakness and temperature loss at the lower extremities, urinary difficulties, diarrhea, postural dizziness and weight loss. The upper extremities were involved later during the disease progression. Neurological examination revealed loss of pain and temperature sensation in a glove and stocking distribution and distal weakness. All but one exhibited orthostatic hypotension. Three patients presented carpal tunnel syndrome. On electromyographic examination there was evidence of denervation in the muscles of the lower limbs. The conduction velocities were slightly below the normal range. Rectal and/or sural nerve biopsies revealed the presence of amyloid deposit. Molecular analysis showed that all patients were heterozygotes for TTR Met30 mutation. Five patients underwent orthotopic liver transplantation (OLTx) from 1993 to 2001. Three of them showed remarkable improvement especially of their autonomic symptoms and muscle strength. They gained weight and their dysesthesias also subsided. Follow up examination showed no evidence for disease progression. Two patients died of post-operative complications.

Conclusion: Familial Amyloidotic Polyneuropathy that occurs on the island of Crete is due to the TTR Met30 mutation. Haplotype analysis that is in progress may help to elucidate the origin of this mutation in relation to other populations. Our results regarding the liver transplantation corroborate those of other groups suggesting that this is the only effective treatment currently available for FAP.

P561
**Chronic inflammatory demyelinating polyradiculoneuropathy. Two cases with spinal cord compression.** O. Nascimento, C. Soares, E. Segurasse, R. Domingues, Universidade Federal Fluminense (Niterói – Rio de Janeiro, BR)

Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) is a peripheral nerve disorder probably due to an immunological disturbance. It evolves slowly, either in a steadily progressive or in a relapsing and fluctuating course. Weakness is mainly in lower limbs proximally and distally. The electromyography (EMG) findings of demyelination probably define the illness. The cerebral spinal fluid (CSF) protein is often elevated. Sometimes enlarged and firm nerves are found. A small number of cases have been described with spinal cord compression due to hypertrophic spinal roots.

Two patients (females, 66 and 67 years old) with long standing CIDP are described. In the first one the evolution was characterized by remission and relapsing course. The second patient had a progressive course. Their CSF and EMG examinations were typical of CIDP. Both patients had an evolution of 10 or more years. They presented a progressive cervical spinal cord compression. They had an asymmetrical spastic weakness of the four limbs with Babinski sign, withdraw reflexes, a sensory level on the trunk and the arms and bladder disturbance. The Magnetic Resonance Image of the cervical spine done in one patient showed hypertrophic roots deforming the cord. In the second patient the necrosy study showed enlarged roots with inflammatory infiltrates and onion-bulb formations.

Enlarged nerves may occur in CIDP with long evolution. The enlarge-

ment is probably due to recurrent demyelination and subsequent remyelination in the peripheral nerves and spinal roots. Our two patients with CIDP presented after 10 years of the disease a cervical spinal cord compression syndrome due to hypertrophic roots. The neurologist must be aware of the possibility of development of spinal cord compression in patients with a long standing CIDP.

P562

**Guillain-Barré syndrome in childhood: clinical and electrophysiological study.** N. Siala, A. Gargouri, N. Zaouchi, M. Ghorbel, A. Sammoud, S. Bousnina, R. Gouider, Paediatric Hospital, Razi Hospital (Tunis, Manouba, TN)

Objective: To determine the epidemiological, clinical and electro-physiological characteristic of Guillain-Barré syndrome in childhood in Tunisia and the outcome after follow up.

Patients and methods: We studied retrospectively the medical records in 83 cases. 31 cases were controlled prospectively to identify clinical and electrophysiological evolution.

Results: Mean age was 6 years (14 months–15 years), half of them having less than 5 years. A preceding infection was noted in 72 % of cases. Onset by sensory disturbances was noted in 30 %, and by weakness of the legs in 94 %. In all cases motor deficit was noticed. Respiratory involvement appeared in 40 %, Total clinical recovery was observed in 74 % while residual deficit was observed in 23 %. Death occurred in 7.2 %.

The electromyographic examination disclosed a demyelinating pattern in 71 % and an axonal form in 25 %. In 4 % we were not able to classify the electrophysiologic pattern. Control EMG realized in 31 % of patients after a period of 3.3 years (4.5–96 months) show a slight correlation between clinical and electrophysiological signs.

Conclusion: Guillain-Barré syndrome is known to have usually a benign outcome; nevertheless it was accompanied in 23 % of our patients by permanent deficit. A weak correlation was found between clinical and electrophysiological evolution.

P563

**Severe polyneuropathy in Sézary syndrome (leucaemic variant of an erythrodermic cutaneous T-cell lymphoma): report of a case with clinical course over 15 months.** U. Hofstadt-van Oy, H. Kirchen, J. M. Schröder, Krankenhaus der Barmherzigen Brüder, Universitätsklinikum RWTH (Trier, Aachen, D)

Sézary syndrome is a cutaneous T-cell lymphoma (CTCL) presenting with erythroderma, pruritus, circulating atypical T lymphocytes and lymphadenopathy. Neurological complications are rare, one case associated with peripheral neuropathy was reported, whose cause remained unclear.

Case report: A 80-year old former vine-dresser developed over six months a progressive distal weakness with frequent falls. Seven years ago he began to experience erythroderma and pruritus, a skin biopsy and the finding of atypical lymphocytes in the blood proved the diagnosis of a CTCL of Sézary-type. For six years ultraviolet light A (PUVA) and extracorporal photochemotherapy in intervals was given, interferon alpha und external corticoids were given also. On admission the patient had a flaccid distal tetraparesis with areflexia and gait ataxia due to sensory loss. The cranial nerves were intact. The skin showed generalized redness with palmoplantar hyperkeratosis and traumatic defects of greater skin areas on the back. Haemorrhagic conjunctivitis was also seen. Lymph nodes were enlarged. The leucocyte count showed 14 % Sezary-cells. Electroneurography demonstrated diminished amplitudes of motor compound action potentials (MCAP) and sensory nerve action potentials (SNAP) of ulnar and median nerves bilaterally, and loss of MCAP and SNAP of peroneal, tibial and sural nerves. The motor and sensory nerve conduction velocities of the ulnar and median nerves were diminished (25 m/s, amplitudes 30–60 % of normal values), the distal motor latencies and F-waves of these nerves were severely prolonged. Electromyography showed denervation and chronic neurogenic changes in a distal distribution. Since the neurographic criteria of the adhoc committee of the American Academy of Neurology (AAN) for chronic inflammatory demyelinating polyneuropathy (CIDP) were fulfilled, a course of intravenous immunoglobulins was applied. No response was observed.

A sural biopsy revealed massive endo-, peri- and epineurial, mainly perivascular lymphomatous infiltration and axonal loss.

Therapy with chlorambucil was begun, therapy with (PUVA) and extracorporal photophoresis was continued. Over 15 months weakness and ataxia improved, today the patient is able to walk 1000 m with a walking-aid.

Conclusion: In a case of CTCL with Sézary syndrome a severe polyneuropathy of the neuronal type occurred which was caused by extensive lymphomatous infiltration of peripheral nerves. Remission was achieved through escalation of therapy with chlorambucil and continuous application of PUVA and extracorporal photochemotherapy.

P564

**Guillain-Barré syndrome: a retrospective review of 35 cases.** C. Sánchez Bueno, M. I. Férnandez Barriuso, J. M. Callejo, J. Masjuán, J. C. Alvarez Cermeño, J. C. Martínez Castrillo, Hospital Ramón y Cajal (Madrid, E)

Objective: We describe the etiology, clinical features, neurophysiological findings and prognosis of Guillain-Barré syndrome patients diagnosed in our department.

Methods: A retrospective review of Guillain-Barré syndrome cases diagnosed from 1996 to 2000, according to Asbury et al. criteria, in the Department of Neurology of Ramon y Cajal Hospital.

Age, sex, etiology, clinical findings, biochemical parameters of cerebrospinal fluid (CSF), therapeutic management and sequelae measured according to modified Rankin scale were recorded.

Results: We registered 35 cases of Guillain-Barré syndrome, 22 were men and 13 women, aged 15 to 82 years (mean 54.3). The etiology was filiated in 22 cases: a mild viral respiratory infection was recognized in 30 %, 6 % were related to Lyme disease,in other 6 % an infection due to Epstein-Barr virus was found, and another 6 % after surgery. In 13 cases (37 %) we couldn't find a determinant cause. The neurophysiological findings showed four different patterns: 17 % axonal damage, 23 % demyelinization, 46 % mixed pattern and in 9 % the Miller-Fisher variant, 5 % could not be classified.The CSF exams showed different degrees of protein concentration, in 40 % more than 1 g/dl, in 34 % less than 1 g/dl, and in 26 % the CSF was normal. Some CSF exams (11 %) showed mild pleocytosis. Many patients received intravenous inmunoglobulins during five days (63 %). About 23 % of patients needed ventilatory support and intensive care. Twelve patients didn't receive any specific treatment. Other therapies given included steroids and plasma exchange.We registered major secuelae (Rankin 3 or more) in 13 patients, moderate dissability (Rankin = 2) in 11 and mild sequelae 1n 11 (31 %).

Conclusions: We found a predominant mixed pattern in neurophysiological studies with both, axonal damage and demyelinization (46 %), and a relationship between mixed and axonal patterns with moderate-severe sequelae, Rankin of 2 or more.We did not find association between higher protein concentration in CSF and major disability.

P565

**POEMS syndrome with central nervous system demyelination.** M. Díaz-Sánchez, A. Herrero, J. Mera, J. López-Jiménez, J. Masjuan, Hospital Ramón y Cajal (Madrid, E)

Introduction: POEMS syndrome is a rare multisystem disorder known for its signs, from which it also takes its acronym: polyneuropathy, organomegaly, endocrinopathy, monoclonal protein and skin changes. This polyneuropathy belongs to the group of chronic inflammatory demyelinating polyradiculoneuropathies (CIDP). The pathogenesis of this syndrome remains unknown, but the presence of monoclonal protein in the serum of patients suggests that immune dysfunction contributes to it.

Case Report: a 67 years old patient presented a history of progressive anorexia, weight loss, weakness and paresthesias in extremities (initially in both legs). Neurological examination revealed a sensorimotor polyneuropathy, involving predominantly the lower limbs. In addition to neurological findings, the patient had mediastinal and supraclavicular adenopathies, and hepatosplenomegaly. Serum protein immunoelectrophoresis revealed a peak of monoclonal IgG lambda gammapathy. Electrophysiological evaluation showed a predominantly demyelinating sensorimotor polyneuropathy. Pathological findings in the lymph nodes were compatible with Castleman's disease. In the context of a sudden confusional state a cranial magnetic resonance imaging (MRI) was performed and disclosed diffuse alteration of cerebral white matter highly suggestive of demyelination.

Discussion and conclusions: the peripheral neuropathy is a common feature in patients with POEMS syndrome. This polyneuropathy which resembles CIDP, usually precedes other abnormalities. Sporadic reports have emphasized the clinical association between CIDP and central nervous system demyelination, but as far as we know not in the context of POEMS syndrome. Cranial MRI should be done to patients with POEMS syndrome in order to verify this new feature.

**P566**
**Brachial amyotrophic diplegia in a HIV-type 1 positive patient.** G. Galassi, A. Ariatti, P. Nichelli, A. Barbieri, University Hospital (Modena, I)

Brachial amyotrophic diplegia (BAD) is defined the isolated upper limb weakness attributed to neurogenic atrophy, sparing lower extremity, respiratory and bulbar muscles. Although such phenotypes may be seen in number of conditions, previous reports pointed out that the label of BAD should refer to patients with slowly or nonprogressive neurogenic atrophy of upper limbs remaining stable at least 18 months after initial presentation. A 50 year old man with unremarkable past history noticed after minor cervical spine trauma progressive weakness and wasting of left shoulder and arm which was followed within few months by gradual involvement of contralateral in relatively symmetric pattern. There were no symptoms in lower limbs, neck, bulbar muscles nor spincter disturbances. On examination, patient exhibited a peculiar posture of both hands hanging loosely at his sides, recalling the "man in the barrel" phenotype. There were visible fasciculations and marked atrophy in upper limb proximal muscles. Reflexes could not be elicited in upper extremities but they were normal in the lower. Cranial nerves and sensory examination were unremarkable. Routine blood tests and search for anti GM1 antibodies were negative. Creatine kinase was found mildly elevated (361mU/ml, normal below 195). Test for HIV-1 was positive. Plasma RNA level was 144.921 copies/ml with CD4+lymphocyte count equal to 403/mm$^3$. Electrophysiology showed in both sides median and ulnar low amplitude of compound muscle action potential, mildly reduced velocity without evidence of conduction block. Sensory conduction study was unremarkable. On electromyography, there were denervation potentials, loss of motor units, acute and chronic neurogenic changes in muscles innervated by C5-C8 myotomes. Cervical magnetic resonance was normal. Patient neurological disability failed to show progression. The case reported exhibits notable aspects: clinical and electrophysiological abnormalities fully restricted to upper limbs, lack of significant demyelinating features, absence of anti GM1 antibodies and of clinical signs related to immunesuppression due to HIV-1 infection. In our opinion, the possible differential diagnosis were multifocal acquired motor axonopathy or a slowly progressive lower motor neuron disorder.

**P567**
**Extracorporeal immunoadsorption: potential treatment for multifocal motor neuropathy. A case report.** C. Khamis, Lebanese Hospital Geitawi (Beirut, LBN)

Case presentation: A 48 years old female presented with 9 years history of progressive asymmetrical weakness of the arms predominant on the left side distally with muscular atrophy and no sensory signs. She had electrophysiological evidence of multifocal motor conduction blocks. MRI of both brachial plexuses showed abnormalities more evident on the left side; this was diffusely swollen with increased signal intensities mainly of the lower brachial plexus on T2-weighted images. As the patient was diagnosed of having MMN, she received intravenous immunoglobulin (IVIG) maintenance therapy 1 g/kg/day for 2 days every 6 weeks which resulted in a nearly complete recovery of muscular strength. However, a year later, she developed tolerance requiring more frequent administration of IVIG with only partial relief of her symptoms. A decision was made to treat the patient with ECI. This was conducted as follows: three weekly treatments for two weeks, then two weekly treatments for two weeks and then one weekly treatment for two weeks. One month after the beginning of the immunoadsorption therapy the patient showed remarkable improvement in her symptoms with near complete recovery of muscular strength. Six months after ECI the patient was still in clinical remission. ECI appeared to be an effective treatment for MMN, this for a very long time. This treatment needs to be further investigated in larger studies in order to estimate objectively its efficacy.

**P568**
**Mononeuropathy multiplex caused by cyclosporine, and improvement following substitution by Tacrolimus, in a liver transplant recipient.** C. Pascual, C. Gaig, J. Valls, Hospital Clínic (Barcelona, E)

Introduction: Cyclosporine and tacrolimus have improved substantially the survival of the graft in several types of transplants. Their main side effects are nephrotoxicity and hepatotoxicity. Neurotoxicity appears in 10–42% of the cases, depends on the series. The cyclosporine has been connected mainly with posterior leucoencephalopathy and pontine myelinolisis, which are usually reversible partially or totally after stopping or adjustment of dose. Sometimes patients report pain and other annoying sensations in fingers the first weeks of the treatment that disappear quickly spontaneously.

Clinical case: Sixty-four year old woman, with idiopathic liver cirrhosis, without other diseases, was liver transplanted. She was treated with corticoides and cyclosporine. There were no postoperative complications. The cyclosporine levels kept within correct range. The first weeks after transplant, arterial hypertension was detected. Both transaminases and cholestasis markers kept continuously two or three times over the normal rates, bilirubin values were normal. In the following months the transaminases kept high, coming to a normal level the colestasis markers.

After two weeks of being transplanted, the patient began to notice paresthesias and sharp pain in top of fingers and feet. These sensations became harder and more constant in a progressive way, growing proximally in the left arm and leg. The right patellar reflex was absent. There was no loss of strength. In the eighth month after the liver transplant electromyographical tests showed alterations compatible with mononeuritis multiplex. The treatment with Cyclosporine by Tacrolimus was substituted. In a month the transaminases were normalized and the dose of antihypertensive drugs could be diminished. The sensitive disturbances improved but did not disappear completely. Known causes of mononeuropathy multiplex were ruled out. Electromyography of control in the sixth month after changing immunosuppressive therapy does not imply changes concerning the previous one.

Discussion: In patients treated with cyclosporine it is uncommon to find alterations of peripheral nervous system on a sustain way. Our patient showed sensitive progressive symptoms, more compatible in the beginning with polyneuropathy, that became slowly more asymmetrical. Mononeuropathy multiplex was shown by neurophysiological explorations. Other causes which could justify them were ruled out. We think a reasonable hypothesis would be to consider ciclosporine as the responsible, taking into account that it appeared in the context of the beginning of the therapy, progressed slowly as long as it continued, and exist a slight tendency to a subjective recovery after conversion to tacrolimus. We think that the possibility of a clinical or subclinical affectation of peripheral nervous system due to cyclosporine, probably by myelinotoxicity, must be considered.

**P569**
**Miller-Fisher syndrome and MRI evidence for a brainstem involvement: a case report.** S. Vlaski-Jekic, I. Kuzmanovski, N. Tufai, Clinic of Neurology (Skopje, MK)

Some authors reported Miller-Fisher syndrome (MFS) as a rare variant of Guillain-Barré syndrome (GBS). Others argued about the central origin of the pathological process and they approached this syndrome to the Bickerstaff brainstem encephalitis (BBE). We report a case where clinical features and investigation data are consistent with both, an acute cranial, limb and dysautonomic peripheral polyneuropathy, and an acute localized brainstem encephalitis.

Three weeks after an upper respiratory infection, a 35 year-old woman complained of a headache, nausea, vomiting, diplopia, bilateral tinnitus and reduced hearing, and an increasing impairment of balance, making her unable to stand and walk. Several days later, asymmetrical facial palsy, dysarthria, dysphagia, trigeminal and distal limb paresthesiae developed and urinary retention occurred. On examination she was fully conscious and manifested a prominent truncal, limb and gait cerebellar ataxia, lateral gaze palsy and upward gaze paresis, as well as bilateral asymmetrical lower motor neuron facial weakness, bilateral sensory loss in trigeminal territory, partial bulbar palsy, tachycardia and urinary and bowel retention. There was no limb weakness or objective sensory dysfunction, except for a slight distal legs diminution of vibration sense. Tendon reflexes were brisk, and left extensor plantar response was present.

General laboratory investigations, including haematological, biochemical, serological and radiological studies were normal. Findings of cranial computed tomography (CT), electroencephalography (EEG) and CSF investigations were within normal limits. Electromyography (EMG) of distal and proximal limb muscles showed no evidence of denervation. Neurophysiological studies demonstrated normal peripheral motor nerve conduction velocities. Sensory evoked potentials (SSEPs) registered a complete conductive block on the brainstem level. MRI was performed one month after the disease onset. It showed a posteriolateral pontine lesion on each side anterior to the fourth ventricle, hyperintense on T2 weighted images. The illness remained static for about ten days, and then it began to improve when high doses of Methylprednisolon were administered. Clinical recovery completed in three months, and MRI findings resolved five months later.

It seems real to estimate that an individual nosological entity exists, clinically manifesting ophthalmoplegia, ataxia and areflexia syndrome (OAAS), in which CNS and PNS involvement concomitantly occur.

P570
**Multifocal motor neuropathy with conduction block and cranial nerve involvement.** G. Galassi, R. Suozzi, G. Tassone, University Hospital (Modena, I)

Multifocal motor neuropathy (MMN) is a chronic immune mediated demyelinating neuropathy characterized by slowly progressive painless asymmetrical weakness usually affecting to a greater extent the arms than the legs and distal more than proximal muscles. GM1 antibodies are found in high titer in 22%–84% of cases. Electrophysiological findings are conduction block (CB) not at usual sites of compression, abnormal temporal dispersion and F-waves, normal or near-normal sensory velocity. We describe a patient affected by MMN with CB exhibiting unusual progression and clinical features.This 63 year old woman was referred for evaluation of one year and half duration weakness which began in right upper limb and progressed to contralateral and to lower extremities. On first admission (July 2001) neurological examination showed normal cranial nerves and sensory testing, moderate asymmetric weakness mainly proximal in the upper extremities and distal in the lower. There were atrophy of small hand muscles and weak deep reflexes. She denied sensory symptoms. There were normal cranial nerves and no upper motor neuron signs. Laboratory tests including creatine kinase, tumour markers, spinal fluid (CSF), anti-GM1antibodies were unremarkable. Electrophysiological study was consistent with MMN with proximal and distal CB. Patient underwent intravenous immunoglobulin (IVIg 0.4 g/kg/daily for five days) and plasmapheresis without significant improvement. In October 2002, her conditions rapidly worsened: she became quadriplegic, unable to swallow, to chew and to protrude her tongue. Admitted to intensive care unit because of respiratory failure, tracheostomy was performed. Repeated searches for anti GM1 antibodies gave negative results. CSF was normal. IVIg (0.4 g/kg/daily for five days) was administered twice with partial benefit although severe disability persisted. IVIg were maintained at the dose of 2 g/Kg each 4–6 weeks. On conduction, features of demyelination and of axonal loss persisted in the nerves of the arms. CB was detected in the ulnars between Erb's point and axilla. The case presented has notable features: the monophasic progression to quadriplegia within two years and half, the acute worsening in absence of precipitating events and of systemic illness, the severe involvement of cranial nerves and respiratory muscles. The response to IVIg is in favour of an immune mediated disorder despite the absence of antiGM1 antibodies.

P571
**Aetiology of small fibre neuropathy.** E. Moravcova, J. Bednarik, University Hospital Brno (Brno, CZ)

Background. Small fiber neuropathy (SFN) is reported to be associated with a spectrum of diseases or conditions that are partly different from those associated with large fiber neuropathy. The study offering proportion of different aetiologies of SFN is, however, lacking.

Goal of the study was to evaluate the aetiology of a group of SFN patients in comparison with mixed (i. e. small and large) fibres neuropathy (MFN).

Methods. A group of 120 consecutive patients sent to the Department of Neurology during 3 years (from 1999 to 2001) with subacute or chronic clinical symptoms of burning, painful dysesthesias in the feet were evaluated clinically and electrophysiologically with quantitative thermal threshold testing (TTT) and routine conduction study (CS) and needle EMG. Seventy-six patients with abnormal TTT and excluded central nervous system lesion were divided into isolated small fiber neuropathy group (31 cases) and mixed fiber neuropathy group (45 cases). SFN cases displayed no motor or sensory loss (with the exception of absent ankle reflexes in some cases), and showed normal CS and needle EMG. Abnormal autonomic tests (heart rate variability and sympathetic skin response) were found in 54% of SFN cases.

Both groups were extensively evaluated for possible aetiology. Beside routine biochemical, haematological and immunological blood tests we also examined a battery of antineuronal antibody tests (anti-GM1, anti GQ1b, anti-GD1b, anti-MAG, anti-sulfatides). Chronic alcohol abuse was verified by a battery of laboratory markers: gamma-glutamyl transferase, mean corpuscular volume and carbohydrate-deficient transferin.

Results. Diabetes mellitus (DM) and chronic alcohol abuse were dominant aetiologies in SFN group (45%; 16%), while further causes were sparse and included monoclonal gammopathy, amyloidosis, critical illness, nephropathy, paraneoplastic mechanism, chemotherapy and B12 deficiency. The spectrum and proportion of causes in the MFN were similar to SFN group. The duration of DM was significantly shorter in the SFN group. We were not able to disclose aetiology in 26% of SFN cases and 24% of MFN cases. Four SFN patients showed slight (up to three-fold) increase of antineuronal antibody titers (including one idiopathic case). A family history of neuropathy was not found in the SFN group.

Conclusions: Subacute and chronic small fiber neuropathy is probably not an independent entity, at least from etiological point of view. Involvement of small somatic fibres is often mixed with those of autonomic and large somatic fibres, but in some cases it may serve as the initial damage. Dominant causes of both small and large fiber polyneuropathies in developed countries are diabetes mellitus and chronic alcohol abuse. About one fourth of cases remains idiopathic. The possibility of autoimmune aetiology of idiopathic subacute or chronic SFN is improbable.

The study was supported by the IGF Brno, grant No15/2000.

P572
**The puzzle of adult-onset unclassified neuropathies: severe chronic idiopathic axonal polyneuropathy. A distinct entity?** L. Quaranta, M. Sabatelli, F. Madia, G. Lippi, A. Conte, M. L. Mereu, P. Tonali (Rome, I)

Unclassified neuropathies still represent a challenge for neurologists, since in about 20–30% of patients no cause can be identified after extensive investigations.

In this context some patients sharing peculiar features have been reported as to be grouped in a single entity termed Chronic Idiopathic Axonal Polyneuropathy (CIAP). The onset occurs in middle to late adulthood, there is a predominant sensory involvement and progression is slow, usually not disabling. Electrophysiologic and pathological studies are consistent with a primary axonal injury.

We report on five patients (four males and one female) affected by adult-onset chronic "idiopathic" axonal neuropathy characterized by an unusual clinical course leading to severe disability and finally to death in two of them.

Onset occurred in the fifth–seventh decades and was characterized by sensory-motor symptoms and signs in both lower and upper limbs with distal distribution. Over a 2–14 years of follow-up the disease showed a progressive course with severe muscle weakness and atrophy of semidistal-distal muscles and involvement of proximal muscles in all cases. Two patients died due to respiratory failure after 5 and 8 years since the onset.

All cases were sporadic and extensive laboratory examination excluded identifiable causes of neuropathy. Nerve biopsy was carried out in all of them showing a severe axonal neuropathy. Amyloid deposits were not observed in nerve sections nor in abdominal fat aspirate. Therapies with steroids and IVIg were attempted in all patients without any response.

Drawing the nosology of acquired adult-onset axonal polyneuropathies is difficult to date since the etiology is still obscure in most cases. Our findings suggest that among this not well defined group of diseases a different subtype exists showing a severe outcome. Whether or not this condition represents a distinct entity, remains to be elucidated.

P573
**Intra-individual variability of thermal threshold assessment.** E. Moravcova, J. Bednarik, University Hospital Brno (Brno, CZ)

Background. Thermal threshold testing method (TTT) is a quantitative sensory test based on psychophysical principles of the thermal threshold, tolerance, the relation between the supraliminar stimulus and the response. A paucity of data referring to the intra-individual variability of this psychophysical test is available. The goal of this study was to assess trial-to-trial variability of threshold values in normal subjects and in small fiber neuropathy (SFN) patients.

Methods. A thermal threshold for cold and warm sensation was established repeatedly within 1 week in a group of 30 healthy volunteers and in 58 SFN patients with abnormal introductory thermal threshold values assessed by TTT. Thermal threshold was established in two locations (the thenar of the hand and the dorsum of the foot) using the electrodiagnostic unit Nicolet Viking IV, software Thermal Sensory Analyser of the Medoc company and the Peltier's contact thermal stimulator. All individuals were examined with 3 various algorithms: two reaction time inclusive methods (RTI – Limits I and II) and one time exclusive method (CS – the random variant of Levels). A trial-to-trial variability of threshold values was expressed as the day-to-day variation coefficient (VC) calculated as the difference between the two threshold values divided by the difference between the first threshold value and the neutral temperature (32 °C) x 100 (expressed as the percentage).

Results. In normal subjects the upper limit for the VC expressed as the 90th percentile varied in various tests between 43 and 82% in the upper extremities, and between 55 and 111% in the lower extremities independently of the type of used algorithm, age, sex, and tested region. All SFN patients showed at least one abnormal thermal threshold value in both

examinations. In SFN patients the absolute differences (expressed in °C) between repeated threshold values increased, but the VC didn't increase significantly in comparison with normal subjects, varying between 62 and 94% in the upper extremities, and 25 and 100% in the lower extremities (90th percentile). In most tests, however, the VC calculated for the cold threshold were significantly higher in SFN patients in comparison with warm threshold values.

Conclusions. The intra-individual variability of the threshold values is considerable, which is not surprising with respect to the psychophysical character of the test, and is comparable with other quantitative sensory tests. The selective increase in trial-to-trial variability of cold threshold values must be taken into consideration in longitudinal follow-up of patients with SFN.

The study was supported by the IGF Brno, grant No15/2000.

P574

**Long term effect of liver transplantation on met 30 TTR familial amyloid polyneuropathy.** D. Adams, D. Samuel, A. Kreib, M. Nakazato, V. Planté, H. Bismuth, G. Said, CHU de Bicêtre (Le Kremlin Bicêtre, F)

Background: Liver transplantation (LT) has been proposed to treat TTR-FAP but its long term effect on the neuropathy is not known, and mechanisms to explain worsening of neuropathy after LT remain unclear.

Objectives: To report long term effect of LT on met30 TTR Familial amyloid polyneuropathy (FAP) and analyse influence of variant TTR in CSF after LT.

Methods: In a monocentric study, we evaluated prospectively and periodically FAP patients before and after LT. Evaluation included functional (Norris) score, extension of superficial sensory loss, muscle testing, electrophysiological evaluation of sensory and motor nerves of the four limbs, search for postural hypotension. We measured variant met30TTR in serum before and after LT and variant met30TTR in CSF.

Results: 70 patients underwent LT between 1993 and 2001. The mean age was 40 y. (range from 22 to 68). The overall survival rate at 5 y. was 71%. In patients with Norris score > 55/81 and without urinary incontinence, the survival rate reached 92%. Twenty six pts with the met30TTR variant were followed for more than 4 years (mean 6.5 years; 4–10 yrs). Six patients had a severe sensory-motor neuropathy (SMN) that required aid for walking (Group Ia), 15 pts a moderate SMN (group Ib) and 5 patients a pure sensory neuropathy (GroupII). In Group Ia, neuropathy worsened in 4 pts. Walking difficulty progressed in 2 pts including one who became bedridden and eventually died, weakness progressed in hands in 4. In group Ib, neuropathy remained stable in 12/15 patients but worsened in 3 with increased walking difficulty, one of them became bedridden. Neuropathy remained stable in group II. There was no improvement of motor function. Manifestations of autonomic dysfunction remained unchanged. Neuropathic osteoarthropathy developed in 5 pts (20%) in hip (n = 1), knee (n = 2), and feet (n = 2) which was severe in 3 leading to walking disability. Ocular manifestations developed in 4 pts. Level of serum met30TTR decreased by a mean of 98% after LT with a durable effect, ranging from 0.06 to 0.48 mg/dL after LT. Level of met30TTR in CSF remained at a high level ranging from 0.34 to 1.55 mg/dL after LT.

Conclusions: LT stops progression of the neuropathy in 73% of patients with met30TTR FAP. Increased CSF met30TTR levels released by choroid plexuses may explain progression of the neuropathy in the others.

# Poster Session 4

## Cerebrovascular disorders

P575

**Lack of association of genotypes of three candidate genes for early carotid atherosclerosis in 418 German males.** J. Metrikat, M. Albrecht, F. Weber, Institute of Aviation Medicine (Furstenfeldbruck, D)

Background: Atherosclerosis is considered to be a complex multifactorial disorder caused by genetic, environmental and behavioural factors. Carotid artery intima-media thickness (IMT) is a widely accepted marker for the extent of early atherosclerotic changes. The aim of this study was to assess the relationship between IMT and polymorphisms of three candidate genes which have been associated with cardiovascular disease before.

Methods: High resolution ultrasound in B-mode of the carotid arteries was performed in 418 young, healthy, German males (mean age 44 ± 8 years). Both carotid arteries of each subject were scanned in multiple planes in a standardized fashion to locate maximum thickness of the common carotid artery (CCA) and its bifurcation. Maximum and mean IMT measurements of both sides were obtained. The investigator was blinded for the person's genotypes. All subjects were genotyped for angiotensinogen (AGT M235T), vitamin D receptor (VDR BsmI) and interleukin 6 promoter (IL-6–174G/C) polymorphisms by PCR and RFLP (AGT, VDR) or TaqMan (IL-6).

Results: Genotype distribution of all polymorphisms was in Hardy-Weinberg equilibrium. In this sample mean CCA IMT was 0.71 ± 0.13 mm, mean IMTmax was 0.91 ± 0.49 mm. There was no association between genotypes of the three candidate genes and IMT.

AGT: MM (n = 143) IMTmax = 0.9 ± 0.43 mm; MT (n = 231) IMTmax = 0.92 ± 0.52 mm; TT (n = 44) IMTmax = 0.93 ± 0.57 mm, ANOVA p = 0.92.

VDR: bb (n = 153) IMTmax = 0.89 ± 0.46 mm; Bb (n = 184) IMTmax = 0.94 ± 0.56 mm; BB (n = 81) IMTmax = 0.91 ± 0.49 mm, ANOVA p = 0.61.

IL-6: GG (n = 130) IMTmax = 0.86 ± 0.36 mm; GC (n = 202) IMTmax = 0.96 ± 0.59 mm; CC (n = 86) IMTmax = 0.87 ± 0.39 mm, ANOVA p = 0.12.

Conclusion: None of the investigated candidate genes showed a strong association with early carotid atherosclerosis in young German males. We therefore conclude that single nucleotide polymorphisms may just have weak effects on the pathogenesis of carotid atherosclerosis. However, further studies including larger numbers of subjects and tested candidate genes are still necessary.

P576

**Stroke incidence in a large centre of chemical industry, 1994–1999.** M. Kolesnikov, Nizhny Novgorod State Medical Academy (Nizhny Novgorod, RUS)

Background and Purpose: The present study was conducted to investigate the incidence of stroke in residents of the city of Dzerzhinsk, Nizhny Novgorod region, Russia (approximately 280 000 subjects) which is one of the largest centers of chemical industry in the country, for the period of 6 years. This study was designed to evaluate whether the chemical industry pollution is a risk factor for stroke or not in order to define several measures to prevent stroke and to increase the understanding of the origin of stroke in a defined Russian population.

Methods: All residents of Dzerzhinsk who had an incident (first-ever) stroke from January 1, 1994 through December 31, 1999, were registered. Immediate notification containing information on all new cases of stroke came to the Stroke Unit of the Municipal Hospital # 3. Those notifications came from primary care physicians, neurologists, inpatient and outpatient clinics, pathologists and forensic-medicine experts. The definition of stroke was based on standard criteria.

Results: The annual incidence rate of stroke increased from 292/100 000 population in 1994 to 497/100 000 in 1999. At the same time 49 chemical shops and factories were closed in Dzerzhinsk during this 6-year study period because of economic problems. Thus the economic decline caused the improvement of ecological situation. For example, the complex index of air pollution declined on 50 percent during the study period. The incidence rate of cancer which is traditionally considered to be one of the markers of the environment state increased from 289/100000 in 1994 to 322/100 000 in 1999 – not at all as significantly as stroke incidence rate did.

Conclusions: Stroke incidence rates in Dzerzhinsk are among the highest in the world. Economic factors influenced these rates much more significantly than ecological factors did. A lot of people were sacked from their jobs because of economic crisis. Thus, they could not afford modern drugs for stroke prevention. Moreover, the customary system of medical examination of the chemical plant workers was destroyed. The system of stroke prevention has to be improved in Dzerzhinsk.

P577

**Is hypoplasia a risk factor for vertebral artery dissection?** C. Zanferrari, C. Bertolino, R. Silvano, F. Granella, D. Mancia, Institute of Neurology (Parma, I)

Background: Spontaneous cervical artery dissections are thought to have an underlying structural defect of the arterial wall, although the exact type of arteriopathy remains unclear. Some morphological features such as arterial redundancies, increased arterial distensibility and/or significant diameter changes have been described in carotid dissection. Conversely, only a few morphological studies have been performed in vertebral artery dis-

section (VAD). Changes of vertebral artery size might represent a possible morphometric parameter which suggests underlying arterial wall abnormalities.

Aim: To measure the size of vertebral arteries and assess the presence of hypoplasia by ultrasonographic study in patients with vertebral artery dissection (VAD) and in age-matched healthy subjects.

Patients and Method: Color-flow duplex sonography was used to assess the diameter of the vertebral artery in 15 patients (7 M and 8 F; mean age 42 ± 12 years, range 23–62 years) with a diagnosis of VAD confirmed by angiography and/or MRA and in 15 age-matched healthy subjects. In both patients and controls, the linear length between the adventitia layers seen on sagittal and transverse planes was considered as the vessel diameter. In all subjects, the diameter was measured at two standardized points: in the pre-transverse tract (V1) and in the inter-transverse segment (V2) between C3 and C4 levels. The diameter of the dissected artery was compared with that of the contralateral one and with that of healthy subjects. Cut-off limits were > 3 mm for normal diameter, between 3 and 2 mm for reduced size, and < 2 mm for vertebral hypoplasia.

Results: Dissection involved the distal segments V3 and/or V4 in 67 % of patients and the proximal segments V1 and/or V2 in 33 % of patients. One patient had a bilateral dissection involving the V2 segment. The mean diameter of dissected arteries (2.4 ± 0.8 mm) was significantly lower ($p < 0.001$) than that of the opposite site (3.4 ± 0.5 mm) and of controls (3.3 ± 0.4 mm). Moreover, the site of dissection (distal or proximal) did not influence these results. Overall, in 8 of 16 patients (50 %) the dissected vertebral artery was hypoplasic (< 2 mm), whereas in 5 of 15 (33 %) it showed a reduced size (between 3 and 2 mm).

Conclusions: The prevalence of hypoplasia was significantly higher in dissected vertebral arteries than in healthy vessels. These findings suggest that vertebral hypoplasia could be considered a potential risk factor for artery dissection.

**P578**

**Seasonality and frequency of the appearance of strokes.** T. Vassileva-Stoeva, V. Dosheva, E. Hadzipetrova, Medical University (Plovdiv, BG)

Background: the cerebrovascular diseases are among the crucial problems of bulgarian health services because the country ranks at one of the first places in the world of stroke morbidity and mortality. In contrast to the common concept of unfavourable influence of risk factors for frequency, severity rate and mortality of cerebral stroke, there are few surveys about seasonality and the published results are contradictory.

Aim: to study the seasonality of appearance of strokes using material of the Clinic of cerebrovascular diseases of the Medical University-Plovdiv, Bulgaria.

Material and Methods: a retrospective clinically-based study of all patients (1046) treated of cerebral stroke within a 3-year period [1999–2001] was made. Four seasonal groups were formed: winter, spring, summer and autumn. Two undergroups were formed in each seasonal group: hemorragic and ischemic stroke. The obtained results are compared by the methods of alternative analysis.

Results: of all studied patients (1046) 600 (57.36 %) are men and 446 (42.69 %) are women. 280 (26.77 %) are with hemorrhagic stroke and 266 (73.23 %) are with ischemic. There are no significant differences in the seasonal variation in ishemic strokes. There is significantly lower frequency in hemorrhagic strokes ($p < 0.001$) during summer (15.71 %) compared towinter (30 %), spring (27.50 %), autumn (26.79 %).

Conclusion: The retrospective clinically-based study shows a statistically considerable higher frequency of hemorrhagic strokes in winter, spring and autumn, in contrast with summer.

**P579**

**The association of Chlamydia pneumoniae – inflammatory markers in patients with acute ischaemic stroke.** C. Falup-Pecurariu, I. Varga, D. Minea, C. Timu, M. Anghel, M. Radoi, University Transilvania (Brasov, RO)

Recent studies have shown the role of inflammation and chronic infections in development and progression of the atherosclerotic lesions. These may be considered as chronic inflammatory disease of arterial wall.

The arguments for involvement of Clamydia Pneumoniae in atherosclerosis are: sero-epidemiological, immunohistochemistry, electronic microscopy, animal models, in vitro experiences and indirect by antibiotic therapy. The most clear arguments were proven by detecting this agent in the atherosclerotic plaques.

The aim of this study is to assess how Chlamydia Pneumoniae infection influences the evolution of inflammatory markers in patients with ischemic stroke.

Patients and method: in a prospective study we have enroled 50 patients with acute ischemic non-cardioembolic stroke. We have documented by using ELISA method the presence of anti-Chlamydia Pneumoniae antibodies (IgG and Ig A), and the serum levels of interleukin-6, tumor growth factor beta, fibrinogen and C-reactive protein. We followed up the incidence of Chlamydia Pneumoniae infection in our patients and the evolution of these parameters related to the presence or absence of the anti Chlamydia Pneumoniae antibodies.

Results: in the study group the incidence of Clamydia Pneumoniae antibody was 34/49 (69.3 %) at the beginning of the study, 26/45 (57.8 %) at 1 month, 36/44 (81.8 %) at 6 months. In the control group the incidence was 11/32 (34.4 %).

At the beginning the incidence of Chlamydia Pneumoniae antibody and hypercholesterolemia was correlated with high level of IL-6. At 30 days at patients with hypercholesterolemia the inflammatory factors (PCR, fibrinogen, IL-6) was significantly higher independently of presence of Chlamydia Pneumoniae antibody.

Conclusion: the incidence of Chlamydia Pneumoniae antibody was higher in ischemic stroke than in control group at the beginning of the study but was not correlated with high level of inflammatory markers at 30 days.

**P580**

**Cerebral $CO_2$-reactivity impairment is assessed at microcirculatory and macrocirculatory levels by NIRS and TCD in.patients with vascular dementia of small vessel origin.** G. Panczel, C. Rozsa, T. Magos, Z. Nagy, National Stroke Center, Jahn Ferenc Hospital (Budapest, HUN)

Background: Near-infrared spectroscopy (NIRS) measures concentration changes of chromophores like total- and oxyhemoglobin (Hb, HbO) and volumes of total hemoglobin (HbVol)-parameters that characterize cerebral blood perfusion and oxygenation. We aimed to assess impairment of vasoreactivity at both the large vessel (by transcranial Doppler/TCD/) and the microcirculatory levels (by NIRS) in vascular dementia.

Methods: 24 patients with dementia, multiple vascular risk factors and MR changes of pronounced vascular encephalopathy and 14 age-matched controls were included in the study. After 10 minutes of rest a 2-minute hypercapnic challenge was introduced by a closed respiratory system. In this period the systolic (Vs), diastolic (Vd) and mean (Vm) cerebral blood flow velocities (CBFVs) were monitored in the left middle cerebral artery by TCD, the Hb and HbO concentration and Hb volume (HbVol) of the left frontolateral region by NIRS, the heart rate and the blood pressure by a BP-monitor, the $pCO_2$ of expired air by a capnograph.

Results: The Hb and HbO concentrations, the HbVol and the CBFVs increased to a maximum and remained around this value in the 2-minute period in controls but this increase was significantly smaller in patients. Mean changes were as follows. Controls: dHb (CI95): 0.19 (0.39), dHbO:1.94 (0.8), dHbVol: 2.18 (0.84), dVs:30 (9.1), dVd:15 (5.7), dVm:20 (6.8). Patients: dHb (CI95;p): −0.25 (0.23; = 0.05), dHbO:0.95 (0.37; < 0.05), dHbVol: 0.62 (0.44; < 0.05), dVs:11.3 (5.8; < 0.05), dVd:7.95 (3.4; < 0.05), dVm:9.95 (3.99; < 0.05). $CO_2$-reactivity was severely impaired in patients with baseline Vm < 40 cm/s and was relatively normal if baseline Vm was above this threshold.

Conclusion: Vascular dementia of small vessel origin was associated with a significant restriction of $CO_2$-reactivity assessed both at small-and large vessel levels. Impairment of $CO_2$-reactivity is in association with baseline CBFV. NIRS has temporal resolution high enough to allow dynamic measurements of vasoreactivity.

**P581**

**The role of systolic blood pressure below 140 mmHg in acute stroke outcome. Should it be treated?** B. Fuentes, E. Díez-Tejedor, R. Merino, A. Frank, P. Barreiro, La Paz University Hospital (Spain, E)

Background: Recent data from IST point to low systolic blood pressure (SBP) as an independent prognostic factor of poor outcome in acute ischemic stroke (IS). Previous studies have suggested the benefit of hypertensive therapy in acute IS. Our goal is to evaluate the presence of SBP < 140 mmHg in the first 48h from onset, its influence on outcome and hypertensive therapy in cases with neurological impairment (NI) associated to low SBP.

Methods: Prospective study. Inclusion criteria: IS < 24 hours. Exclusion criteria: TIA, previous dependence, unconsciousness, severe or fatal concurrent disease. SBP and Canadian Stroke Scale (CSS) were determined every 8 h during the first 48 h. NI was defined as a decrease in 1 point in CSS. Patients with NI associated to SBP < 140 and NIHSS > 4 with SBP threshold were selected for hypertensive therapy. Outcome at 3 months

was evaluated by CSS and modified Rankin scale (mRS). Chi-square, t-student, Fisher and U-Mann Whitney tests were carry out.

Results: 81 patients included (mean age 70,9 ± 10,9 years). Mean time from stroke onset: 6 ± 5.8h. 60 patients (74 %) presented SBP < 140 in the first 48 hours, from which 8 % suffered neurological impairment. Only one patient fulfilled hypertensive treatment criteria. A significant improvement in CSS (up 3 points) was demonstrated in this patient. No significant differences between patients with SBP < 140 or > 140 in length of stay and outcome at 3 months were detected (CSS p = 0.823, mRS p = 0.944).

Conclusions: SBP < 140 is a common finding in acute IS but only in few cases determines NI. Our data do not support the opportunity of systematic hypertensive therapy in IS, only in selected patients. More studies are needed to determine the profile of patients prone to develop NI with low SBP in which hypertensive therapy could be useful.

P582

**Aetiopathogenic heterogeneity in Sneddon syndrome.** A. García Pastor, J. Rodriguez Vico, F. Díaz Otero, J. A. Villanueva Osorio, S. Gimenez Roldan, Hospital Gregorio Marañón (Madrid, E)

Background: Sneddon Syndrome (SS) is defined as the association of livedo reticularis and stroke. Although the etiopathogenicity of SS is unknown, some cases associate antiphospholipid antibodies (aPL). Cerebral angiography usually shows multiple occlusions of the distal branches of cerebral arteries.

Purpose: Study of the etiopathogenic features of SS.

Methods: Analysis of 9 patients with SS diagnosed in our institution during the period 1995–2002. All patients underwent laboratory tests including inmunological investigation (aPL determination, and other antibodies), EKG, transthoracic echocardiography, and cranial MRI. A cerebral angiography or angio-MRI was performed in 8 patients, 5 patients underwent a cerebral SPECT and a skin biopsy was performed in 4 patients.

Results: All patients were women, mean age: 39 [32–49], 8 patients suffered a stroke or TIA. In one case the first neurological manifestation was vascular dementia. 5 patients were smokers or had previous history of headache, 4 patients presented high blood pressure, Raynaud's phenomenon was observed in 3 patients. aPL were present in 3 cases. Cardiac examination disclosed a cardioembolic source in 3 cases. Multiple occlusions of the distal branches of cerebral arteries in angiography was only observed in 3 patients, 2 patients showed occlusion of great cerebral arteries, in one patient a vasculitic pattern was observed. Cerebral SPECT showed a disproportion between perfusion defects and ischemic lesions. Skin biopsy only showed unspecific changes and did not help to confirm the diagnosis.

Discussion: Occlusive vasculopathy of distal branches of cerebral arteries seems to be the typical finding in SS. We only found this angiographic pattern in 3 patients. Other mechanisms to explain stroke were identified in our patients (cardioembolism, great vessel disease, vasculitis). Clinical SS may probably include different etiopathogenic entities.

P583

**Primary antiphospholipid syndrome in 34 Iraqi neurological patients.** S. Al-Fahad, H. Al-Azzawi (Baghdad, IQ)

Primary Antiphospholipid Sy. is a well known cause of intravascular thrombosis & neurological involvement, it is no uncommon cause of stroke specially under the age of 40Y.

Methods: 34 patients were diagnosed between January 1998 and October 2002, they fulfilled the clinical & laboratory requirement for the diagnosis, (proposed in Sapporo 1998).

Results: 28 patients were females, the age ranged between 19–45 years, with a mean of 33 years. The neurological presentation was stroke in 23 (68 %), TIA in 4 (12 %), multiple strokes in 4 (12 %), pseudo bulbar palsy in 2 (6 %), diffuse cerebral in one (3 %), Pseudo tumor cerebri in 6 (18 %), migraine in 4 (12 %), epilepsy in 2 (6 %), & optic nerve ischemia in one (3 %).

The neurological manifestations during the course showed stroke in 25 (75 %). The left cerebral stroke in 12 (35 %), right cerebral in 8 (24 %), vertebro basilar in 6 (18 %). TIA in 5 (15 %), migraine in 6 (18 %), Pseudo tumor in 6 (18 %). 3 patients had catastrophic form.

The non neurological manifestations: DVT in 12 cases 36 % (2 of them had pulmonary embolism), abortions & intra uterine deaths in 12 (36 %), livedo reticularis in 4 (12 %), peripheral arterial disease in 3 (9 %), bleeding gum & menorrhagia in 7 (21 %), arthralgia in 3 (9 %), myocardial infarction in one, & renal vein thrombosis in one.

The laboratory data were prolonged aPTT in 16 (47 %) patients, anticardiolipin Ab. In 27 (80 %), KCT in 11 (33 %), positive VDRL in 3 (9 %) & low platelet count in 3 (9 %), high ESR in 12 (35 %).

Conclusion: we feel that the suspicion of Primary Antiphospholipid sy. must be kept in mind in cases of cerebrovascular disease in female under 40years, pseudotumor cerebri, & migraine.

## Dementia/Higher function disorders

P584

**Galantamine demonstrates consistently small 'number needed-to-treat' in patients with 'advanced moderate' Alzheimer's disease.** S. Schwalen, G. Hammond, M. Davidson, Janssen Cilag, Chaim Sheba Medical Center (Neuss, D; High Wycombe, UK; Tel Hashomer, IL)

Introduction: Galantamine (Reminyl) is a new drug with unique modulating properties on the nicotinergic receptor. Controlled trials have demonstrated the efficacy of galantamine in Alzheimer's disease (AD) across all evaluated domains. Subgroup analyses using pooled data show that galantamine use is associated with significant improvements irrespective of patient gender, age or disease stage. We performed a number needed to treat (NNT) analysis to give further details of the value of galantamine for patients with 'advanced moderate' AD and compared this with NNTs for anticonvulsants.

Methods: Data were pooled from 3 placebo-controlled studies of 5–6 months' duration including 705 patients receiving galantamine 24 mg. Of these, 178 with baseline ADAS-cog scores > 30, and 86 with baseline MMSE scores ≤ 14 were included and compared to the corresponding placebo groups (N = 183 and 101 respectively). Efficacy was defined as the proportion of ADAS-cog responders (with improvements ≥ 0, ≥ 4 and ≥ 7 points) and from patients 'improved or unchanged' according to the Clinician's Interview-Based Impression of Change plus caregiver input (CIBIC). NNT is an efficacy measure: the lower the NNT the more effective the drug. A NNT of 4 for an ADAS-cog improvement ≥ 4 would mean that 1 out of every 4 patients responds with an improvement of at least 4 points. NNT values for galantamine were compared to those of commonly used anticonvulsants using 50 % seizure reduction as the response criterion.

Results: Galantamine and placebo groups were similar in terms of all baseline characteristics eg ADAS-cog, MMSE, gender and age. In patients with baseline ADAS-cog scores of > 30 the NNT was 3 for ADAS-cog improvement of ≥ 0 points, 4 for improvements of ≥ 4 and ≥ 7 points, and 5 for CIBIC 'improved or unchanged'. In patients with baseline MMSE ≤ 14 the NNT was 3 for patients with ADAS-cog improvements ≥ 0 and ≥ 4 points, 5 for those with cog improvement ≥ 7 points, and 4 for CIBIC 'improved or unchanged'. The NNT for a 50 % seizure frequency reduction of the anticonvulsants lamotrigine or gabapentine is 9.

Conclusion: The consistently small NNTs for galantamine for cognitive and global outcomes for patients with 'advanced moderate' AD strengthen the conclusion that galantamine is highly effective. Treatment for dementia appears to be at least as effective as treatment for other neurological disorders such as epilepsy, therefore the treatment of AD patients is justified.

P585

**Cognitive impairment and cranial magnetic resonance imaging in acute carbon monoxide poisoning.** D. Aygun, L. Incesu, Z. Doganay, H. Sahin, E. Kelkitli, Ondokuz Mayis University (Samsun, TR)

Background: Acute carbon monoxide (CO) poisoning may cause cognitive impairment (i. e. loss of memory, attention, mental processing speed, dementia), loss of consciousness, coma, and death. We would like to assess cranial magnetic resonance imaging (MRI) findings in CO-poisoned patients and their relationship to cognitive functioning, to investigate the effects of acute exposure to CO on mini-mental-state examination [(MMSE), cognitive functions] scores, and to determine the frequency of lesion seen on MRI after acute CO-poisoning.

Methods: Ten consecutive CO-poisoned patients were studied. Each patient, as soon as CO-poisoning was suspected, was treated with supplemental oxygen and other supportive measures. After stabilization and initial management, the patients underwent MMSE in addition full neurologic examination. Following the clinical assessment MRIs were obtained from each patient. Chi-square and independent t-test were used for statistical analysis.

Results: Mean age of our patients was 28.6 ± 11.7 (from 17 to 48). Male/female ratio was 1. Thirty percent (n = 3) of CO-poisoned patients had a pathologic MRI finding. In these patients, the localization of lesions included the globus pallidus, putamen, caudate nucleus, thalamus, cortex, centrum semiovale, and hippocampus. Duration of loss of consciousness

correlated with both MMSE (cognitive impairment) and MRI lesion ($P = 0.047$). While in the patients with MRI lesion, mean duration of loss of consciousness was $910 \pm 10$ minutes, it was $29.28 \pm 40$ minutes in the patients without MRI lesion. Of our patients, 20 % had amnesia on admission. These cases had a normal MRI. Glasgow coma scale (GCS) score was higher in the patients (mean: $14.5 \pm 0.7$) without MRI lesion than those (mean: $11.3 \pm 2.8$) with lesion ($P = 0.01$). There was a significant relationship between MMSE and MRI. That is, the patients with MRI lesion had significantly lower MMSE score than those without lesion (mean MMSE score: $11.6 \pm 9.4$ versus $26 \pm 1.9$; $P = 0.01$). One patient was unconscious and intubated on day 1. This case died as a result of cardiac arrest at the forth day of poisoning. The most important limitation of our study was a small number of patients.

Conclusions: Acute CO-poisoning can result in brain lesion showed by MRI, and a decreasing in MMSE scores (cognitive impairment). MMSE appears to be useful in assessing the cognitive impairment due to acute CO-poisoning. Cranial MRI should be obtained from patients with lower MMSE score.

**P586**

**Monocyte chemotactic protein-1 gene A-2518G polymorphism in Alzheimer's disease.** C. Fenoglio, D. Galimberti, C. Lovati, A. Gatti, B. Corrà, I. Guidi, M. Tiriticco, P. Baron, G. Conti, C. Mariani, N. Bresolin, E. Scarpini, IRCCS Ospedale Maggiore, Ospedale L. Sacco (Milan, I)

Monocyte Chemoattractant Protein-1 (MCP-1) serves as potent in vitro microglial and macrophage chemoattractant. So far, the importance of MCP-1 in Alzheimer's disease (AD) pathogenesis has been demonstrated: astrocytes and oligodendrocytes produce MCP-1 upon stimulation with amyloid peptides. Further more, MCP-1 was found immunohistochemically in mature senile plaques and reactive microglia in brain tissues of AD patients. Recently, a biallelic A/G polymorphism in the MCP-1 gene regulatory region at position -2518 has been found, influencing the level of MCP-1 expression in response to an inflammatory stimulus. This may in part explain the different severity of pathological conditions among individuals who are affected by the same inflammatory disease. The distribution of the A-2518G polymorphism in the MCP-1 gene was determined in 140 Northern Italian patients with probable AD, diagnosed according to NINCDS-ADRDA criteria, as well as in 90 healthy subjects, and correlations between the presence of the polymorphism and the levels of MCP-1 in serum were evaluated. Genomic DNA was isolated from whole blood, A-2518G polymorphism was determined by PCR-RFLP assay. Allelic and genotypic frequencies were obtained by direct counting. Hardy Weinberg equilibrium was tested by using a $chi^2$ goodness-of-fit test. MCP-1 serum levels were determined by specific ELISA and comparisons between groups were performed using the non parametric Mann-Whitney test. The frequency of the A/G polymorphism in both our AD and control populations was similar to the one reported for the Caucasian population. Stratifying by ApolipoproteinE genotype or gender, no difference in allele frequency was observed. Nevertheless, MCP-1 levels in serum were increased in patients carrying at least one mutated allele compared with non carriers ($996.1 \pm 39.7$ pg/ml vs $863.8 \pm 49.2$ pg/ml; $P = 0.03$) and, notably, they were markedly increased in G/G homozygous ($1153.9 \pm 128.5$). The presence of A-2518G polymorphism in MCP-1 gene doesn't seem to be a risk factor for sporadic AD in Italian population, either by itself or interacting with the e4 allele. However, it might have a role in AD pathogenesis: in fact, the presence of this mutation correlated with higher levels of serum MCP-1, which can contribute to increase the inflammatory process occurring in AD.

**P587**

**Assessment of memory functions in mild cognitive impairment.** B. Cangoz, K. Selekler, Hacettepe University (Ankara, TR)

Objectives: the main goal of present study is to determine which memory tests, or combination of tests, are best for detecting mild cognitive impairment (MCI) from healthy control subjects. Secondary goal is to invastigate the correlation between memory tests score and Functional Activities of Daily Living Questionnaire score.

Method: Participants: this study includes 20 MCI patients (10 female, 10 male) and 20 healthy control subjects (10 female, 10 male) matched according to age, sex, education and manual preference. In the diagnose phase, three scales were applied by clinical trainers: 1. Mini Mental State Exam: MMSE, 2. Dementia Rating Scale (DRS) and 3. Functional Activities Questionnaire (FAQ).

Measures: in the test phase, five neuropsychological tests that depend on memory were applied: 1. Auditory Verbal Learning Test (AVLT), 2. Wechsler Adult Intelligence Scale-Revised (WAIS-R) Vocabulary Subscale, 3. Visual-Aural Digit Span Test-Adult Form (VADS), 4. Word Stem Completion Test (WSCT) for implicit memory and 5. Benton's Facial Recognition Test.

Design and Analyses: according to two group experimental design, MCI and healthy control subjects were compared by t-test. Inter-correlations for all memory tests and scales were computed.

Results: according to the results, patients with MCI were significantly inferior to healthy control subjects on AVLT and WAIS Vocabulary Subscale. However, the other tests did not discriminate MCI and control groups. AVLT total score with high correlation with AVLT subtest scores and correlation between WCST and Benton's Facial Recognition Test scores were statistically significant.

Discussion: AVLT and WAIS Vocabulary subscale discriminated patients with MCI from healthy control subjects.

**P588**

**Early magnetic resonance imaging and immunoglobulin treatment in an adult case of Rasmussen's disease.** H. Kajosch, V. Calomne, I. Declercq, F. Piéret, M. Gille, Clinique Sainte-Elisabeth (Brussels, B)

Rasmussen's disease (RD) is a childhood chronic focal encephalitis of unknown origin, usually presenting as an intractable epilepsy with progressive cognitive decline and hemiparesis. Adult forms of RD are less frequent and seem to have a better prognosis. A 63 year-old woman presented with a partial status epilepticus, followed by a subacute frontal cognitive decline. The epilepsy was rapidly controlled with valproate and phenytoine. The magnetic resonance imaging (MRI) was normal at the initial stage and revealed several weeks later cortical hyperintense lesions in the right insular and medial frontal lobes on T2, FLAIR, and diffusion weighted-images. The laboratory data were unremarkable, including metabolic screening, autoimmune, antiviral, and antiborrelia serologies. The cerebrospinal fluid examination was normal, except for a slight increase of the 14-3-3 protein. The open surgical frontal biopsy showed a nonspecific chronic encephalitis. A significant cognitive improvement was demonstrated after 4 monthly cures of immunoglobulin (IVIg) therapy. This case illustrates the usefulness of MRI in diagnosis and the efficacy of IVIg therapy in the early stage of adult RD. Focal cortical hyperintense lesions on MRI are often present in the early stage of RD, but are not specific. They may also be observed in some viral encephalitis, Creutzfeld-Jakob's disease, and other conditions leading to cortical laminar sclerosis.

**P589**

**Which type of dementia is more common in Qatar?** A. Hamad, M. Ibrahim, E. Al Sulaiti, Hamad General Hospital (Doha, QA)

Objective: Qatar lies along the east cost of the Arabian Peninsula. Its population is 600,000. The native Qataries around 25 %. The majority of them are below 60 years old. The aging population is expected to rise sharply. No data about prevalence of dementia are available, but because of the high prevalence of the DM & HTN it is believed that vascular dementia is predominant.

Background: To determine the different type of dementia among Qatari population. Such study is important, as each type of dementia needs different type of management and health care.

Method: Retrospective hospital base study; files of patients diagnosed as dementia were reviewed. Dementias and its subtypes were defined according to the DSM IV criteria. All patients were screened by modified MMSE and rated according to the clinical dementia rating scale (CDRS). In all patients psychiatric interview, physical, neurological, neuroradiological examination and comprehensive laboratory tests were done.

Result: 281 files were reviewed. 186 were excluded; 53 expatriates. 113 missing vital data. 93 cases fulfill the criteria, 22 (24 %) had Alzheimer disease (AD), 18 (18 %) had mixed AD and VD, 17 (18 %) had VaD, 6 (6 %) had mixed AD and Parkinson disease. 30 (34 %) had dementia due to other medical condition. CDRS was severe in 70 % and moderate in 18 % of AD patients while CDRS was severe in 50 % and moderate in 35 % of VaD. Small artery disease was found in 50 % of VaD patients.

Conclusion: AD is the commonest type of dementia among Qataries (49 %) followed by vascular dementia (37 %). Other types of dementia were rare (14 %). These findings follow the western pattern rather than the eastern. A community based epidemiological study is needed to confirm this finding and to show the prevalence of each type of dementia. Such study is important, as each type of dementia needs different type of management and health care.

P590
**Concentration of human growth hormone and insulin-like growth factor-1 in patients with dementia. Preliminary report.** P. Kobylański, A. Klimek, Regional 'Copernicus' Hospital (Lodz, PL)

The etiopathogenesis of degenerative diseases is still unknown. Maybe the growth factors play an important role in all these processes. The population becomes older and that is why the problem of dementia becomes more and more substantial. It is interesting to explore the role of growth factors in these processes. 5 patients with vascular dementia and 5 with Alzheimer disease were examined in the study. The diagnose was established based on neurological examination, psychological tests and neuroimaging (both CT or MRI). There were 3 men and 2 women in the age of 55 to 74 years (mean age 66) in the group with vascular dementia. The Alzheimer disease was diagnosed in 2 men and 3 women in the age ranged 72 to 85 years (mean age 78). The serum concentration of human growth hormone (hGH) and insulinlike growth factor -1(IGF-1) were examined. In the group with vascular dementia the level of hGH ranged between 0.05 to 2.8 micrograms/ml with the mean concentration of 1.04 micrograms/ml. The IGF-1 serum concentration ranged between 180.8 to 426 micrograms/ml with the mean level 297.6 ± 58.7.

In the group with Alzheimer disease the hGH mean concentration was 0.28 micrograms/ml (ranged 0.13 to 0.63). The IGF-1 mean concentration ranged 154.35 to 464.1 with mean value of 274.0 micrograms/ml ± 74.0.

The hGH concentration was normal in all samples but mean IGF-1 level was higher in both groups. The normal values for IGF-1 were established by laboratory as 61 to 174 micrograms/ml for women and 84 to 202 for men. In both groups only two samples revealed the normal IGF-1 concentration and the rest were higher about 50%.

Conclusion: These data may suggest the IGF-1 plays important role in dementia. It is necessary to continue the study in larger groups of patients.

P591
**Developmental prosopagnosia: a clinical and neuropsychological study.** A. J. Larner, J. J. Downes, J. R. Hanley, D. Tsivilis, M. Doran, Walton Centre for Neurology and Neurosurgery, University Department of Psychology (Liverpool, UK)

Objective: To report the clinical and neuropsychological findings in a patient with developmental prosopagnosia.

Results: A man in his thirties gave a history of lifelong difficulty identifying people by their faces, despite otherwise normal physical and cognitive development. Examples included failure to: identify the faces of fellow pupils when a schoolboy; identify familiar customers in the work environment; recognize his wife in the street unless she was wearing familiar clothes; identify his children when collecting them from school. However, in his work as an optician, he was easily able to recognize different makes of spectacle frame.

Neurological examination was unremarkable: normal visual acuity, visual fields (confirmed with automated perimetry), fundoscopy; no achromatopsia. Neuropsychologial assessment showed above average intelligence, fluent reading, and no obvious perceptual difficulties. He scored in the normal range on the Graded Naming Test and the Boston Naming Test, but was impaired on the Boston Facial Recognition Test.

Discussion: Prosopagnosia, the failure of facial recognition, is most often seen following cerebrovascular injury, commonly bilateral occipital-temporal lesions. Some cases are associated with progressive focal atrophy of the right temporal lobe. Few cases of developmental or congenital prosopagnosia have been reported. In cases of acquired prosopagnosia, pathology is unlikely to respect functional boundaries, whereas developmental prosopagnosia seems to represent a selective impairment of neuropsychological function.

Conclusion: Developmental prosopagnosia is rare, but such patients provide a unique opportunity to explore the functional neuropsychological substrates of facial recognition.

P592
**The beneficial effects of donepezil in patients with moderate to severe Alzheimer's disease are independent of baseline dementia severity.** H. Feldman, S. Gauthier, J. Hecker, B. Vellas, Y. Xu, J. Ieni, E. Schwam, University of British Columbia, McGill Centre for Studies in Aging, Memory Disorders Study Unit, Centre de Geriatrie, Pfizer Inc, Eisai Inc (Vancouver, Verdun, CAN; Daws Park, AUS; Toulouse, F; New York, Teaneck, USA)

Objective: To determine if the treatment effects of donepezil in moderate to severe Alzheimer's disease (AD) vary as a function of baseline dementia severity, as measured by the standardized Mini-Mental State Examination (sMMSE).

Background: Although donepezil has demonstrated benefits in patients with moderate to severe AD (MSAD; Feldman et al. (2001) Neurology), it has not been determined if donepezil's effects are consistent across the full range of baseline dementia severity (sMMSE 5–17) in the MSAD study.

Methods: 290 patients with moderate to severe AD (sMMSE score 5–17) were randomized to donepezil (n = 144) or placebo (n = 146) in this 24-week, double-blind, placebo-controlled trial. Outcome measures included global (Clinician's Interview-Based Impression of Change with caregiver input), cognitive (sMMSE and Severe Impairment Battery), functional (Disability Assessment for Dementia), and behavioral assessments (12-item Neuropsychiatric Inventory). ANOVAs and ANCOVAs were performed on Week 24 change from baseline scores for each measure (last observation carried forward; LOCF), with terms for treatment (donepezil or placebo), dementia severity (baseline sMMSE score), and severity x treatment interaction. In the ANCOVA analysis, baseline sMMSE score was treated as a continuous variable ranging from 5 to 17. In ANOVA, sMMSE scores were dichotomized into severe [5–12] and moderate AD [13–17] subgroups.

Results: There was no imbalance in baseline patient demographic characteristics or assessment scores between treatment groups. Mean sMMSE scores (± SD) at baseline were 11.9 ± 3.56 for the donepezil group and 12.1 ± 3.75 for the placebo group. Mean sMMSE scores (± SD) for the severe AD subgroup were 9.0 ± 2.19 for donepezil and 8.9 ± 2.19 for placebo, and for the moderate AD subgroup were 15.0 ± 1.52 and 15.4 ± 1.53, respectively. Treatment effects were significant for each outcome measure ($P < 0.01$) at Week 24 LOCF, for both analyses (continuous and discrete). There was no treatment x severity interaction for any measure at Week 24 LOCF for both continuous ($P > 0.25$) and discrete ($P > 0.10$) analyses.

Conclusions: Donepezil's treatment effects were consistent across the full range of dementia severity in patients with moderate to severe AD and were independent of baseline disease severity in analyses using either continuous or discrete variables. These data suggest that donepezil is efficacious across the spectrum of more advanced AD.

## Epilepsy

P593
**Treatment of complex partial status epilepticus with Lamotrigine.** A. Kowalik, J. Strauss, G. Daxer, H. Wiethölter, Bürgerhospital (Stuttgart, D)

Although convulsive status epilepticus (SE) is considered a medical emergency, morbidity and mortality of the complex partial status epilepticus (CPSE), especially in the critically ill, is often underestimated. The pleomorphic clinical presentation of CPSE with a variety of differential diagnosis and a common delay in medical treatment show the value of an early EEG and the therapeutic trial of antiepileptic drugs (AEDs). The first-line treatment of CPSE includes benzodiazepines (BZDs) and phenytoin. A delayed or poor response is often seen in elderly or critically ill patients. Given the rapid-onset of CNS and cardiovascular-depressant effects of AEDs used to treat CPSE, it is possible that these drugs may increase mortality by causing respiratory failure or hypotension.

Purpose: To demonstrate that lamotrigine (LTG) is effective as first-line or add-on therapy in patients with CPSE.

Methods: In a retrospective study we examined 13 patients with CPSE (age range 57 to 86). Diagnosis was established by clinical presentation (impaired responsiveness, cognitive disturbances and absence of convulsions) and EEG (delayed background activity, rhythmic focal delta activity and focal epileptic discharges). None of the patients previously had a diagnosis of epilepsy. All patients received a single dose of 25–50 mg LTG. In most patients LTG was increased to an average dose of 100 mg. Seven patients initially had BZDs without response and thus were given TLG. Six patients received LTG only. Clinical presentation and EEG was evaluated 24 h after administration of LTG.

Results: All patients showed significant clinical and electroencephalographical improvement after administration of lamotrigine. LTG was well tolerated with no relevant adverse effects, especially no skin reactions, even in patients initially receiving 50 mg LTG.

Conclusion: CPSE often goes unrecognized and may be resistant to treatment with BZDs and other AEDs. Lamotrigine can be highly effective as a single or add-on drug in patients with CPSE and is safe as shown in this retrospective study of 13 patients. To accurately establish treatment of CPSE with LTG, a well designed, prospective clinical study is necessary.

P594
**Seizures due to cerebrovascular disease.** M. Kilinc, N. Dericioglu, A. Yilmaz, G. Celiker, S. Benli, U. Can, S. Saygi, A. Ciger, Baskent University, Hacettepe University (Ankara, TR)

The incidence of epilepsy after cerebrovascular disease (CVD) is reported fairly low: 6–8 %. However, because of the high incidence of stroke (200 per 100,000) in relation to the incidence of epilepsy (30 to 50 to 100,000), CVD is one of the most common causes of epilepsy, particularly in the elderly. Hauser and Kurlend found that 30 % of seizures with onset in patients older than 60 years were due to CVD.

In order to show the relationships between imaging data and those derived from clinical and EEG examination we evaluated a series of patients with CVD and seizures.

The patients referred to our EEG laboratory with a petition form defining CVD as the probable cause of epileptic seizures were included in the study. The files of these patients were examined retrospectively for the exact time of the seizure (early if within the first 2 weeks, late if afterwards) with respect to a CVD (either ischemia or hematoma), the type of seizure activity (focal, generalized – including secondary generalization – or complex partial), EEG activity (normal, diffuse slow, focal abnormalities), antiepileptic drugs, and seizure recurrences. Excluded were the patients who had epileptic seizures before the CVD, who had another possible cause for the development of seizure activity (as subdural hematoma or sinus thrombosis), and the ones who were lost from control.

157 patients (58 female, 99 male; mean age 59 – between 16 and 89 –) were included in the study.

Among these patients 134 (85.4 %) had ischemic CVD while 23 (14.6 %) had hematoma.

After the CVD 38 (24.3 %) of the patients developed seizures in the first 2 weeks' time while 119 (75.7 %) developed seizures later.

As for the type of the seizures, 113 (71.9 %) developed generalized seizures, 40 (25.4 %) focal seizures, 3 (1.9 %) complex partial seizures, and 1 (0.6 %) epilepsia partials continue.

EEG was normal in 36 (22.9 %). There were diffuse slow waves in 22 (14 %), focal slow waves in 78 (49.6 %), focal spikes in 19 (12.1 %), and periodic lateralizing epileptic discharges in 2 (1.2 %).

141 (89 %) of the patients were prescribed an anti-epileptic drug; however 67 (38.2 %) of these patients developed recurrent seizures in spite of the therapy; 56 of these patients who developed recurrent seizures had ischemic CVD (41.7 % of the ischemic patients) while 11 had hematoma (47.8 % of the hematomas).

Among the patients with recurrent seizure activity 28 (41.7 %) developed only one more seizure, 15 (22.3 %) once to twice a year, 6 (8.9 %) two or four times a year, and 18 (26.8 %) more than four times a year.

16 (42.1 %) of the patients with early seizures, and 51 (42.8 %) with late seizures had recurrences.

In conclusion, most of these patients developed seizures after the first two weeks of the cerebrovascular disease; most of them had generalized seizures; the most repeatedly demonstrated EEG abnormality was focal slow waves. Although seizure recurrences were observed, monotherapy was effective in most of the cases.

P595
**Cognitive impairment and relationship with demographic and clinical factors in patients with epilepsy.** R. Mameniskiene, D. Jatuzis, G. Kaubrys, V. Budrys, Vilnius University Hospital (Vilnius, LIT)

Impairments of memory and other cognitive functions are common among patients with epilepsy. During last years there are wide discussions about epilepsy-related factors that could negatively influence cognitive functions. Only few studies evaluated long-term recall abilities that are especially important for daily functioning of patients.

Aims of the study: to evaluate cognitive functions in Lithuanian adult patients with epilepsy; to assess the influence of demographic factors, clinical characteristics of epilepsy and treatment with antiepileptic drugs (AED).

Patients and methods: 121 patients with epilepsy and 93 control persons were investigated during 2000–2002. A self-assessment of memory by patients and controls was performed using a 10-point rating scale. All patients underwent a full neurological examination, neuroimaging studies and interictal electroencephalography (EEG). Working memory, delayed recall, long-term verbal, verbal-logical and non-verbal memory, attention, concentration, speed of motor executive functions, and visual-constructive abilities were evaluated by the 11 cognitive tests battery.

Results: 71 (58.7 %) patients with epilepsy and 26 (28 %) control persons reported having memory impairment. Self-reported memory assessment did not correlate with the demographical factors. The highest correlation ($r = 0.407$) and significant dependence (beta = 0.314; $p < 0.001$) was estimated between the self-reported memory complaints and depression in epilepsy group. Poorer ranking of memory by control persons was more dependent on advancing age. Cognitive tests were performed significantly worse by patients with epilepsy compared to controls ($p < 0.001$). Patients with longer duration of epilepsy performed worse long-term tests of verbal ($p = 0.003$), logical ($p < 0.05$), non-verbal visual-constructive ($p < 0.001$) memory. Patients with temporal lobe complex partial seizures had more impaired long-term memory ($p < 0.05$) than patients with temporal lobe simple partial seizures. Frequent seizures were associated with impairment of delayed recall ($p < 0.05$), long-term memory ($p \leq 0.001$), concentration and working memory ($p < 0.001$). Generalized or focal temporal epileptiform activity was associated with poorer performance on cognitive tests for learning, delayed recall, long-term memory, and attention ($p < 0.05$). Concentration of attention and speed of motor executive functions were more impaired in subgroup of patients using few AED ($p = 0.019$), however, the performance of memory tests was not significantly associated with the number and type of medications ($p > 0.1$).

P596
**Prevalence of depressive disorders and manic/hypomanic symptoms in patients with epilepsy.** I. Barbov, G. Kiteva-Trencevska, I. Petrov, L. Pijanmanova-Karovska, Clinical Center Skopje (Skopje, MK)

Purpose: Depressive symptoms are common in patients with epilepsy (PWE), but the prevalence of bipolar symptomatology is unknown. Neurological factors, including site and lateralisation of seizure focus, may be important for development of depression. Mood effects of antiepileptic drugs can be complicated, however, as many of these drugs have also been reported to cause depression as an adverse effect.

Method: We conducted a survey of subjects selected from Macedonian general population. Our group of PWE ($n = 98$) were treated in the Clinic of neurology Skopje and Neurological Unit Strumica, because of an initial seizure or due to frequent seizures. Minnesota Multiphase Person's Inventory (MMPI) can indicate a likelihood of bipolar disorder based on presence and severity of depressive and manic/hypomanic symptoms. Depressive and bipolar disorders and their clinical forms in our group of patients were determined with clinical evaluation according to DSM IV (Diagnostic and statistical manual of mental disorders IV edition) criteria and by means of Hamilton's scale for evaluation of depressive levels.

Results: The frequency of depressive disorders in our group of patients was 28.6 % (major depression 17.9 % and dysthymia 82.1 %). This was higher than the rates for the general population (8.7 %). Depressive disorders are more frequent in patients with localization-related epilepsy than in idiopathic epilepsy (59 % vs. 41 %). Left-sided seizures foci have a higher association with depressive symptoms than right-sided foci (61 % vs. 39 %). Bipolar disorders had been diagnosed in 9.2 % of PWE, higher than in general population (1.2 %). PWE had substantially higher positive responses to all surveyed manic/hypomanic symptoms, with 8.1 % of PWE reaching a diagnostic threshold on the MMPI compared with 2.1 % in the general population.

Conclusion: Depressive and bipolar disorders, and manic/hypomanic symptoms are more common in epilepsy than in general population. Depressive disorders are more frequent in patients with localization-related epilepsy with left-sided seizures foci.

P597
**Transcranial magnetic stimulation in the evaluation of the activating effect of sleep deprivation on EEG.** E. Slusarska, K. Niedzielska, M. Niewiadomska, M. Baranska, W. Lojkowska, A. Wierzbicka, C. Glazowski, W. Lechowicz, L. Wolkow, D. Ryglewicz, Institute of Psychiatry and Neurology (Warsaw, PL)

Background: Sleep deprivation (SD) is considered a valuable EEG procedure facilitating epileptiform discharges in patients with epilepsy. However, it remains controversial whether the activating effect of SD is due to increased neuronal excitability or simply to induction of sleep. Transcranial magnetic stimulation (TMS) may give us some information about the level of neuronal cortical excitability and inhibition.

The aim of the study was to assess the influence of SD on EEG and on the values of cortex motor treshold (MT) and silent period (SP) evoked by TMS in patients with first ever epileptic seizures.

Methods: We studied 25 patients (aged 17–40) with newly diagnosed epilepsy so far untreated. TMS was performed using Magstim Model 200, applying single pulse technique, in wakefulness. Routine EEG and EEG after SD were performed immediatly after TMS in all patients. Mean values

of MT and SP before and after SD were assessed. MT values were compared with our own standards.

Results: Routine EEG revealed changes in 21 patients: groups of theta waves uni- or bilateral in 16 patients, with sporadic sharp waves in 8 cases and abortive generalised spike-wave or sharp-wave discharges in 5 cases. After SD epileptiform discharges occurred in EEGs of 12 patients: generalised in 6 patients and localised with or without secondary generalisation in 6 patients. In all cases EEG activation was observed only in lighter stages of NREM sleep. Mean MT values before SD for the right (43.2 ± 7.7) and left hemisphere (45.0 ± 6.3) and after SD (43.2 ± 7.3 and 44.7 ± 9.4) respectively did not change significantly; the differences in mean MT values between the epileptic group and healthy controls were statistically not significant as well. Mean SP duration increased slightly after SD in the right hemisphere (143.8 ± 55.0 vs. 161.7 ± 64.5) and decreased in the left one (163.8 ± 67.7 vs. 151 ± 83.2), but these differences were not significant ($p = 0.27$, $p = 0.03$). Mean MT and SP values in the group of patients with positive EEG activation were also not statistically different before and after SD.

Conclusions: In the examined group of patients MT and SP values obtained with TMS did not change significantly after SD. Epileptiform discharges were provoked in 12 out of 25 patients only in NREM sleep after SD. According to our results the activation of epileptiform discharges was connected rather with facilitating effect of sleep, than with SD itself.

**P598**

**L-2-hydroxyglutaric aciduria: three adult cases.** H. Karatas, B. Bastan, S. Saygi, A. Ciger, Hacettepe University (Ankara, TR)

L-2-Hydroxyglutaric aciduria (L-2-OHGA) is an autosomal recessively inherited neurometabolic disorder of childhood, characterized by slow and progressive neurological dysfunction with cerebellar ataxia, pyramidal signs, intellectual decline, seizures, extrapyramidal symptoms and macrocephaly. Although it is a childhood metabolic disorder, adult cases are also rarely reported. MRI images reveal characteristic subcortical white matter abnormalities. The diagnosis is made biochemically by increased concentration of L-2-OHGacid in body fluids.

We report three adult cases, two sisters and a boy, whose diagnoses were established in adulthood. Clinical and MRI findings were quite different from each other.

Case1; 22-year-old female was admitted to our clinic with seizures. She had postural instability and bilateral hand tremor for 1 year. Neurological examination (NE) revealed macrocephaly, dysarthria, intentional tremor, mild ataxia. MRI images showed bilateral, symmetrical dentate nucleus, basal ganglia, frontal and temporal subcortical white matter hyperintensities on T2-weighted images (T2WI). We thought of L-2-OHGA as a prior diagnosis with clinical and MRI findings. The diagnosis was confirmed by detection of increased level of L-2-OHGacid in urine (1770 mmol/molkre) by urine organic acid analysis.

Case 2; 34-year-old female, who is a sister of case 1, had cervical dystonia and tremor most prominently on hands for 15 years. She had mild mental-motor retardation, titubation and cervical dystonia in NE. L-2-OHGacid excretion was 1020 mmol/molkre in urine. Cranial MRI showed bilateral dentate nucleus and severe basal ganglia involvement on T2WI.

Case 3; 20-year-old male has suffered from epileptic seizures for 2 years. He had mental and motor retardation. There were patellar and achilles clonus in the NE. His parents had consanguinity of first degree. Cranial MRI showed bilateral dentate nucleus, cerebral white matter, globus pallidus and internal capsule hyperintensities on T2WI. Metabolic diseases were thought in the differential diagnosis and the definite diagnosis was established with urine organic acid analysis (L-2-OHGacid 3760 mmol/molkre).

L-2-OHGA shows wide range of clinical spectrum. Patients who had mild and variable symptoms like seizure and dystonia can not be diagnosed up to adulthood. Even in case of mild clinical presentation we must think of this disorder in the guide of typical MRI findings. Urine organic acid screening is necessary for the diagnosis.

**P599**

**Epilepsy and developmental venous anomalies (venous angiomas): report of 12 cases.** C. C. Turk, N. Dericioglu, S. Saygi, Hacettepe University (Ankara, TR)

Introduction: Developmental Venous anomaly (DVA) is the most common vascular malformation and usually accepted as a benign lesion. It is commonly encountered in the evaluation of patients with headache and seizures. In this study we tried to answer the question whether the localisation of DVAs is concordant with the suspected epileptogenic area in patients with seizures and DVAs.

Methods: Among the patients with epilepsy admitted to Epilepsy Unit of adult neurology clinic at Hacettepe University Hospitals.The ones having DVA on their MRIs during the last ten years included to the study. Patients with DVAs associated with any other anomalies except developmental cortical dysplasias (DCD) were excluded. Clinical, interictal - ictal EEG findings and MRI data were reviewed retrospectively. Ictal semiology and/or EEG were used for lateralisation - localisation of epileptogenic area. If there is concordance for lateralisation and/or localisation between the DVAs and clinical- EEG data, age of onset and prognosis of the epilepsy were evaluated.

Results: In 8/12 (66%) of patients, DVA lateralisation and/or localisation is concordant with epileptogenic area. In 3/12 patients, MRI showed DCD at the same localisation with DVAs. In 4/12 (25%), epileptogenic area localisation could not be done with scalp EEG and ictal semiology, so we were not able to say whether the DVAs could be responsible for the seizures. Among the cases showing concordance, mean age at onset was 12.87. Febrile convulsions were reported in 3 of them. (3/8, 37.5%) In the follow up, 4/8 (50%) of patients were seizure free with antiepileptic medication. Mean duration of follow up was 70.5 months in the concordant patients.

Conclusion: Although DVAs are usually accepted as an incidental finding in MRIs, when it is found in the epileptic patients' MRI, lateralisation of the epileptogenic area can be on the same side of the DVAs in 2/3 of them and the associated DCD should be searched carefully in the MRIs.

**P600**

**Incidence of epileptic seizures in chronic alcoholism.** F. Vecchio, E. Menegazzo, C. Fattorello S. (Mirano, I)

Objective: The aim of our study was to evaluate the incidence of epileptic seizures (E. S.) alcohol related in a series of patient (pz) with chronic alcoholism who were recovered in our Hospital during the last 12 years; the second aim was to characterize the type of the seizures.

Materials and methods: We evaluated 590 pz (354 males and 236 females, mean age 51.7) with anamnestic history of alcohol abuse of at least 10 years, recovered in our Hospital for an E. S. All pz underwent, besides routine blood and instrumental exams, to alcoholhemia, ammoniemia, EEG and to brain CT with m. d. c. Pz with anamnestic risk factors for E. or affected by primitive Epilepsy (E.) were excluded. The EEG were distinguished in normal, with focal abnormalities and with widespread abnormalities.

Results: We observed that 158 pz (26.7%), 102 males and 56 females, showed one or more E. S. In all pz the E. S. were generalized. In all pz alcoholemia was normal or lower than 50 mg/ml., hepatic indexes were altered and presented macrocytic anemia, hyperammoniemia and normal glycemia. The EEG was normal in 462 pz, with widespread abnormalities in 120 pz (among these 70 presented E.S and the EEG registration was made during the intercritic phase), with focal abnormalities in 18 pz (9 of them with E. S.). CT brain scan showed brain atrophy in 164 pz (27.8%); 20 of them presented an E. S.

Conclusions: Our data confirm the high incidence of E. S. in chronic alcoholism; the percentage of the alcoholist pz that presented E. S. (26.7%) is in agreement with the literature data (3.7%–36%) such as the type of the seizures that in our pz were all generalized. The EEG didn't show specific abnormalities. The brain atrophy that was documented from CT scan was not a predictive factor for E. Even when alcoholism is obvious, it is important to exclude other causes of E. with an accurate clinical and instrumental investigation.

**P601**

**Hemiconvulsion-hemiplegia-epilepsy syndrome: aetiological, clinical, EEG and neuroradiological evaluation.** M. Yaman, Z. Ünlüsoy, N. S. Yeni, N. Karaagaç, I. U. Cerrahpasa Medical School (Istanbul, TR)

HHE syndrome was first defined by Gestaut et.al. It is characterised by hemiconvulsion, and following ipsilateral hemiplegia and epilepsy. Although head trauma, and CNS infections such as meningitis take place in etiopathogenesis febrile convulsions are more common. The differences in etiopathogenesis make differences in clinical presentation. In order to emphasize on these differences 38 patients followed by our epilepsy out patient clinic who met the HHE diagnostic criteria were investigated for their clinical presentation and etiopathogenesis retrospectively. Among the patients the ones whose follow up was not sufficent who did not have imaging and symptomatic HHE were excluded.

The remaining 14 patients who was diagnosed as idiopathic HHE were included in the study. Of these patients perinatal history, hemiparesis, febrile convulsions, epileptic seizure, EEG, imaging features were investi-

gated. The clinical features of these patients and their response to therapy were found to be varied. According to these results we tried to emphasize, once more, on the importance of follow-up and treatment of childhood febrile convulsions in the aspect of chronic epileptic syndromes.

## Extrapyramidal disorders

P602
**Low dose of olanzapine in the treatment of dementia with Lewy bodies.** C. Lucetti, R. Ceravolo, G. Gambaccini, S. Bernardini, C. Berti, G. Dell'Agnello, U. Bonuccelli, Clinical Neurology (Pisa, I)

Objective: To evaluate the efficacy of different dose (2.5, 5, 7.5 mg) of olanzapine in patients with Dementia with Lewy Body (DLB).

Methods: Eighteen patients meeting the criteria for DLB were enrolled; 7 were randomized to 2.5 mg/die, 6 to 5 mg/die, and 5 to 7.5 mg/die of olanzapine. Patients were evaluated at baseline and after 3, 6, 12 months of olanzapine with Unified Parkinson's Disease Rating Scale (UPDRS) for parkinsonism, and the Neuropsychiatric Inventory (NPI) and the Brief Psychiatric Rating Scale (BPRS) for psychosis, and the Mini Mental State Examination (MMSE) for cognitive impairment.

Results: Olanzapine was discontinued in two patients because of adverse effects (increased confusion and sedation); sixteen patients completed the study period. No decrement in MMSE scores was reported in any of the three subgroups. An exacerbation of parkinsonism was observed in the subgroup of patients with 7.5 mg/die of olanzapine. An amelioration of BPRS scores was found in all three subgroups ($p < 0.01$). Patients with 5 and 7.5 mg/die of olanzapine showed a reduction of NPI scores compared with the basal value ($p < 0.05$).

Conclusions: Olanzapine is an effective well tolerated neuroleptic drug, mainly at the dose of 5 mg/die in the treatment of patients with DBL.

P603
**Results of deep brain stimulation in Parkinson's disease and essential tremor: a follow-up study.** T. Glöckler, U. Sommer, J. Koy, M. Sauermann, H. Reichmann, Universitätsklinik Dresden (Dresden, D)

Between 1997 and 2002, we treated 26 patients with Deep Brain Stimulation (DBS), 13 of them were implanted within subthalamic nucleus (STN) and 12 within ventralis intermedius nucleus (VIM) on one or both sides. The VIM-group consisted of 2 patients with essential tremor (ET) and 10 patients with tremordominant Parkinson's disease (PD). The remaining 14 patients got bilateral STN-electrodes because of PD and motor complications or dyskinesias. Duration of disease ranged from 3 to 34 years. Male patients [19] dominated over female [7]. The aim of this investigation was to assess the efficacy and safety of deep brain stimulation during a one-year postoperative period.

Examination parameters were motor scores within UPDRS III and activities of daily living (ADL) within UPDRS II pre- and postoperatively during off- and on-state as well as postoperative side effects. Further we performed an extensive neuropsychological examination before and one year after surgery.

Presurgically all Parkinson patients greatly improved from off- to on-period (about 63 %). After surgery the improvement in UPDRS III scores was significant from off-stimulation/off-medication state to on-stimulation/off-medication state (approximately 56.9 %). Additionally there was a 18 % improvement in motor symptoms in on-stimulation/on-medication state. We saw also a significant improvement in ADL (50 %). Our preliminary neuropsychological data showed no significant difference between pre- and postoperative state.

Stimulation induced dyskinesias, mild oculomotoric disturbances and gait ataxias were the most common side effects in STN-stimulated patients. Another undesirable effect were levodopa withdrawal symptoms. Transient paresthesias and dysarthria appeared in the VIM-group.

In conclusion we saw a significant benefit concerning the motor function and the quality of life on the one side and no impairment of the neuropsychological function or any severe side effects on the other side.

P604
**Objective assessment of tremor severity in patients with essential tremor.** M. Rudziñska, S. Bukowczan, A. Izworski, M. Marona, K. Banaszkiewicz, A. Szczudlik, Collegium Medicum of Jagiellonian University (Cracow, PL)

Background: Subjective clinical methods of tremor rating by means of spirals and handwriting are commonly used in clinical practice. For more detailed assessment of efficacy of anti-tremor drugs, more objective and reliable method is needed. Electronic registration of tremor with digitizing tablet and computer spectral analysis would be useful and sensitive method for assessment of tremor severity.

The aim of the study was to evaluate a new method of quantitative tremor registration by means of spectral analysis of drawing a spiral on a digitized tablet by comparing it to other methods of measuring tremor severity such as subjective clinical measures, objective functional performance tests and impact of tremor on patients' lives.

Methods: Upper limbs tremor in both hands was assessed in 56 patients with essential tremor (mean age 50.6, 29 women, mean disease duration 12.4 years). The results of new technique automatically analysing data from drawning Archimedean spirals with a subject sited comfortable at a digitizing tablet were correlated with subjective clinical measures (rating tremor in spirals) and objective functional performance test (modified volumetric method, Gibson maze test) and impact of tremor on patients' lives (Activities of Daily Living Self-questionnaire-ADLS and Assessment of Handicap Self-questionnaire-AHS).

Results: Intensity of tremor assessed by the index of acceleration in the XY axis in spectral analysis data from digitizing tablet correlates well with the tremor scores from spiral drawings of both dominant and non-dominant hands (Spearman's rank correlation analysis, $R = 0.71$ and $R = 0.64$ respectively, $p < 0.05$) and modified volumetric methods ($R = 0.59$, $p < 0.05$ and $R = 0.43$, $p = 0.009$), Gibson maze test ($R = 0.48$, $p < 0.0002$ and $R = 0.25$, $p = 0.5$) and Activities of Daily Living Self-questionnaire-ADLS ($R = 0.42$ $p = 0.002$ and $R = 0.32$ $p = 0.02$).

Conclusion: Spectral analysis of spiral-drawing on the graphic digitizing tablet is a reliable method of quantitative assessment of tremor intensity in essential tremor patients.

P605
**Clinimetrics in monitoring diagnosis and treatment of Parkinson's disease – multicentre study.** W. Pakszys, D. Kadzielawa, J. Kotowicz, W. Kulinski, M. Glod, P. Grieb, Parkinson's Monitoring Treatment Lab, Warsaw University, Polish Academy of Science (Warsaw, PL)

Because of significant progress in diagnosing and pharmacology as well as rehabilitation treatment of patients with PD, were undertaken multicenter clinimetrics study. To meet these needs we have elaborated a Chart for Periodical Examinations (possible 250 data, manifold gathered) a graphic as electronic version. With Industrial Institute of Electronics/dr Witold Kornacki, P. W. and team/was made Manual Portable Tremorometer/Actograph. This device permits clinimetrically assessed energy and potency of tremor correlated with PDScales as Websters, UPDRS, Hoehn-Yahr, England-Schwab's and others. Preclinical dgns were confirmed by DaTSCAN (123J-10FUPANE) [6, 1]. The treatment was correlated with pharmacokinetics/pharmacodynamics studies and neurorehabilitation complex treatment (kinesy-cognitive functions complex treatment by EEG-Biofeedback therapy).

Conclusions: 1) Early stages of PD confirmed by DaTSCAN- needs a lower quantity of substitution therapy L-dopa/DDJ and presents longer t1/2 and higher Cmax. Tmax was dependent on pharmaceutical form of drugs. 2) Tremorrometry/Actograph Data presents direct correlation with AUC of L-Dopa. 3) Such multicenter study permits to eliminate polytherapy and diminish side effects and thanks of neurorehabilitation presents higher QoL of the patients and spouses.

Supported partially by: Committee of Science Inst., Polish Academy of Sciences. Grant 8T11E 00718.

P606
**Reversed hemichorea and hemiballismus associated with new onset hyperglycaemia.** A. Akçali, C. Tataroglu, A. Ozge, S. Sevim, D. Yalçinkaya, Mersin University Medical Faculty (Mersin, TR)

Unilateral proximal involuntary movements (hemichorea or hemiballismus) involving mainly the upper extremity can be caused by a wide variety of degenerative, metabolic or vascular disorders affecting the subthalamic nucleus. Herein we described the characteristic clinical presentation and imaging findings of a patient with hemichorea and hemiballismus (HC-HB) associated with nonketotic hyperglycemia. A 56-year-old female was admitted of a HC-HB on her left arm and face of ten days duration. Although she had no history of diabetes mellitus (DM), her serum glucose level was elevated to 560 mg/dl with no ketones in urine. CT scan and MRI were normal. Lowering the blood glucose level reversed the movement disorder within 48 hours. After 24 hours of the correction of hyperglycemia her SPECT was performed and showed normal perfusion of the basal ganglions. Pathophysiology of this syndrome is still being discussed for two

decades with limited number of patients. Insulin therapy is advised for the achievement of euglycemia and resolving of the involuntary movements. Although previous case reports had revealed hyperintensities on MRI, in our case it was normal. The SPECT scans taken at the hyperglycemia period had shown hypoperfusion of the basal ganglions, but we were able to perform SPECT after 48 hours of administration and found it normal. Undiagnosed DM should be suspected in patients with acute onset of HC-HB and the movement disorder usually reverses within two days and may not require dopamine receptor antagonists.

**P607**

**Cerebellar hemangioblastoma presenting with writer's cramp: a case report.** L. Capone, R. Gentile, A. Coretti, Regional General Hospital (Bolzano, I)

Background: Writer's cramp (WsC) is a condition defined as focal task-specific dystonia that causes difficulties in writing. A certain number of these patients may have problems with other simple tasks as shaving, combing hair, eating with utensils or lifting a cup. The pathophysiologic hallmark of WsC is the excessive co-contraction of agonistic and antagonistic muscles due to abnormal functioning of the basal ganglia. In the majority of cases the cause is unknown and symptomatic WsC is reported only in few cases of lesions localized in the deep part of brain.

Case Report: A 28-years old left-handed man, with unremarkable medical history presented after 3 months of progressive difficulty with writing. He had noted that his writing was becoming progressively slow, irregular and unreadable. In the 3 weeks before admission he had also begun to complain of balance and speech disorder. On neurological examination there was impaired arms and legs coordination, power and sensitivity were normal, deep reflexes were enhanced on the right side. Plantar were flexor. During handwriting he showed an excessive gripping of the pen, flexion of the wrist and elbow elevation, without tremor. Neurological examination was otherwise normal. Magnetic resonance imaging (MRI) of brain showed a large cystic, sharply demarcated, and noninfiltrative lesion in the left cerebellar hemisphere. There were beginning signs of brainstem compression with light dislocation of the midline structures, without hydrocephalus. The mass was surgically removed and at the histological examination was diagnosed with hemangioblastoma. MRI scan of whole spinal cord was normal and genetic counselling to exclude a von Hippel-Lindau syndrome was performed.

Comment: In this case WsC was for long time the only symptom of a cerebellar hemangioblastoma, even if cerebellum is not generally reported among the sites of lesion that cause secondary dystonias. Based on this experience, we suggest to perform neuroradiological investigations on occurrence of focal dystonia in adult patients.

**P608**

**Botulinum toxin type B for the treatment of type A resistant blepharospasm patients.** J. C. Martinez-Castrillo, A. Mariscal, J. A. Dominguez-Moran, Hospital Ramon y Cajal (Madrid, E)

Objective: We have treated six patients with blepharospasm who were resistant to botulinum toxin type A (BTx-A), with the type B (BTx-B).

Patients and methods: Four women and two men, aged between 51 and 84 years, who suffered from blepharospasm, were treated with BTx-A for 5 to 10 years. They became non-responders on an average of 2.1 years since BTx-A was introduced. Maximum doses used were 80–160 U (Botox, Allerga). Four patients had a positive frontal atrophy test (no response), and in the other two the answer was unclear. BTx-B was injected in 8 points in each patient. The initial dose was 250 U (Neurobloc, Élan) per point. Two patients have received four sessions of BTx-B treatment, three have received three sessions, and one two sessions. In two patients the dose had to be increased to 500 U per point, and in one to 750 U per point.

Results: All patients, except one, had a satisfactory response to BTx-B treatment. The mean duration of this response was 6.4 weeks (range 2–8 weeks). BTx-B injections were remarkably more painful than BTx-A ones.

Conclusions: BTx-B is a useful treatment for patients with blepharospasm who become resistent to BTx-A. This response is shorter than the usual response with BTx-A.

**P609**

**Acute akinesia in Parkinsonism.** A. Thomas, D. Iacono, A. Luciano, M. Onofrj, Neurophysiopathology (Pescara, I)

Acute Akinesia, akinetisches Syndrom, sudden akinesia is an ill-defined complication appearing in the course of Parkinson's Disease (PD), when infectious diseases, bone fractures, gastrointestinal tract diseases occur and is consisting of the acute worsening of parkinsonian symptoms and of transient unresponsiveness to current treatments or to increments of dopaminomimetic treatments. This definition is a tentative one as the Akinetic Syndrome has never had the honour of a consensus agreement in its consistency, and descriptions are merely anecdotally more accurately reported in German language or briefly enunciated in general reviews on the treatment of PD complications.

Nonetheless the German literature claims that a treatment based on the injectable form of amantadine is indicated for the syndrome, even though no comparisons have been made with other injectable treatments.

Furthermore the Akinetic Syndrome, because of its appearance during concurrent gastrointestinal tract or infectious diseases, and in patients with advanced PD and psychiatric complications, might be confused with the hypothermia with akinesia due to withdrawal of L-DOPA, dopaminoagonists or amantadine or with the malignant hypothermia or malignant catatonia due to inappropriate-inadequate administrations of typical neuroleptic drugs.

In the search for a consensus on the syndrome and of adequate indications for its treatment we report our experience with 22 patients and a review of the available literature.

## General neurology

**P610**

**Neurological manifestations in Sjögren's syndrome [1]. Clinical and biological findings in a cohort of 82 patients.** S. Delalande, J. De Seze, A. L. Fauchais, E. Hachulla, T. Stojkovic, D. Ferriby, P. Y. Hatron, P. Vermersch, CHU Lille (Lille, F)

Introduction: Neurological involvement occurs in approximately 20 % of patients with primary Sjögren's syndrome (SS). However, the diagnosis is sometimes difficult and central nervous system (CNS) manifestations have rarely been described.

Aim: To describe the clinical and laboratory features of SS patients with neurological manifestations.

Methods: Eighty-two patients (65 women and 17 men) with neurological manifestations associated with primary SS, as defined by the American-European criteria (Vitali et al. 2002), were studied.

Results: The mean age at neurological onset was 54 years. Neurological involvement frequently preceded the diagnosis of SS (81 % of patients). Fifty-six patients had CNS disorders mostly focal or multifocal. Twenty-nine patients had spinal cord involvement (acute myelopathy [n = 12], chronic myelopathy [n = 16] and motor neuron disease [n = 1]). Thirty-three patients had brain involvement and 13 patients had optic neuropathy. The disease mimicked relapsing-remitting multiple sclerosis (MS) in 10 patients and primary progressive MS in 13 patients. We also recorded diffuse CNS symptoms: seizures (n = 7), cognitive dysfunction (n = 9) and encephalopathy (n = 2). Fifty-one patients had peripheral nervous system involvement (PNS). Symmetric axonal sensorimotor polyneuropathy with a predominance of sensory symptoms or pure sensory neuropathy occurred most frequently (n = 29), followed by cranial nerve involvement affecting trigeminal, facial or cochlear nerves (n = 16). Multiple mononeuropathy (n = 7), myositis (n = 2) and polyradiculoneuropathy (n = 1) were also observed. Patients with PNS manifestations had frequent extraglandular complications of SS. Anti-Ro (SSA) or anti La (SSB) antibodies were detected in 21 % of patients at the diagnosis of SS and in 43 % of patients during the follow-up (mean follow-up: 10 years). Biological abnormalities were more frequently observed in patients with PNS than in those with CNS involvement ($p < 0.01$).

Conclusion: Our study underlines the diversity of neurological complications of SS. The frequency of neurological manifestations revealing SS and negative biological features, especially in the event of CNS involvement, could explain why it is frequently misdiagnosed. Screening for SS should be systematically performed in cases of acute or chronic myelopathy, axonal sensorimotor neuropathy or cranial nerve involvement.

**P611**

**Increased kynurenic acid and IgG in cerebrospinal fluid of patients with polyneuropathy.** B. Kepplinger, H. Baran, A. Kainz, H. Schmid, J. Wallner, LNK Mauer, Veterinary University of Vienna (Mauer, Vienna, A)

There is abundant evidence that the tryptophan degradation is up-regulated in certain cells in inflammatory states, e. g. in microglial cells and in activated macrophages producing toxic tryptophan derivatives in addition

to other cytotoxines (Stone, 1993; 2001). Kynurenic acid (KYNA), a metabolite of tryptophan, is an antagonist of ionotropic excitatory amino acid receptors and acts preferentially at the glycine site associated with the N-methyl-D-aspartate (NMDA) receptor complex and has anticonvulsive and neuroprotective properties (Stone, 1993). In humans KYNA is synthesized by transamination of L-kynurenine. Two enzymes, kynurenine amino transferase I and kynurenine amino transferase II are responsible for KYNA formation. De novo synthesized KYNA is readily liberated into the extracellular milieu where it can exert control over NMDA receptor activity particularly when the modulatory site is not saturated with glycine or other endogenous factors. Alteration of KYNA content in the CNS and periphery may contribute to neuronal impairment and/or degeneration.

By using a HPLC method (Baran et al. 2000) we analysed the levels of KYNA in CSF and serum of patients with Polyneuropathy (PNP), Neuroborreliosis (NBOR) and in corresponding controls (CO) and compared with alterations of immunsystem's marker gammaglobulin. Lumbar puncture was carried out to obtain cerebrospinal fluid (CSF) for differential diagnostic reasons. Blood was drawn and serum prepared for clinical investigations. Samples were obtained from PNP aged 59.9 ± 3.4 [9] years; NBOR aged 51.8 ± 8.6 [11] years and corresponding controls (CO) aged 43.4 ± 3.3 [19] years. The increase of KYNA in CSF was found in NBOR (18.69 ± 3.05 fmol/µl) followed by PNP (5.33 ± 0.17 fmol/µl, $p < 0.05$) as compared to control (3.24 ± 0.17 fmol/µl), respectively. No alteration of KYNA could be seen in serum of PNP patients (29.27 ± 2.91 fmol/µl) and KYNA was moderately increased in NBOR (33.49 ± 4.05 fmol/µl), as compared to control (28.8 ± 2.13 fmol/µl). The IgG levels in CSF and serum of control patients were 1.92 ± 0.16 mg/dl and 981.9 ± 54.7 mg/dl, respectively. In PNP patients IgG was 4.93 ± 0.84 mg/dl ($p < 0.05$, vs. CO) and 1114.9 ± 123.0 mg/dl in CSF and serum, respectively. Whereas in NBOR patients IgG was 2.97 ± 0.44 and 1004.6 ± 114.2 mg/dl, respectively.

In summary, patients suffering from different types of PNP exhibit significant enhancement of KYNA levels in CSF. Do alterations of KYNA metabolism occur in the central nervous system of PNP patients and is there a central co-affection with autochthonic IgG production in PNP?

Baran H, (2000) J Neural Transm 107: 1127–1138.; Stone TW, (1993); Pharmacol Rev 45: 309–379.; Stone TW, (2001) Progress in Neurobiology 64: 185–218.

Supported by Multiple Sklerose Forschungsgesellschaft Wien, Austria and in part by FWF Austria Project P15371.

**P612**

**Nervous system complications of systemic autoimmune diseases.** V. Mastorodimos, M. Mamoulaki, M. Spilioti, H. Kritikos, D. Boumpas, A. Plaitakis, University Hospital of Crete (Heraklion, GR)

Background and Objective: Limited data exist on systemic autoimmune diseases that present with initial neurologic symptoms. To our knowledge no systematic studies have been performed about their relative frequency and presenting features.

Materials and Methods: We reviewed the charts of the patients with neurologic symptoms that were admitted to our hospital, a tertiary, referral center for the island of Crete (population of approximately 700,000) between 1/1/1997 and 31/12/2002. The diagnosis of a systemic autoimmune disorder was established, according to American College of Rheumatology (ACR) diagnostic criteria. All patients underwent head CT/MRI and screening for systemic autoimmune disorders (RF, CRP, ANA, Anti-ds DNA, ANCA, C3, C4, IgG/IgM anti-cardiolipin antibodies (aCL), IgG/IgM anti-beta2 glycoprotein antibodies, lupus anticoagulant).

Results: 9 patients (7 females and 2 males) were recognized out of 2973 that were admitted during the last 5 years. The age of the patients ranged between 24 and 50 years old. Four patients fulfilled the ACR criteria for Systemic Lupus Erythematosus (S. L. E.) and three of them had secondary antiphospholipid syndrome. Of these 4 patients one presented with a hemispheric ischaemic stroke, one with hemichorea, one with seizures and one with demyelinating syndrome. The four patients that were diagnosed with primary Antiphospholipid Syndrome (APS) were females and had cerebrovascular disease. Two of them had vertebrobasilar system infarcts, the former in the distribution of posterior inferior cerebellar artery (PICA) and the latter, a 39 year old female in puerperium, a 'top of the basilar artery' stroke. From the rest one had a combination of hemorrhagic and ischaemic hemispheric lesions and one multiple transient ischaemic attacks (TIAs). In addition the latter two had recurrent migrainous headaches. One patient presented with seizures and despite normal neurologic examination and cranial CT/MRI he subsequently proved with Wegener granulomatosis diagnosis. The mean time from the initial neurologic manifestation until the final diagnosis was approximately 2 months (range 0.5–6 months).

Conclusions: In a tertiary, regional referral center the most common diagnosis in patients presenting with a neurological event is the APS, either primary or secondary to SLE. Young (< 40 years old) females presenting with initial manifestations of TIAs or ischaemic stroke, in the absence of the usual risk factors, should undertake a thorough screen for systemic autoimmune disease.

**P613**

**The cerebellum plays a role in the modulation of cortical motor output following electrical stimulation of the sciatic nerve in the rat.** N. Oulad Ben Taib, M. Laute, M. Pandolfo, M. Manto, Hôpital Erasme (Brussels, B)

A sustained somatosensory stimulation changes the features of the response of the rodent motor cortex. Whether the cerebellar pathways could modulate this effect is unknown. We analysed the possible roles of the cerebellum on the modulation of cortical motor output associated with electrical stimulation of the sciatic nerve in the rat. We analysed the response evoked by electrical stimulation of the right motor cortex before (basal condition) and after peripheral electrical stimulation of the left sciatic nerve in 8 control rats. In addition, we investigated the effects of (a) administration of ethanol (20 mM) in left cerebellar nuclei (n = 5 rats), (b) administration of tetrodotoxin (10 µM), a sodium channel blocker, in left cerebellar nuclei (n = 5 rats), (c) electrical stimulation by deep cerebellar stimulation (frequency 100 Hz) on the left side (n = 5 rats), (d) electrical stimulation of cerebellar nuclei on the right side (100 Hz) in 6 rats. Peripheral stimulation: all the animals received 1 hour of electrical stimulation. Trains of stimulation consisted of 5 stimuli (duration of 1 stimulus: 1 msec) at a rate of 10 Hz. Stimulation of the motor cortex: peak-to-peak amplitudes in responses of the left calf muscle were analysed. The intensity used was 130% of motor threshold. In control rats, peripheral electric stimulation was associated with an increase of motor response of 141.6 ± 7.9% ($p < 0.001$). Administration of ethanol in the cerebellum prevented the enhancement of the response ipsilaterally. Mean ± SD of motor responses was 105.7 ± 6.2% of baseline measurements following stimulation of the sciatic nerve ($p = 0.36$). The same observation was made following the infusion of tetrodotoxin (mean ± SD of motor responses: 107.1 ± 7.4% after peripheral stimulation, $p = 0.19$) and following electrical stimulation of the cerebellum on the left side (mean ± SD of motor responses: 104.3 ± 8.5% after peripheral stimulation, $p = 0.40$). However, electrical stimulation of cerebellar nuclei on the right side did not impair the modulation of cortical motor output by sciatic nerve stimulation (mean ± SD of motor responses: 148.4 ± 5.8% after peripheral stimulation, $p < 0.001$). We demonstrate that the cerebellum plays an unsuspected role in this form of short-term neural plasticity.

**P614**

**Posterior reversible encephalopathy syndrome – aetiology and MR appearance.** S. Popovic, D. Kozic, R. Semnic, M. Lucic, R. Irizarry, Institute of Oncology, University of Miami (Sremska Kamenica, YU; Miami, USA)

Purpose: To identify etiologic factors and to evaluate MR characteristics and lesion distribution in posterior reversible encephalopathy syndrome (PRES).

Patients and methods: Five patients were included in the study. The most prominent symptoms were severe headaches and transitory blindness.

Results: Four adults and 1 child were affected. Two patients were treated for neoplastic disorder, one patient with a history of lung transplantation was treated with immunosuppressive agents while hypertension was most likely the cause of symptoms in 2 patients. Bihemispheric, relatively symmetric lesions were identified predominantly in parietal and occipital lobes, however frontal regions were not spared in all patients. Bilateral involvement of thalami was also noted. No zones of restricted diffusion were found in a patient in whom this sequence was performed. Complete recovery was confirmed in patients who underwent control MR examination.

Conclusion: PRES is acute, dramatic, but reversible clinical condition with relatively symmetric bihemispheric involvement. Our study shows that frontal regions and thalami can also be affected. Absence of restricted diffusion is helpful to exclude acute infarct from differential diagnosis.

**P615**

**Hyperhomocysteinemia in Parkinson's disease.** D. Religa, K. Czyzewski, M. Styczynska, B. Peplonska, M. Chodakowska-Zebrowska, K. Stepien, B. Winblad, M. Barcikowska, Karolinska Institutet, Polish Academy of Sciences, Central Laboratory CSK/MSWiA (Stockholm, S; Warsaw, PL)

Objective: Hyperhomocysteinemia is a common finding in the psychogeriatric population. It has been seen that Parkinson's disease (PD) pa-

tients treated with levodopa have higher levels of homocysteine in the blood. The aim of this study was to examine homocysteine levels in treated and untreated PD patients and test the relationship between homocysteine and levodopa treatment. We focussed on the influence of doses and duration of therapy and genetic, such as C677T methylenetetrahydrofolate reductase (MTHFR) polymorphism and environmental factors, such as vitamin B12 and folic acid, on homocysteine levels.

Methods: Sixty levodopa-treated patients with PD, 55 untreated PD patients and 100 elderly controls without PD were studied using standard diagnostic criteria, genotyping, biochemical analysis and statistical techniques. PD patients were at Hoehn and Yahr stage 3.0 or less.

Results: We found that levodopa-treated PD patients had elevated homocysteine plasma levels as compared to the controls ($p < 0.001$) and it depended on levodopa doses ($p < 0.01$). However the most important factor influencing the homocysteine levels was the duration of PD ($p < 0.001$). The frequency of alleles C677T MTHFR gene does not differ between PD and controls, however C allele has a protective role for levodopa-induced hyperhomocysteinemia ($p < 0.05$). The high levels of homocysteine were also connected with cognitive impairment seen in PD patients.

Conclusions: Hyperhomocysteinemia is associated with duration of PD and levodopa treatment. Cognitive impairment and dementia in PD population can be, at least partially, due to hyperhomocysteinemia. The C677 allele of MTHFR gene, as well as high levels of vitamin B12 and folic acid, have protective effect for levodopa-induced hyperhomocysteinemia. We recommend the supplementation of folic acid and vitamin B12 in PD patients to prevent or treat hyperhomocysteinemia in order to avoid toxic effect of homocysteine on the brain.

**P616**

**Experimental allergic encephalomyelitis facilitates recovery following traumatic axonal injury in the spinal cord.** S. Grigoriadis, R. Lagoudaki, A. Kalpatsanidis, E. Polyzoidou, N. Grigoriadis, E. Shohami, P. Selviaridis, V. Kontopoulos, I. Milonas, Aristotle University of Thessalonica, Hadassah Hebrew University (Thessalonica, GR; Jerusalem, IL)

Passive immunization with T-cells against Central Nervous System (CNS)-associated myelin antigens has recently been found to offer neuroprotection following CNS trauma, leading to the concept of protective auto-immunity. However, limited research exists about whether actively induced CNS autoimmunity may offer any similar benefit. In this study, the kinetics and the effect of endogenously anti-myelin activated T-cells that invade CNS following spinal cord injury (SCI), were investigated. Experimental Allergic Encephalomyelitis (EAE) was actively induced in Lewis rats following immunization with Myelin Basic Protein (MBP). In vivo BrdU incorporation from activated T-cells was used as a marker. BrdU was injected on days 5, 6, 7 post-induction (DPI) in all EAE induced animals. On DPI 8, a hemi-compressive injury was induced by a transient extradural application of an aneurysm clip at the T8 spinal level. Control animals were sham operated. In a group of naive animals, BrdU was injected for 3 consecutive days and SCI was induced on the next day. According to this protocol, the pure percentage of invaded T-cells that are attributed to EAE or SCI could be evaluated. The animals were clinically evaluated during the following 4, 6, 8, 13 and 30 days. Cryostat spinal cord sections were stained for Hematoxylin-eosin, Nissl, Bielschowsky and BrdU immunohisto-chemistry. SCI resulted in spastic paralysis of one hind limb, ipsilateral to the trauma in all but sham injured animals. Recovery from SCI was significantly better in EAE animals. Activated mononuclear cells were selectively accumulated at the side of the injury, whereas in animals with EAE only, perivascular infiltrations were randomly distributed throughout the spinal cord. Axonal loss was less in the EAE group following SCI. Sham operated animals presented neither cell infiltration nor any neurodegenerative process. Our findings indicate that actively induced autoimmunity against CNS myelin antigens may protect spinal cord pathways from mechanical injury. A neuroprotective, additional to the neurodestructive role of the inflammatory process within the CNS, may be attributed to the secretion of neuronal growth factors from the inflammatory cells and/or to the immunoregulatory role that some of these cells may have.

**P617**

**Hashimoto's encephalopathy presenting as generalized tremor.** C. Gaig, V. García-Gil, R. Sànchez del Valle, J. L. Molinuevo, A. Iranzo, Hospital Clinic (Barcelona, E)

Introduction: Hashimoto's encephalopathy is a rare entity related to Hashimoto's thyroiditis which is clinically characterized by confusional state or transient stroke-like episodes and the presence of antithyroidal antibodies. Its pathogenesis is unknown although an autoimmune cerebral dysfunction is suspected.

Patient: A 73 year-old woman presented progressive generalized tremor and unsteady gait during a week that progressed to important mental confusion, somnolence and disorientation. She had no history of severe diseases except diabetes type II. On examination she was drowsy, disoriented, inattentive with severe intentional four limbs tremor and ataxia.

Results: Standard blood tests, urinary exams, electrocardiogram and chest radiograph were normal. Brain CT and MRI were unremarkable. EEG showed symmetrical delta rhythm with anterior triphasic-like waves. Lumbar puncture revealed high protein levels without pleocytosis and normal glucose with negative microbiological cultures and viruses PCR. Thyroidal function analyses disclosed slight increase in TSH (6.103 mU/L, normal range, 0.4–4), normal T4 (1.08 ng/dL, normal range 0.8–2), and the presence of high titres of antithyroglobuline and antiperoxidase antibodies (813 IU/mL and 193 IU/mL respectively, normal is lower than 35 and 40). Treatment with intravenous methylprednisolone (1 gr per day during three days) was associated with an important clinical improvement characterized by resolution of the tremor and confusional state with normalization of the EEG activity.

Conclusion: Hashimoto's encephalopathy is a treatable entity whose clinical picture may start with generalized tremor.

**P618**

**Recurrent spontaneous hypothermia and a traumatic lesion of the corpus callosum: a variant of Shapiro's syndrome?** D. Hemelsoet, J. De Bleecker, University Hospital Ghent (Ghent, B)

Background: Recurrent hypothermia (core temperature < 35°C) can be caused by a number of neurological, metabolic and drug-induced disorders. A rare form of spontaneous hypothermia is known as Shapiro's syndrome in which recurrent episodes of hypothermia are associated with agenesis of the corpus callosum. The pathophysiology of the syndrome is unknown. Diencephalic epilepsy, a reset of the hypothalamic thermostat or a biochemical cause (by neurotransmitters) have been proposed. The specific role of the corpus callosum remains unclear.

Patient and methods: We investigated a 49-year-old man with a history of a frontal syndrome and tetraparesis due to a brain contusion after a car crash 10 years before. The patient was admitted because of recurrent episodes of confusion, drowsiness and aggressive behaviour. On admission a patient with stupor, severe hypothermia (a body temperature of 32°C) and bradycardia (36 bpm) was found. The patient was investigated by conventional MRI and blood analysis.

Results: Conventional brain MRI showed extensive frontal lobe atrophy with destruction of the anterior third of the corpus callosum. No hypothalamic lesions were seen. Other possible causes and contributing factors in hypothermia were excluded. Severe hypernatremia, mild anemia, thrombocytopenia and renal insufficiency were found. Natremia and thrombocytopenia were fluctuating together with body temperature. Hormonal investigation showed decreased growth hormone levels, elevated prolactin levels and normal thyroid function. An EEG showed no epileptic activity. After warming up the patient, body temperature rose but remained between 30°C and 35°C. After empirical treatment with clomipramine temperature normalized. Biochemical (except hormonal) abnormalities and bradycardia recovered together with body temperature.

Conclusion: In our patient with recurrent hypothermia and various biochemical abnormalities a posttraumatic anterior corpus callosum lesion was found. Structural abnormality of the corpus callosum in this patient suggests a traumatic variant of Shapiro's syndrome. In recurrent hypothermia of unknown cause the corpus callosum should be investigated by MRI. The pathogenesis of the thermoregulatory failure remains unclear. In our case we presume that corticohypothalamic fibers were disrupted due to the traumatic lesion.

**P619**

**Clinical pharmacokinetics of pregabalin in healthy volunteers.** C. W. Alvey, H. N. Bockbrader, J. A. Busch, B. W. Corrigan, G. Haig, E. L. Posvar, L. L. Radulovic, E. J. Randinitis, J. C. Strand, D. L. Wesche, Pfizer Global Research and Development (Ann Arbor, USA)

Purpose: To characterize pregabalin pharmacokinetics following single- and multiple-dose administration and to determine the effect of food on pregabalin pharmacokinetics in healthy volunteers.

Methods: Five studies were performed to assess pregabalin pharmacokinetics. Study 1 was a single dose (1- to 300-mg) study in 29 subjects, Studies 2 (25- to 300-mg q8h and 300 mg q12h) and 3 (300-mg q8h) were single- and multiple-dose studies in 45 and 12 subjects, respectively, and Studies 4 (100-mg) and 5 (150-mg) were single dose studies to examine the effect of food on pregabalin pharmacokinetics in 11 and 14 subjects, re-

spectively. Serial blood samples (all studies) and urine collections (Studies 1 and 2) were obtained for the quantification of pregabalin in plasma and urine. Noncompartmental methods were used to determine pregabalin pharmacokinetics.

Results: Following single- and multiple-dose administration, the drug was rapidly absorbed with peak plasma pregabalin concentrations occurring between 0.7 to 1.4 hours postdose. Plasma pregabalin concentrations increased proportionally to dose following both single- and multiple-dose administration. Pregabalin oral bioavailability was approximately 90% (based on urinary excretion data) and was independent of dose and frequency of administration. Food reduced the rate of pregabalin absorption resulting in lower and delayed Cmax concentrations; however, the extent of drug absorption was unaffected. The delayed absorption is not expected to be clinically significant. Pregabalin elimination half-life was approximately 6 hours, and steady-state was achieved within 1 to 2 days of repeated administration. Pregabalin plasma clearance, once corrected for oral bioavailability, was essentially equivalent to renal clearance indicating that pregabalin undergoes negligible non-renal elimination.

Conclusions: Pregabalin has linear and predictable pharmacokinetics that lend to its ease of use. Systemic exposure to pregabalin is proportional to dose following single- and multiple-dose administration. Pregabalin can be given without regard to meals, and steady-state is achieved rapidly following repeated administration. Since pregabalin undergoes negligible metabolism, renal function is an important determinant of the pharmacokinetics of pregabalin.

**P620**

**The association of Charcot Marie Tooth-1A and transitory central nervous system inflammation mimicking spinocerebellar ataxia-case report.** I. Dujmovic, N. Stojsavljevic, S. Knezevic, R. Trikic, D. Keckarevic, J. Drulovic, S. Apostolski, Institute of Neurology, PCR Centre (Belgrade, YU)

Hereditary neuropathies have been anecdotally reported to be associated with signs of central nervous system (CNS) affection. We report a case of a 40-year old female with a history of pes cavus from the early childhood who started developing slowly progressive deafness at the age of 15. Her family tree showed autosomal dominant mode of inheritance. At the age of 26, she experienced transitory episode of double vision and right eyelid ptosis. Fourteen years later, in addition to already developed clinical signs of sensorimotor polyneuropathy and bilateral sensorineural deafness, the examination revealed ophthalmoplegia with the right gaze induced diplopia, bilateral pyramidal and cerebellar signs and moderately ataxic gait. At that time, the clinical presentation was temporarily misdiagnosed as spinocerebellar ataxia. The patient also had a transitory manic expression. Nerve conduction studies showed a marked reduction in the motor (< 20 m/s) and less significant slowing of sensory conduction velocity. Cerebrospinal fluid (CSF) findings disclosed mild elevation of protein level (0.5 g/L) and moderate pleocytosis (54 lymphocytes/mm$^3$). Oligoclonal bands were not detected. The results of serum and CSF immunological and microbiological analyses were normal or negative. Brain Magnetic Resonance Imaging showed two focal hyperintensity lesions on T2W: in the brainstem (pons and medulla oblongata) and in the left fronto-temporal deep white matter. Genetic analysis confirmed the presence of 17p11.2 duplication in the region coding for peripheral myelin protein 22 (PMP22) thus implicating the diagnosis of Charcot Marie Tooth (CMT)-1A. We observed spontaneous regression of all symptoms and signs of CNS dysfunction during the following 2 months. Our case presents the association of CMT-1A with CNS inflammatory disorder of unknown etiology.

**P621**

**Amnesic syndrome and persistent fever after basilar aneurysm embolisation.** C. Gerace, M. R. Fele, S. Galgani, S.Camillo Hospital (Rome, I)

In the last ten years results of embolization using GDC suggest that endovascular treatment could offer significant improvement in the management of patients with cerebral aneurysms eliminating craniotomy. However little is known about possible neurologic complications related to this treatment.

We describe a patient with amnesic syndrome and persistent fever after embolization procedure.

A 60 year old woman had a six months history of double vision and visual field defect, without headache or other symptoms suggesting intracranial bleeding. Neurological examination was normal except bilateral temporal defects on visual field exam. MRI showed a giant unruptured aneurysm of the tip of basilar artery. The embolization was performed with detachable coils and the procedure was well tolerated with no bleeding and complete aneurysm occlusion. Some days after treatment the patient became febrile and confused with poor response to antibiotic therapy. Blood and urine culture were negative and the patient had no symptoms of general disease. A lumbar puncture yielded 200 cells, the glucose level was 40 mg/ml, the protein level was 60 mg/ml; CSF was sterile on culture. Cognitive status suggested an amnesic syndrome: the patient was awake and responsive, but had an impaired ability to conserve and recall events and other informations occurred in the last weeks. She was not able to learn new informations and her replies to specific questions were often only fantasies. MRI disclosed a large T2 hyperintensity in the area near the embolized aneurysm involving diencephalic region. After two months since embolization the patient was still febrile and confused, without any significant change in her neurological status. Fever improved only with steroid therapy.

The neurologic complication observed in our patient may be related to embolized aneurysm.Tissue damage around the aneurysm may appear after embolization procedure and can be caused by several mechanisms. Increased aneurysm mass effect after endovascular treatment with coils could generate compression on the nervous and vascular structures with oedema and neural dysfunction. Amnesic disorder in fact may be due to diencephalic dysfunction. Aseptic flogistic reaction to "coiled" aneurysm could explain fever in our patient as suggested by the significant improvement with steroid therapy and CSF findings.

**P622**

**Generalised botulism after administration of Botulinum toxin A or food-born intoxication?** K. Mitosek-Szewczyk, H. Fota-Markowska, T. Hasiec, School of Medicine (Lublin, PL)

Botulinum toxin type A (BT/A) is commonly used nowadays in the treatment of patients with local muscle spasticity, spasmodic torticollis and blepharospasm. The toxin, in therapeutic doses, is considered to be effective and safe, however there were described cases of generalised botulism-like syndrome after intramuscular injections of botulinum toxin type A. Nevertheless the most frequent reason of botuline intoxication is consumption of food that contains botulinum toxin type A, B, E and rarely F. In Poland the most frequent cause of botulism is the toxin type B. We present the case of food-born botuline intoxication in 65 year old woman, who was treated with BT/A because of blepharospasm. The patient received injection of total 250 units of BT/A, that was administered in orbicularis oculi muscle of left eye. Following five days nausea and vomiting of chyme, general weakness, xerostomia and hoarseness appeared. The next day they appeared: vision disturbances, photophobia, bilateral weakness of visual acuity and double vision, and then belly meteorism and obstinate constipation. In spite of patient treatment in Lublin Neurology Department, the symptoms intensified and muscle weakness, swallowing problems and respiration disturbances appeared. Blood tests towards the presence and identification of botulinum toxin confirmed botuline intoxication. The patient was transferred to the Department of Infectious Diseases. During examination was stated: pale, mask face, mydriasis, asymmetric pupils, no pupil reaction for light and accommodation disturbances, impaired ocular movement with bilateral ptosis, lack of palatine reflexes, xerostomia, belly meteorism and shallow respiration with limiting movement of chest and diaphragm. Moreover was stated: suppression of peristaltic movement and lack of diuresis. This typical clinical picture also confirmed the presence of botulism. Clinical improvement, including regression of intercostal muscles palsy, return of peristaltic movement and diuresis and regression of visual disturbances, was achieved after administration of 150 ml of botulinum antitoxin. In laboratory diagnostics, during biological tests on mice with antitoxin neutralisation, the presence of botulinum toxin type B was stated, which excluded intoxication with BT/A. In detailed epidemiological investigation it was appointed that probable source of intoxication was home-made preserved meat the patient consumed five days after BT/A injection.

Conclusion: Therapeutic administration of botulinum toxin may cause the appearance of generalised symptoms of botulism. Nevertheless, the cardinal reason of botulism is the consumption of food infected with botuline.

## Neuro-immunology

**P623**

**Autoantibodies to a 108-kd protein in relapsing-remitting autoimmune agrypnia.** G. Frisullo, G. Della Marca, M. Mirabella, M. Caggiula, G. F. Mennuni, P. A. Tonali, Institute of Neurology (Rome, I)

We describe a 55 year old woman presenting multiple nerve palsy, several episodes of total insomnia (agrypnia) and nocturnal respiratory crises associated to dysautonomic symptoms, leading to respiratory failure, severe hypotension and coma. During these crises the PSG showed a continuous 9–11 Hz activity pattern associated with sporadic polymorphic theta activity and isolated sharp waves on temporal leads of both hemispheres. Immunosuppressive treatment and plasma exchange were followed by disappearance of respiratory crises and restoration of sleep architecture. Clinical improvement and the presence of oligoclonal IgG bands suggested autoimmune pathogenesis of the syndrome Anti-Hu, anti-Ri, anti-Yo, anti-glutamic acid decarboxilase (GAD), anti-islet cell (ICA), anti-potassium channel (VGKG), and anti-amphiphysin autoantibodies were negative. Immunohistochemical staining showed increased binding of serum and CSF on GABAergic synapsy-rich neuronal cells, in particular on cerebellum (granule cells), brainstem and hyppocampus.

We tested our patient purified IgG by western blotting on monodimensional gels of homogenate of mouse cerebral cortex, cerebellum and brainstem.

A band with an apparent molecular mass of 108 kd was recognised by patient purified IgG, mainly on extracts of cerebellum cortex.

Further studies are being made in order to identify the target protein.

**P624**

**Levels of dopamine receptors mRNA are decreased in lymphocytes from multiple sclerosis patients.** M. Giorelli, P. Livrea, M. Trojano, University of Bari (Bari, I)

Objective: To assess wether the dopamine network of lymphocytes is involved in the pathophysiology of Multiple Sclerosis.

Background: A growing number of experimental evidences suggest that catecholamines modulate T cells activation in Multiple Sclerosis (Zoukos et al. 1992; Rajda et al. 2002). Dopamine is synthesised by the blood mononuclear cells (PBMCs) and can counteract activation-related functions of lymphocytes in an autocrine-paracrine manner. Dopamine has at least five G-protein coupled receptor subtypes, D1-D5, which have been divided into D1-like (D1R and D5R), and D2-like (D2R, D3R, D4R) subtypes. To date, only the D3R, D4R, and D5R have been detected in PBMCs.

Design/Methods: We studied the expression of dopamine receptors (D3R, D4R, D5R) mRNA in PBMCs from eighteen [18] stable Relapsing Remitting (RR) MS patients and from eleven [11] age and sex-matched healthy controls.

To address this issue, we used the semi-quantitative RT-PCR method employing the housekeeping gene beta-actin as internal standard (DR/bA).

Results: The D3R mRNA was detected in 2/18 (11.1 %) MS patients and in 9/11 (81.8 %) controls. The mean D3R/bA ratio was $0.07 \pm 0.13$ (min = 0; max = 0.38) in MS patients and $0.7 \pm 0.15$ (min = 0.3; max = 0.8) in controls ($p < 0.001$).

The transcript for the D4R mRNA was present at low basal levels in all (100 %) controls. In MS patients, the D4R mRNA was up-regulated (2 folds) in 8/18 individuals (44.4 %) and absent in the others (no differences in disease duration and EDSS score between these two groups). The average D4R/bA did not differ significantly ($p = 0.46$) between MS patients and healthy individuals.

The mean D5R/bA was lower (1.5 to 8 folds) in MS ($0.3 \pm 0.3$) (min = 0; max = 0.65) than in controls ($0.62 \pm 0.3$) (min = 0.34; max = 1.1) ($p < 0.05$).

The levels of dopamine receptor mRNA in MS patients did not depend on disease duration and EDSS score. Reduction in D3R and D5R mRNA was observed in all MS patients as compared to the matched controls.

Conclusion: We studied RR MS patients in a cold phase of disease (stable). Thus, the reported abnormal expression of dopamine receptors mRNA in PBMCs would be pre-existent to a possible relapse initiation. As dopamine has predominant down-regulating effects on lymphocytes functions, decreased levels of dopamine receptors may lead to failure of the peripheral dopaminergic inhibition of T cell activation in MS patients. Lacking this control, lymphocytes more easily can start differentiation upon antigen recognition. This may lead to CNS invasion of self-directed lymphocytes and, possibly, to relapse beginning. Further studies exploring the immunological role of dopamine receptors on lymphocytes from MS patients are needed. Indeed, availability of agonists/antagonists for dopamine receptors might open novel paths to therapeutic intervention.

**P625**

**Age- and sex-dependent differences in the inducible nitric oxide synthase mRNA expression in the striatum of C57Bl/6 mice following toxic degeneration caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.** I. Joniec, A. Ciesielska, A. Przybylkowski, I. Kurkowska-Jastrzebska, A. Czlonkowska, A. Czlonkowski, Medical University, Institute of Psychiatry and Neurology (Warsaw, PL)

Nitric oxide (NO) plays an important role in cell signalling in the nervous system and is implicated in memory and synaptic plasticity. Under conditions of excessive NO formation, it can act as a neurotoxin and may contribute to the pathogenesis of several neurodegenerative disorders including Parkinson's disease (PD).

The nitric oxide-synthesizing enzyme nitric oxide synthase (NOS) is present in the brain in three different isoforms: neuronal-nNOS, endothelial-eNOS and inducible iNOS.

High levels of NO, synthesized by iNOS, the enzyme with the highest enzymatic activity, may be cytotoxic to other cells, most likely due to peroxynitrite formation. iNOS is induced by various cytokines. The increased density of pro-inflammatory cytokin-producing glial cells in the substantia nigra of patients with PD was documented. Several studies indicate that estrogen can modulate iNOS expression and/or activity, and may also have the influence on the production of cytokines.

Within this context, we investigated the influence of age and gender on iNOS gene expression in the striatum of C57BL/6 in young and old (3 and 12-month old) male and female mice in a murine model of PD induced by MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The animals were sacrificed on 6 h, on 1, 3,7,14, 21 days after MPTP intoxication. The levels of mRNA for iNOS were assayed by RT-PCR method. In the control group baseline levels of mRNA for iNOS were minimal in both young and old male and female mice.

In young and old male mice iNOS mRNA expression rapidly increased and was higher than in controls as early as at 6h after MPTP intoxication; peaked at 24h, next significantly decreased at 3 day, and was observed till 14th day. In young and old female mice iNOS mRNA expression was observed later than in males: it started from 24h reading peak value, but was still higher than in control till 14th day of experiment. At 21st day the level of iNOS mRNA decreased to the level noticed in control in all groups of animals. We observed differences in the expression of iNOS mRNA in the young animals in comparison to the aged ones. In aged mice, the expression of iNOS was higher than in young at every time points after MPTP intoxication.

In conclusion, iNOS mRNA expression is age and gender-dependent: different levels of iNOS mRNA were observed in young and old animals; the pattern of iNOS mRNA expression was different in males and females.

**P626**

**Meningoencephalomyelitis following anti-cholera vaccination.** E. Karakoc, E. Demirci, G. Nurlu, E. Tan, O. Saribas, Hacettepe University (Ankara, TR)

Various neurologic complications were previously attributed to vaccines in the literature; in particular, viral and live-attenuated ones. Additionally, immunogenicity of cholera vaccine is obviously described in several case reports in the literature. Neurologic complications such as leukoencephalomyelitis or transverse myelitis caused by cholera vaccine were reported from Italy in 1974. There were no other similar reports on this subject from another country at that period, however. A 42-year-old female patient was referred to our clinics for evaluation of progressive paraparesis and urinary incontinence. Her early development was uneventful until the age of 3 years when she had left side dominant paraparesis following rabies vaccination in 1963. At the age of 12 years, in 1972, she encountered another postvaccinal complication of meningoencephalomyelitis with cholera vaccine from which she recovered completely in a 3-month period. Among vaccinees in the same school, two other encephalomyelitis cases, none of which could survive, were diagnosed concomitantly. She began to suffer from weakness in left lower limb and urinary incontinence four years ago. Current clinical examination revealed findings of loss of convergence in the left eye, left-sided mild proximal paresis, bilaterally increased deep tendon reflexes, and bilateral Babinski sign all of which were sequelae of abovementioned episodes. Only finding that had not been noted in the past examinations was loss of pain and touch sensation below the fifth thoracic level on the left side. Serum and cerebrospinal fluid investigations including viral serological tests, oligoclonal bands, vasculitic

and immunological markers were completely within normal limits. The only positive finding was antinuclear antibody positivity in low titers. Additionally, normal electrophysiological investigations including brainstem, visual, motor and somatosensory evoked potentials excluded other possible diagnoses. Magnetic resonance imaging of brain and spinal cord seemed to be stable, being consistent with few millimetric hyperintense lesions and thoracal spinal cord atrophy. In conclusion, we would like to emphasize another endemic postvaccinal encephalomyelitis due to cholera vaccine in 1972 in our country.

**P627**

**Idiopathic late onset cerebellar ataxia responding to immunotherapy.** L. Bataller, I. Bosca, E. Fages, T. Sevilla, Hospital University La Fe (Valencia, E)

Background: Patients suffering from idiopathic late onset cerebellar ataxia (ILOCA) often have a progressive invalidating course. However, there is increasing evidence that some patients with ILOCA may have an autoimmune disorder, and therefore benefit from immune suppressant medications.

Objectives: To identify clinical and laboratory features that define a subgroup of patients with ILOCA that may benefit from immune modulating treatments.

Patients/methods: We considered candidates for receiving immunosuppressive treatment those patients with ILOCA, with at least two years of follow up, fulfilling at least one of the following criteria: i) acute onset of symptoms and/or relapsing/remitting course, ii) clinical features of progressive myoclonic ataxia (excluding mitochondrial disease), iii) presence of laboratory tests of autoimmune background (antigliadin antibodies, organ specific antibodies). Treatments employed included: intravenous immunoglobulins, corticosteroids, cyclosporin and/or azathioprin. Periodic clinical evaluations were performed with videorecording using a modified version of the international cerebellar ataxia rating scale (ICARS). A functional scale was also used.

Results: Eight out of 60 patients affected by ILOCA fulfilled the inclusion criteria. They included: 4 patients with clinical profile of progressive myoclonic ataxia and relapsing course; 1 patient with antigliadin antibodies; 3 patients with organ specific antibodies. Response to treatments administered led to: almost full recovery in three patients; partial improvement in 3; and only slight improvement in 2 cases.

Conclusions: A subgroup of patients affected by ILOCA have an autoimmune background (13% in our series). These patients may benefit from immunosuppressant treatments.

**P628**

**Congenital angioneurotic oedema with predominantly cerebral symptoms.** Z. Kazibutowska, E. Motta, J. Jarzab, B. Wludarczyk, B. Rogoz, Medical University of Silesia (Katowice, PL)

Angioneurotic edema related to deficit of inhibitor C1-esteraze (C1-INH), can be either congenital or acquired. We present a rare case of congenital deficit of C1-INH in a 10-year-old girl with dominant brain symptoms.

First symptoms of the disease which lasted few days were abdominal pains, vomiting, provoked by stress or infection, appeared when she was 5-year-old. At the age of 8, after few minor injures, a unsymmetrical edema of upper and lower limbs appeared and lasted 2–3 days. We have then found out – that the concentration of C1-INH in the serum was low – 0,106 g/l (normal range 0,15–0,35); the C4 component of the complement was 4,5% (normal range 10–40). When she was 8,5 years old, while having infection of upper air ways, a loss of consciousness happened and was accompanied by left-sided convulsions. It was proved neurologically that only bilateral strabismus appeared and analysis of cerebrospinal fluid revealed pleocytosis 44/3. MR of the head disclosed not characteristic demyelinating or inflammatory changes in both hemispheres with distinct regression after 10 days. All tests for rheumatic diseases, collagenosis, boreliosis, brucelosis, tuberculosis, viral diseases were negative. During next 10 months seizures appeared, as well as head dizziness, transient pyramidal left or right-sided paresis, accompanied by abdominal pains, vomiting and diarrhoea or by edema of the limbs. Repeated head MR did show not characteristic changes in both brain hemispheres (with alternate edema) and in cerebellum with a tendency to their regression.

Angio CT did not reveal any substantial abnormalities. A check-up with the use of spectroscope MR suggested metabolic distemper.

A control check-up confirmed low concentration of C1-INH in the serum. After including first the epsilon-aminocapronic acid and next tranexamic acid long term symptoms remission was achieved.

Conclusion: In our female patient we recognise congenital angioneurotic edema with brain manifestations. A possibility of such illness of the brain has next to remittent-relapsing brain symptoms, other symptoms of angioneurotic edema appear.

**P629**

**Myositis in a patient with unusual combined immunodeficiency syndrome.** B. Rosche, M. Jacobsen, P. Barth, B. Hemmer, Neuroimmunology Group, Center of Pathology (Marburg, D)

We describe a patient with recurrent opportunistic infections and severe and progressive myositis affecting predominantly the proximal leg muscles. Analysis of muscle enzymes suggested involvement of both skeletal and heart muscle. Muscle biopsy demonstrated cellular infiltrates and extensive skeletal muscle destruction at two different time points. T cell infiltrates in the muscle were predominantly CD8+. Immunological studies revealed NK and B cells deficiency in the patient. Furthermore, CD8+T cells largely outnumbered CD4+T cells in the peripheral blood. TCR-analysis by spectratyping and flow cytometry revealed clonotypic expansion of CD8+T cells. Following treatment with steroids and immunesuppressive drugs, muscle enzymes normalized and the patient's muscle weakness stabilized. After the development of side effects, the treatment was discontinued resulting in further progression of clinical symptoms and elevation of muscle enzymes. Overall, the unusual findings in blood and muscle together with the immediate response to immunosuppression suggest an autoimmune process as the cause of disease in this patient.

**P630**

**Cerebellar subacute degeneration and sensory neuropathy in a patient without malignancy and with an unidentified anti-neuronal nuclei antibody.** G. Santuccio, R. Nemni, E. Calabrese, L. Sanvito, M. Sgandurra, N. Canal, Don Gnocchi Foundation (Milan, I)

Background: Neurological paraneoplastic syndromes (NPS) are not-metastatic neoplasm complications with a subacute onset that precedes about 6–12 months the discovery of a malignancy of reproductive system in women and lung microcitoma in men. These syndromes are associated to antibodies (Abs) anti-onconeural antigens (Ags) that could have a pathogenetic role. Some of them have been associated to subacute cerebellar degeneration (SCD). In women of age 50, SCD is reported to have a paraneoplastic origin in about 2/3 of cases.

Objective: to describe a 51-year-old woman affected by a SCD with serum and liquoral Abs to an unidentified onconeural antigen.

Methods: A neurological evaluation, haematological routine, anti-onconeural Abs, electrocardiogram, chest-ray, evoked potentials, cranial magnetic resonance imaging (MRI), cerebro-spinal fluid (CSF) analysis, electromyography (EMG) and a complete screening for occult malignancies, were performed both at baseline and at follow-ups.

Results: in 12.95 the patient complained of numbness in left lower limb and gait unbalance with gradual worsening. Later she reported weakness at lower limbs with fatigue during walking. In 9.96 she was admitted in neurological department, where neurological exam showed opsoclonus associated to neuropathic and cerebellar signs. In few days she rapidly worsened with important weight loss, marked ataxia and weakness at 4 limbs. Research for malignancies was negative. She was treated with intravenous (iv) Methylprednisolone, 1 g/day for 10 days, with gradual neurological improvement. Laboratory tests showed the presence of serum and CSF anti-neuronal nuclei Abs (ANNA) with a molecular weight of 54 kDaltons, and the absence of anti-disyalogangliosides Abs. B12 vitamin and folic acid levels were normal. CSF analysis showed oligoclonal bands not present in the serum. Cranial MRI was negative for parenchimal lesions and cerebellar atrophy. EMG showed a mild axonal neuropathy at all 4 limbs. The diagnosis was SCD and subacute sensory neuropathy (SSN) in patient with ANNA Abs. She improved until 12.97, then she was steady. Last neurological control of 1.03 showed brainstem signs, sensory and cerebellar ataxia with walking possible with bilateral aid.

Conclusions: we described the clinical features of a 51-year-old woman who developed a SCD and a SSN. These syndromes suggest a paraneoplastic origin but no neoplasms were found. The good response to iv high-dose steroids further suggests an autoimmune pathogenesis. We think that our patient is affected by an autoimmune neurological syndrome with Abs against ANNA which mimics a paraneoplastic syndrome.

## Infection

**P631**

**Expression of CXCL9 and CXCL10 during experimental herpes-simplex virus encephalitis.** J. Sellner, F. Martinez-Torrez, P. Rau, F. Dvorak, Y. Zhou, A. Krick, P. Schramm, S. Heiland, J. Haas, U. Meyding-Lamadé, University of Heidelberg (Heidelberg, D)

Background and Goals: Herpes-simplex-Virus Encephalitis (HSVE) still remains a life-threatening disease even with early initiation of antiviral therapy. There is evidence for a vigorous compartimental immune response in the brain during acute HSVE. Interferon-gamma (IFN-g) was shown to govern the immune respone during the very early phase of the disease. Subsequently, the IFN-g inducible chemokines CXCL9 (MIG, monokine induced by interferon-gamma) and CXCL10 (IP-10, interferon-gamma inducible protein of 10 kDa) were studied during HSVE. Recently, it was indicated that these chemokines regulate neuroviral inflammation with recruitment of leukocytes and orchestrate antiviral clearance but are also involved in the development of long-term tissue abnormalities.

Methods: Following an experimental model mimicking human HSVE, all mice but a negative control group (n = 6) were intranasally inoculated with 10^6 pfu HSV-1 strain F. Subsequently, the infected animals developed acute encephalitis and standard T2- and diffusion-weighted MR scans were obtained on day 3, 7 and 10 post inoculation. At each timepoint, 5 animals were sacrified and brains were extracted microsurgically. The mRNA expression of CXCL9 and CXCL10 was determined by a quantitative real-time PCR using GAPDH as an internal reference (relative units (ru)). Brain viral load was quantitated with a nested PCR.

Results: In negative controls, no abnormalities were shown on MRI and low levels of both chemokines were found (CXCL9 0,53 ru + 0,68, CXCL10 0,86 ru + 0,5). In acute encephalitis we found a sequential regulation of mRNA expression of CXCL9 (day3 5,38 ru + 7,76; day7 39,53 ru + 72,67 and day10 6,81 ru + 10,80) and CXCL10 (day3 20,18 ru + 44,31; day7 ru 54,11 + 118,17 and day10 3,38 ru + 5,24). At all timepoints the mRNA expression of both chemokines was significantly increased compared to healthy controls (CXCL9 $P < 0,01$, CXCL 10 $P = 0,01$). Abnormalities on MRI were moderate at day3 and peaked at day7 as well as the virus copies in the brain.

Discussion and Conclusion: We found a sequential expression of both chemokines during acute encephalitis. The mRNA expression was significantly increased in acute HSVE and correlated well abnormalities on cranial MRI and brain viral load. These data suggest a key role of the chemokines CXCL9 and CXCL10 in the pathophysiology of HSVE. Future studies will have to elucidate the exact mechanisms of the neuroprotective and antiviral effect as well as the participation in the development of chronic-progressive tissue abnormalities in HSVE.

**P632**

**Immunization with a non-pathogenic HSV-1 strain prevents clinical and neurochemical signs of experimental HSV-1 encephalitis.** J. Weidenfeld, A. Itzik, O. Barak, Y. Asher, R. Yirmiya, Y. Becker, T. Ben-Hur, Hadassah-Hebrew University Hospital (Jerusalem, IL)

Aim: To examine whether immunization with a non-pathogenic strain of herpes simplex virus -1 (HSV-1) may prevent the clinical signs and neurochemical changes induced by a pathogenic HSV-1 strain.

Introduction: We have previously reported that intracerebroventricular (ICV) inoculation of rodents with a pathogenic HSV-1 strain induced acute encephalitis, manifested by fever, motor hyperactivity and aggressive behavior. These clinical signs were associated with hypersecretion of the pituitary-adrenal hormones ACTH and corticosterone (CS) and increased production of brain prostaglandin E2 (PG-E2). Inoculation with a non-pathogenic strain did not induce clinical, behavioral or neurochemical changes.

Methods: Out-bred male adult Sabra rats were immunized by 2 intraperitoneal inoculations with the non-pathogenic HSV-1 intratypic recombinant strain R-15. One month later, the immunized and control rats were challenged by ICV inoculation with the pathogenic HSV-1 strain Syn17 + . Fever and motor activity were measured continuously by biotelemetric transmitters. Aggressive behavior was evaluated by a clinical score (ranging 0–2). Serum ACTH and CS, and the ex-vivo production of PG-E2 by brain slices were measured 4 days after inoculation, using RIA.

Results: Inoculation of strain Syn17 + to control rats induced fever (peaking up to 40C), marked elevation of motor activity and aggressive behavior (scoring 1.6 ± 0.4). Serum levels of ACTH and CS increased by approximately 5-fold and brain PG-E2 production by 2.5-fold. The mortality in this group was 100 %. Immunization with strain R-15 prior to challenge with Syn17 + completely prevented the fever, motor hyperactivity and aggressive behavior, and markedly attenuated the ACTH, CS and PG-E2 responses. An in-vitro assay showed the presence of neutralizing antibodies to HSV-1 Syn17 + in the sera of R-15 immunized rats. Immunization with R-15 completely prevented the mortality induced by strain Syn17 + .

Conclusion: These results indicate that immunization with HSV-1 strain R-15 protects rats from lethal HSV-1 encephalitis and prevents its clinical and neurochemical manifestations.

**P633**

**Clinical and magnetic resonance imaging findings of HIV-negative syphilis patients.** B. Bilgic, C. Gurses, B. Topcu, N. Yesilot, M. Kurtuncu, H. Hanagasi, H. Sahin, G. Akman-Demir, H. Gurvit, O. Coban, M. Emre, Istanbul University, Ondokuz Mayis University (Istanbul, Samsun, TR)

Syphilis is an infectious disease caused by Tropenema pallidum. Due to the sensitivity of the microorganisms for many antibiotics especially penicilins, it had become a rare disease until acquired immunodeficiency syndrome (AIDS) emerged. Central nervous system may be involved at any stage of infection in about 5 % to 10 % of untreated patients and manifestation of the CNS involvement is rich and neuroradiological range is wide.

In this study based on clinical and laboratory evaluations, 5 patients were diagnosed as neurosyphilis. Two of them were admitted to hospital with chronic vision loss while two of them were admitted with dementia symptoms and one had only cranial neuropathy. Interestingly, only one patient declared primary stage skin lesions. VDRL and TPHA were positive both in serum and cerebrospinal fluid (CSF) and serum HIV antibody was negative for all patients. MRIs of the 2 patients (one with cranial nerve involvement alone and one with vision loss alone) were totally normal. Global cerebral atrophy was observed in demented patients and beside this finding, one demented patient's MRI revealed bilateral medial temporal signal abnormality mimicking herpes encephalitis. Bilateral white matter signal abnormalities were seen in the MRI of the other patient with vision loss. All patients received a 14-day-course of intravenous penicillin.

There are not a wide number of MRI studies on neurosyphilis with HIV [-] patients and the present ones are all case studies. The differences in the clinical and MRI findings of these five patients with neurosyphilis should draw our attention to the fact that neurosyphilis should be thought of while doing differential diagnosis.

**P634**

**Herpes simplex encephalitis: diagnostic and clinical features in 12 patients.** M. Rodriguez-Yañez, F. Diaz-Otero, B. Castaño, A. Esquivel, M. L. Martinez-Gines, C. de Andres, J. A. Villanueva, S. Gimenez-Roldan, HGU Gregorio Marañon (Madrid, E)

Background: Herpes simplex encephalitis (HSE) is a severe neurological disease with high mortality. Early diagnosis and treatment with antiviral therapy can improve the prognosis.

Purpose: To identify symptoms at onset and complications.

Methods and patients: We reviewed charts from 17 patients suspected of having HSE at admission from Jan-1996 to Dec-2000. Five patients proved to have other conditions. The diagnosis was confirmed in 9 cases by polimerase chain reaction (PCR) in cerebrospinal fluid (CSF) studies.

Results: In 12 patients (8 males, 4 females) whose mean age was 58.9 years. Disturbance of consciousness (91.7 %), fever (91.7 %), headache (41.7 %), behaviour changes (33.3 %) and seizures (33.3 %) were the most frequent symptoms at onset. Computerized tomography (CT) at admission (mean time 3.1 days from clinical onset) was normal in 58.3 % of the cases. Control CT (mean time 8.3 days from clinical onset) showed abnormalities in 80 %. Magnetic resonance images (MRI) showed temporal and frontal lobes hyperintensity in T2-weighted sequences in 9 of 10 patients. All patients received intravenous acyclovir (4.5 days from onset). 8 patients suffered seizures during evolution. Only one patient died.

Conclusions: 1. Clinical data suggest the correct diagnosis of HSE. 2. CT scan may be normal early in disease. 3 Early treatment significantly reduces mortality.

**P635**

**Progressive multifocal leukoencephalopathy associated with coeliac disease.** P. Kinirons, K. Keohane, H. Harrington, Cork University Hospital, Mercy Hospital (Cork, IRL)

Introduction: Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by JC virus. It occurs almost exclusively in the immunocompromised. Here we describe an autopsy proven case of PML occurring in association with coeliac disease in the absence of immune deficiency

Case report: a 60-year-old man presented with a two week history of ataxia. Coeliac disease had been diagnosed five years previously on jejunal biopsy and was well controlled on a gluten free diet. Over the following four weeks he underwent progressive neurological deterioration characterised by a fluctuating level of consciousness, swinging pyrexia, generalised rigidity and myoclonic jerking. He had a marked startle response. Reflexes were brisk throughout and both plantar responses were extensor. He died from respiratory failure six weeks after admission. Laboratory tests including white cell count, serum immunoglobulin levels, serum electrophoresis and vasculitis screen were normal. There were normal levels of haemoglobin, iron, vitamin B12, folate and serum protein. CSF testing revealed 8 lymphocytes but was otherwise unremarkable. MRI revealed multifocal white matter lesions throughout the brain and brainstem suggestive of PML. HIV testing was negative. A full autopsy revealed multiple areas of active demyelination in the brain with numerous enlarged oligodendrocytes containing visible viral inclusion bodies. The autopsy was otherwise unremarkable. The conclusion was of PML without underlying immune suppression.

Discussion: Coeliac disease has been reported in association with a variety of neurological conditions including spinocerebellar degeneration, epilepsy, neuropathy, myelopathy, brainstem encephalitis and bilateral occipital calcification. The cause of the association with these disorders is not clear. A case of PML has previously been reported in association with coeliac disease. In contrast to our case, this patient had evidence of malabsorbtion and had a remarkably prolonged course. PML is due to opportunistic infection by JC virus. It is well described in patients with HIV, cancer and those receiving immunosuppressant drugs. The cause of immunosuppression in our patient is not clear. Some reports have suggested that there may be a functional disorder of immunity in patients with coeliac disease. PML should be considered early in patients with celiac disease presenting with unusual neurological syndromes.

**P636**

**Bilateral striatal necrosis associated with cerebral malaria.** M. Vokaer, J.-C. Bier, E. J. Bartholomé, Erasme Hospital (Brussels, B)

Background: Cerebral malaria (CM) is a well defined clinical entity. Metabolic acidosis is a frequent consequence of malaria. Extrapyramidal syndrome is not an usual manifestation of CM. Moreover, striatal necrosis has never been described in case of CM. There are several identified causes of bilateral striatal necrosis. In most of the cases, the implicated pathogenic mechanism is thought to be a disturbance of the mitochondrial metabolism inducing lactic acidosis.

Case Report: A 37-year-old man was hospitalized for tetraparesis and parkinsonism. Two months earlier, five days after his return from Kenya, he presented pyrexia (40C), diarrhoea, hypotension (71/43 mmHg) and anuric renal failure. Peripheral blood smear was positive with a parasitemia of 40 % for Plasmodium Falciparum. Plasma lactate was > 8 mEq/L with a pH of 7,18. Treatment by quinine 1,5 g/day was initiated. Five days after the onset of symptoms, the patient presented generalized seizures and coma. Brain MRI showed a lesion located in the left parietal lobe and bilateral necrotic lesions affecting globus pallidus, subthalamic nucleus and substantia nigra. The patient stayed in coma in the intensive care unit during 4 weeks. Thereafter, he was admitted in our rehabilitation department. Neurological examination revealed saccadic pursuit and several extrapyramidal signs including bilateral upper limbs rigidity, postural tremor and bradykinesia. There was a tetraparesis predominantly affecting the lower limbs with bilateral lower limb hyporeflexia and absence of pyramidal signs. Electrophysiological investigations disclosed an axonal sensorimotor neuropathy and intensive care unit neuropathy was diagnosed.

Discussion: There are only a few reported cases of extrapyramidal syndrome associated with CM. Although metabolic acidosis is part of the main documented causes of bilateral striatal necrosis and is frequently observed in case of malarial infection, striatal necrosis has not yet been reported in case of CM. Systemic hypoxia, hypotension, or drug toxicity are thought to play a role in the generation of such lesions. The bilateral striatal lesions observed in our case are likely due to the association of both malaria-induced lactic acidosis and hypotension.

In conclusion, our case suggests that clinicians should systematically search for extrapyramidal signs and striatal necrosis in patients with CM and lactic acidosis, especially when systemic hypoxia or hypotension are present.

**P637**

**Central nervous system infections by mycoplasma pneumoniae - report of two cases.** R. Chorão, A. Braga Costa, M. R. Silva, R. Jorge, F. Esteves, Hospital de S. Pedro (Vila Real, P)

Introduction: Central nervous system (CNS) infections by Mycoplasma pneumoniae may be those of meningitis or encephalitis, cranial nerve or cerebellar involvement, or transverse myelitis. Prognostic is variable, with some fatal cases.

Case reports: The first case is that of a 68 year-old man, with a rapidly progressive tetraplegia with sensory disturbances and sphincter involvement. He had concomitant signs of atypical pneumonia with respiratory failure. With appropriate antibiotic treatment and advanced support care, he recovered to the extent of a slight to moderate paraplegia, being able to walk with support.

The second case is that of a 46 year-old man with the clinical picture of an acute paraplegia, accompanied by fever and meningeal irritation signs. The clinical situation deteriorated rapidly to brain involvement, with depressed level of consciousness to profound coma, ocular signs and respiratory failure. In spite of advanced supportive measures at the Intensive Care Unit (ICU), the patient did not recover and died.

Cerebrospinal fluid (CSF) revealed mononuclear pleocytosis and elevated protein content in both patients, with low glucose level in the second one. Magnetic resonance imaging showed spinal cord swelling in both cases; the second patient had also signs of midbrain and thalamic involvement. Serologic tests to mycoplasma antibodies were positive in blood (in both), and in CSF (in the second one).

Conclusions: Mycoplasma pneumoniae infections of the CNS are infrequent, and they can appear with unapparent signs of pneumonia at its onset. CSF shows a mononuclear pleocytosis, and antibodies to mycoplasma in peripheral blood or in the CSF, or its culture lead to the diagnosis, which can be a late finding. Treatment can be difficult because most of the drugs that are effective against this agent do not cross easily the blood-brain barrier.

**P638**

**Legionella pneumonia presenting with neurologic manifestations: a case report.** A. Yilmaz, Hu. Lakadamyali, H. Erdogan, Ha. Lakadamyali, M. Kilinc, U. Can, Baskent University (Ankara, TR)

Legionella is a microorganism that would cause both nosocomial and community acquired pneumonias. Legionella infections often cause multisystem involvement; and neurologic manifestations tend to appear concurrently with, or soon after the onset of fever. Cases with neurologic manifestations preceding the development of pneumonia are exceedingly rare. The most common neurologic signs of Legionella disease are disorientation, headache, and somnolence. Less frequent signs are cerebellar dysfunction, hallucinations, agitation or stupor, encephalitis, affective disorders, peripheral neuropathy, pyramidal disturbance, memory loss, seizures, cranial nerve palsies, extrapyramidal disturbances, acute disseminating encephalomyelitis and myositis.

We present a 39 year old male who admitted with neurologic manifestations before the development of Legionella pneumonia. The patient had complaints of headache, fever, slurred speech, dysphagia, weakness of legs and disturbance of gait. On examination he was awake, cooperated, and oriented. He was tachicardic (pulse rate 120/min), and dyspneic (respiratory rate 28/min). The blood pressure was 190/100 mmHg and body temperature was 39 °C. His speech was dysarthric, he had right facial paralysis, and bilateral palatal arch paralysis. There was slight proximal weakness of lower extremities (4 + /5), and he was ataxic. Laboratory findings revealed leucocytosis, elevated liver enzymes, elevated erythrocyte sedimentation rate and creatinin kinase. Chest X-ray showed a nonhomogenous infiltration of the right hemithorax except apical zone. Legionella SG-1 urine antigene was positive and indirect fluorescent antibody titer was found to be increased by fourfold. Cranial magnetic resonance imaging showed contrast enhancement of basal meninges. Lumbar punction revealed elevated cerebrospinal fluid pressure. The protein, and glucose content of the cerebrospinal fluid were within normal limits, no cell or microorganism could be demonstrated. Electroneuromyography showed normal nerve conduction velocities. The patient was put on antimicrobial therapy with klarithromycine and rifampicine. All of the patient's neurologic manifestations disappeared one week after the beginning of theraphy.

The pathogenesis of the neurologic manifestations in Legionnaires' disease is unknown. It is suggested that the neurologic findings are caused by the toxins of the microorganism or by direct invasion of the nervous system. The prognosis is generally good.

The case was reported because Legionnaires' disease rarely presents first with neurologic manifestations.

**P639**

**Hydrocephalus due to cysticercotic chronic meningitis.** A. Esquivel López, M. Martínez Ginés, J. Villanueva Osorio, Hospital Gregorio Marañón (Madrid, E)

Background: Cysticercosis is the most common parasitic infection of the central nervous system, and is an important public health problem in developing countries, with a variety of neurological manifestations and consequences. This condition is also more frequent in developed countries as a result of immigration from endemic areas.

We report one patient who had a singular clinical manifestation of neurocysticercosis.

Case report: A 79-year-old Ecuatorian woman was hospitalized because of 10-month history of progressive cognitive impairment, gait disturbance and urinary incontinence. She arrived in Spain 15-days before. At admission to our hospital she had a severe frontal disfunction without symptoms and signs of raised intracranial pressure. Routine laboratory test was normal. Cranial computerized tomography scan showed ventriculomegaly without another remarks. Brain magnetic resonance imaging confirmed the hydrocephalus and showed periventricular transependymal edema with craniobasal arachnoiditis. Cerebral spinal fluid (CSF) examinations demonstrated opening pressure of 20 cm $H_2O$, 30 cells/mm$^3$ with 98% lymphocytes, 180 mg/dl protein, 5 mg/dl glucose (with a serum glucose of 110 mg/dl). No micro-organisms were seen on Gram's stain, and culture for bacteria, fungi and mycobacteria was negative. Serological test for anticysticercus antibodies by enzyme-linked inmunosorbent assay was positive both in the CSF and in the serum.

Cysticercotic chronic meningitis was diagnosed after excluding neoplastic and another infectious causes.

The patient received oral albendazole and steroids for 2 weeks. Ventriculoperitoneal shunt was placed after albenzazole therapy. The clinical condition improved within 7days. CSF parameter showed improvement one month later.

Conclusion: Neurocysticercosis remains a major worldwide health problem, unfortunately, the pleomorphism of this parasitic disease creates confusion, and diagnosis is difficult because clinical manifestations are nonspecific, most neuroimaging findings are not pathognomonic, and some serologic tests have low sensitivity and specificity.

Treatment has been revolutionized by the advent of effective chemotherapy, nevertheless, surgery is still indicated in certain situations, especially when the ventricular system is affected.

**P640**

**An unusual case of neurosarcoidosis misdiagnosed as tuberculosis.** E. Karakoc, C. Turk, I. Saatci, E. Tan, K. Varli, Hacettepe University (Ankara, TR)

Sarcoidosis is a systemic disease that can affect any organ or system. Nervous system involvement is seen in 5% of sarcoidosis cases. Central nervous system is usually affected in the very early stages of sarcoidosis while peripheral nervous system in further stages. Diagnosis of neurosarcoidosis is a challenging course particularly when subtle systemic clinical findings ensue. In this report, a young female who was admitted with complaints of headache, vertigo, nausea and instability upon walking and was empirically treated as tuberculous meningitis, but later appeared to have neurosarcoidosis is presented. Throughout clinical history, she was learned to have received combined antituberculous therapy for about one year and prednisolone with a preliminary diagnosis of tuberculous meningitis. Patient suffered from sensorineural hearing loss on the eleventh day of treatment, which was attributed to streptomycin in the therapeutic regimen. Clinical signs and symptoms improved for the first two months under prednisolone treatment until when the diagnosis was thought to be obscure due to recurrence of initial complaints. Her complaints such as headache, nausea and vertigo accompanied by instability were aggravated further, as compared to the previous months, so the patient came to our attention. At presentation, her physical examination showed findings related to meningeal irritation, left sided peripheral facial palsy and extensor plantar reflex on the right. With regard to laboratory work up CSF findings were consistent with aseptic meningitis, tests for acid resistant bacilli both with PCR and culture were negative, as well as anergy to tuberculin skin test. Angiotensin converting enzyme level in serum was detected to be high. Eventually diagnosis of neurosarcoidosis was made with clinical, laboratory and radiological findings. Images of the ischemic lesion in amigdalohippocampal region on diffusion scans and ADC mapping are also presented since it is exceptional to present with ischemic pathologies in the brain in neurosarcoidosis. In patients presenting with meningeal irritation findings, fever, headache and CSF analysis consistent with aseptic meningitis, generally tuberculosis is preliminary diagnosis. But cranial nerve involvement, particularly facial nerve, lesions in hypothalamus and pituitary glands, high ACE levels in serum, anergy to tuberculin skin test in addition to absence of acid resistant bacilli both in PCR and culture indicate another diagnosis like neurosarcoidosis. Usage of steroids in treatment of tuberculous meningitis leads to improvement of symptoms, which could sometimes be misleading about the diagnosis.

## Motor neuron disease

**P641**

**Genetics of amyotrophic lateral sclerosis in Yugoslav families.** Z. Stevic, D. Keckarevic, M. Parton, N. Leigh, A. Nikolic, S. Pavlovic, D. Blagojevic, A. Djarmati, A. Vujic, A. Radunovic, M. Spasic, S. Romac, S. Apostolski, University of Belgrade, King's College London, Institute for Biological Research (Belgrade, YU; London, UK)

Introduction: Mutations in the Cu/Zn superoxide dismutase (SOD1) gene have been linked to approximately 20% of all familial ALS (FALS) and 1–2% sporadic ALS (SALS) patients.

Objective: The present study presented the results of the SOD1 mutations screening in Yugoslav patients with sporadic and familial ALS and describe the clinical features of patients with SOD1 mutations.

Methods: One-hundred and seventy six blood samples from 133 sporadic, 30 FALS patients and 13 asymptomatic relatives of FALS patients were screened for mutations in SOD1 gene. Medical reports of all subjects were studied. The activities of the Cu/Zn superoxide dismutase in erythrocyte lysate in patients with SOD1 gene mutations were also determined.

Results: The most common SOD1 mutation was in exon 5, Leu144Phe. A total of 29 affected members of these families exhibited a relatively not severe phenotype with atypical clinical features including urinary disturbances, sensory and cognitive impairment. The mean age at onset of disease (± SD) was 53.8 ± 9.9 years (range 41–67) with the mean duration of disease (± SD) of 6.6 ± 3.1 years (range 3–20). The novel missense mutation, also in exon 5, Ala145Gly, was found in two families including 9 affected members, earlier onset, and shorter survival. The mean age at onset of disease was 34 ± 10.2 (range 25–53) with the mean duration of disease of 3.8 ± 2.0 (range 2–6). Despite a variability in age at onset and disease duration, the clinical pattern was uniform, with the onset in lower limbs, ascending progression and predominant lower motor neuron involvement in patients. In patients with this mutation we did not find any atypical clinical features.

Decrease of SOD activity was noted in patients with both mutations, as well as in 3 out of 9 asymptomatic carriers with Leu144Phe mutation.

Conclusion: Leu144Phe and Ala145Gly are the only two mutations that so far have been identified in Yugoslavia. This is the first report of the missense mutation Ala145Gly of SOD1 gene in exon 5. SOD activity of red blood cells was reduced in FALS patients with both mutations compared to controls. Moreover this reduction is more prominent in patients with Leu144Phe than in patients with Ala145Gly mutation.

**P642**

**Mitochondrial dysfunction in skeletal muscle of ALS-patients.** T. Grehl, J. Zange, S. Fischer, K. Mueller, J.-P. Malin, Clinic Bergmannsheil, German Aerospace Center (DLR e. V.) (Bochum, Cologne, D)

Introduction: A potential role of mitochondria in the pathogenesis of amyotrophic lateral sclerosis (ALS) is gaining increasing support. Several morphological abnormalities and impaired histoenzymatic respiratory chain activities have been described not only in ventral horn motor neurons but even in muscle biopsies of patients with ALS. Since there is no surrogat factor for the diagnosis of ALS a non-invasive method which reflects this important pathogenetic step in the course of the disease could be of great importance especially for clinical trials. Methods: Ten patients (3 women and 7 men, age range, 31–66 years; mean, 53 years) with sporadic ALS (SALS) were chosen from the population followed up at the ALS centre of the Neurological University Clinic "Bergmannsheil", Bochum. Patients had a disease duration of approximately 2 years and were classified as having probable to definite ALS. All patients had a primary spinal onset and were drug treatment free for three weeks before study entry. Controls were age-matched.

Muscle energy metabolism was monitored by Phospor-Magnetic-Resonance-Spectroscopy (31P-MRS) using a special protocol. Spectra were taken from the calf muscle during rest, isometric foot plantar flexion (30% maximum voluntary test force) performed under ischemic and aerobic

conditions and during the aerobic recovery. Spectra were evaluated for phosphocreatine (PCr), inorganic phosphate (Pi), ATP, intracellular pH (pHi) and the time-constants of aerobic PCr-recovery (_au) after both contractions. Results: Absolute force values at 30% MVC and PCr-consumption during both phases of contraction was significantly lower in patients than in controls. PCr/ATP ratio in resting muscle was higher in patients than in controls. No significant difference was found for _au-values between patients and healthy subjects.

Discussion: In means of 31P-MRS-data the phosphorylation potential represented by _au-values should be diminished due to the assumed mitochondrial defect in skeletal muscle of patients with SALS. But we could not demonstrate any pathological changes in mitochondrial respiratory function. Perhaps the force values have been too low; the very low PCr-consumption during contraction in some patients caused even drop outs in the determination of recovery time constants. On the other hand it has been shown before that the distribution of mitochondrial defects in muscle fibres of ALS patients could be heterogeneous or even absent in early-stage SALS. Furthermore under special unstressed conditions muscle cells in cultures seemed to be able to maintain their energy production despite impairment of mitochondrial electron transfer chain activity. One last point is the clinical heterogeneity of patients with SALS. Perhaps one can find changes in mitochondrial respiration in some patients but in general 31P-MRS seems not to be helpful to design e. g. new therapeutic trials.

**P643**

**Flail arm syndrome: a clinical variant of amyotrophic lateral sclerosis.** A. Czaplinski, A. Steck, P. Andersen, M. Weber, University of Basel, University of Umea, Cantonal Hospital (Basel, CH; Umea, S; St.Gallen, CH)

A 65 year old male patient presented with a six months history of symmetrical, slowly progressive wasting and weakness of his arms but no significant functional involvement of other regions. Past history was unremarkable.

Neurological examination at the time of presentation revealed symmetric, predominantly proximal wasting and weakness of both arms (especially of the infra/supraspinatus and deltoideus) leading to severe functional disability. The upper limbs adopted a characteristic position, with the shoulders slumped, and the arms, forearms, and hands pronated. Bulbar and leg mucles were not affected. Fasciculations were noted in the upper limbs. Deep tendon reflexes were absent in the upper and hyperactive without clonus in the lower limbs. The jaw jerk and abdominal reflexes were normal. Plantar responses were flexor bilaterally. Sensory functions were normal.

EMG showed signs of acute and chronic denervation and fasciculations in all limbs. Motor and sensory nerve conduction velocities were normal with no evidence of conduction block. Motor evoked potentials, cerebrospinal fluid examination and a MRI of the cervical spine were normal. There was no abnormal expansion of trinucleotide (CAG) repeat of the androgen receptor gene and no mutation in the SOD1 gene. IgM Anti-GM1 antibodies were negative. The revised El-Escorial criteria allowed the diagnosis of probable laboratory supported ALS. However, the overall clinical presentation with predominantly proximal muscular atrophy and weakness of the upper limbs and relative sparing of bulbar and leg muscles were consistent with the proposed clinical criteria of the so-called flail arm syndrome.

The term flail arm syndrome was coined 1998 by Hu et al., who described a subgroup of ALS patients with frank signs of lower motor neuron disease in the upper limbs and little or no involvement of bulbar and lower limb muscles in early stages of the disease. The presence of the upper motor neuron signs, different clinical evolution, and genetic testing allow to distinguish the flail arm syndrome from other motor neuron syndromes such as spinal muscular atrophy, Kennedy's disease, multifocal motor neuropathy, and monomelic amyotrophy. The much higher preponderance in males suggests that genetic factors linked to male sex predispose a proportion of patients to develop the flail arm syndrome. In our case as well as in other reported cases prognosis seems to be better than in typical Charcot ALS.

**P644**

**On the clinic, electrophysiology and treatment of neuromyotony (Isaacs-Mertens syndrome).** M. Muradyan, B. Ishpekova, M. Dasakalov, P. Stamenova, Tzaritza Joanna Medical Academy, University Hospital (Sofia, BG)

Neuromyotony is a rare disease, which is clinically characterized by muscular hypertony, myokymias, fasciculations, delayed muscular decontraction of some muscular groups and walking disorders. The constant activity of the muscular fibres shows a typical electromyographic picture. In the present report 18 cases with this syndrome are described, aged from 8 to 71 years. 10 of them are heredofamilial, these are brothers and sisters, the neuromyotonia in these patients being associated with inherited motor-sensory neuropathy (II type), in other 6 the neuromyotonia is associated with polyneuropathy of indeterminate etiology, and in the remaining 2 patients an additional neurological disease is not found. The needle electrode examination revealed an abnormal pattern of motor unit firing, consisting of myokymic discharges, doublets and multiplets, neuromyotonic discharges, and fasciculations. The treatment of the patients is carried out with carbamazepine in a dose of 400 to 1000 mg/24 hours. In all of the patients a considerable or full regression of the neuromyotony manifestations is reported, including the electromyographic symptoms. The temporary interruptions of the treatment have always resulted in recurrence of the clinical and electrophysiological symptoms of a constant muscular activity.

**P645**

**Motor neuron syndrome associated with inclusion body myopathy: two case reports.** A. Del Corona, C. D'Avino, G. Cafforio, M. Falorni, F. Galluzzi, B. Solito, S. Pistolesi, G. Fontanini, G. Siciliano, University of Pisa (Pisa, I)

Inclusion body myopathy (IBM) is a disorder usually presenting in patients older than 50 years, often causing asymmetric proximal and distal limb muscle involvement. Motor neuron disease (MND) is a progressive neurodegenerative disorder involving both upper and lower motor neurons. The pathogenesis of both diseases is still unknown. We report here on 2 cases with clinical presentation of upper and/or lower motor neuron involvement and histopathological diagnosis of IBM.

Case 1: a 68 year old man with a 18 month history of progressive trunk and limb weakness, fasciculations at four limbs, leg spasticity, dysarthria, dysphagia, urge incontinence and cognitive impairment. Electromyography (EMG) showed neurogenic signs and fasciculations on four limb and tongue muscles. Nerve conduction studies were normal. Motor Evoked Potential (PEM) revealed marked pyramidal tract involvement. Serum creatine kinase, cuprum, ceruloplasmin, iron, antiganglioside antibody titer and exercise blood exercise lactate were normal. Cerebrospinal fluid (CSF) examination showed slight increased level of proteins. Brain magnetic resonance imaging disclosed deposits of paramagnetic material in the basal ganglia bilaterally, associated with involvement of the cortico-spinal tract, while dopaminergic receptorial SPECT showed normal basal ganglia marker binding. Muscle biopsy revealed a neurogenic myopathy with rimmed vacuoles.

Case 2: a 64 year old woman with 20 month history of muscle weakness slowly progressing from upper to lower limbs. Neurological examination showed distal muscle weakness and wasting at the upper limbs, deep tendon hypo-areflexia. EMG showed neurogenic signs and fasciculations at four limbs. Nerve conduction studies were normal, but H reflex and F late responses were absent at lower limbs. PEM were normal. Blood antiganglioside antibody titer and thyroid hormones dosage were normal, as was CSF examination. Muscle biopsy revealed a neurogenic myopathy with scattered rimmed vacuoles.

These case reports, although not nosologically defined, confirm the clinical variability of the inclusion body myopathy and at the same time raise questions on the possible mechanisms of neuronal damage in motor neuron syndromes, in particular related to the pathogenic significance of the inclusion figures in this neurodegenerative disorder.

**P646**

**Amyotrophic lateral sclerosis associated with primary hyperparathyroidism.** M. Xifaras, I.E Markakis, E. Alexiou, T. Kostis, M. Vikelis, A. Basta, A. Tsakiris, Piraeus General State Hospital (Nikaia, GR)

Background: Primary hyperparathyroidism (PH) has been associated with a variety of neurological manifestations like disorders of mentation, proximal muscle atrophy with myopathic features and signs or symptoms of myelopathy. Its relation to amyotrophic lateral sclerosis (ALS) remains a controversial issue. Syndromes bearing a superficial resemblance to motor neuron disease usually show improvement or remain stable after successful treatment of the underlying condition. However definite or probable ALS cases (according to the El Escorial criteria) associated with PH show a relentless progression despite adenoma resection.

Objectives: We present a case of clinically definite ALS associated with PH.

Case-history: A 73-year old man presented with a 6-month history of progressive asymmetric leg weakness. On inspection there was atrophy of the right biceps femoris and gastrocnemius muscles. Fasciculations were present in all four limbs. Muscle strength of hip and knee flexors and plan-

tar dorsiflexors was 3/5 on the right and 4/5 on the left. Tendon reflexes were brisk but symmetrical in both upper and lower limbs. Hoffmann's sign was present in the left hand and plantar responses were extensor bilaterally. Language and cognitive functions were intact. There was no evidence of sensory impairment or sphincter abnormalities. Bulbar and cerebellar signs were absent. Magnetic resonance imaging of the spinal cord was normal. Nerve conduction studies were normal except a decreased amplitude of compound muscle action potentials. Needle electromyography revealed fasciculation and fibrillation potentials in the right gastrocnemius and biceps femoris, the right and left quadriceps femoris as well as both the right and left biceps brachii, extensor digitorum communis and abductor pollicis brevis. Laboratory studies were unremarkable except a serum calcium level of 13.1 mg/dl. Serum parathormone levels were 109 pg/ml. A nuclear parathyroid scan revealed a parathyroid adenoma. Despite successful surgical resection the patient's neurological condition continued to deteriorate. Walking became almost impossible and left hand weakness was added after a 6-month follow-up period.

Conclusions: Although the neuromuscular manifestations of PH usually improve following adenoma resection, when the clinical and electrophysiological criteria for ALS are fulfilled, treatment does not alter the progressive motor deterioration.

## Multiple sclerosis

**P647**

**Similar effects of interferon-beta and glatiramer acetate on novel matrix metalloproteinases.** F. Bernal, H. Pischel, H.-P. Hartung, B. C. Kieseier, Heinrich Heine University (Dusseldorf, D)

Interferon-beta and glatiramer acetate (GA) are the two main immunomodulatory drugs used in the treatment of multiple sclerosis (MS). Notably, while both ultimately decrease inflammation in the central nervous system, their mechanisms of action differ. While GA induces a shift in the cytokine pattern of autoreactive T cells, interferon-beta has potent activity at the blood-brain barrier and impairs the trafficking of inflammatory cells into the CNS by reducing the activity of matrix metalloproteinases (MMPs). MMPs represent a family of zinc-dependent endoproteinases implicated in the pathogenesis of MS. Although, there are more than 20 different MMPs known so far, most of the present studies focused on the expression of the gelatinases A and B (MMP-2 and -9). To extend our understanding of the mechanisms of action of standard MS immunomodulatory drugs we investigated their effects on the expression of newly described MMPs: MMP-19 (RASI), MMP-24 (MT5-MMP), and MMP-25 (MT6-MMP). Membrane type (MT)-MMPs have been suggested to be more effective than soluble forms at aiding cellular invasion of basal membranes. MMP-19 may also be an important player, as it exhibits a very broad spectrum of matrix substrates and has also been identified as an autoantigen in rheumatoid arthritis. Peripheral blood mononuclear cells (PBMC) from healthy donors and MS patients were stimulated with interleukin-2 or phytohemagglutinin in vitro and treated with interferon-beta and GA. mRNA expression levels of MMP-19, MMP-24, and MMP-25 were measured by a semiquantitative reverse transcription-polymerase chain reaction assay, and normalised to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Whereas MMP-24 was found to be constitutively expressed, increased mRNAs for MMP-19 and MMP-25 were detectable under stimulatory conditions. Treatment of stimulated PBMNC with interferon-beta and GA markedly reduced the expression of MMP-19 and MMP-25, but did not alter MMP-24 mRNA levels.

Our present data reveal common effects of interferon-beta and GA on the expression of these novel MMPs, widening their range of action.

**P648**

**Brain MRI in late-onset multiple sclerosis.** S. Delalande, E. Michelin, J. de Seze, J. Y. Gauvrit, M. A. Mackowiak, L. Defebvre, D. Ferriby, T. Stojkovic, J. P. Pruvo, P. Vermersch, CHU Lille (Lille, F)

Introduction: Multiple sclerosis (MS) with clinical onset after the age of 50 years is unusual (between 1 and 6 %) and is frequently misdiagnosed. Furthermore, we have frequently observed brain MRI abnormalities in elderly patients.

Aim: To describe brain MRI in late-onset MS and to evaluate the sensitivity and specificity of radiological MS criteria in patients aged over 50 years.

Methods: We evaluated brain MRI of 20 patients with onset of MS after the age of 50 years. We compared these MRI with 26 control subjects matched for age, sex and vascular risk factors. MRI were blindly analyzed by two neuroradiologists in respect to Paty's criteria, Fazekas' criteria and Barkhof's criteria.

Results: The mean age at MRI scan was 58 years. Sensitivity was 90 % for Paty's criteria, 80 % for Fazekas's criteria and 85 % for Barkhof's criteria. Specificity was 54 % for Paty's criteria, 69 % for Fazekas' criteria and 65 % for Barkhof's criteria. Accuracy was 69 % for Paty's criteria, and 74 % for Fazekas' criteria and Barkhof's criteria, respectively. Subcortical lesions were not specific to MS as they were also frequently found in the control group (65 % of control subjects).

Conclusion: The sensitivity of currently used criteria for MS was similar in our cohort compared to those reported in younger patients. Our results underline the poor specificity of Paty's criteria. Barkhof's criteria are less specific in older patients than in young patients. Subcortical lesions are unspecific. We suggest that spinal cord MRI should be systematically performed in late-onset MS because of its higher specificity compared with brain MRI.

**P649**

**HLA-DRB1 and DQB1 alleles in Brazilian patients with multiple sclerosis from the northeast region of the State of São Paulo.** D.B Souza, P. A. Donadi, A. A. Barreira, Hospital das Clínicas – Faculdade de Medicina, USP (Ribeirão Preto, BR)

The prevalence of multiple sclerosis (MS) delineates geographic areas of high, intermediate and low risk. The risk varies with latitude, although race seems to be the most determinant factor. Australia and New Zealand show different patterns of prevalence related with latitude gradient since the populations of these countries are ethnically homogeneous. Multiple sclerosis (MS) does not occur in the Black African population or has a very low frequency. Multiple sclerosis has been associated with the presence of DR15 antigen of the HLA system in certain populations, especially from North Europe. The present study is the first carried out in the northeast region of the State of São Paulo, Brazil, which a has peculiar migration pattern, with the finality of verifying a possible relationship between MS and the alleles of HLA system.

Forty eight [48] patients with MS type relapses and remissions (RRMS) and 5 with primary progressive MS (PPMS) according to Poser's criteria, and 114 healthy blood donors, without neurological symptoms were studied. The DNA was extracted using a salting out procedure and amplified by polymerase chain reaction (PCR) using sequence specific primers (SSP). Statistical analysis was performed using Fisher, Qui-square and Kruskal Wallis test

The DRB1*01, DRB1*15, DRB1*16, DRB1*3, DRB1*12, DRB1*13, DRB1*14, DRB1*07, DRB1*8, DRB1*9 and DRB1*10 alleles, in RRMS and PPMS showed the same frequency as in the control population. There was a significant difference between the DRB1*04 and DRB1*11 alleles, with a lower expected frequency in RRMS. The DRB1*12, DRB1*09 and DRB1*10 were not observed in our patients. The DQB1*05, DQB1*06, DQB1*02 and DQB1*04 alleles exhibited similar frequencies in patients and control individuals.There was a negative association between DQB1*03 allele and MS. The DRB1*01/DQB1*05, DRB1*15/DQB1*06, DRB1*16/DQB1*05, DRB1*0301/DQB1*02, DRB1*04/DQB1*08, DRB1*08/DQB1*04, DRB1*013/DQB1*06, DRB1*14/DQB1*5 haplotypes showed similar frequencies in control individuals and MS patients.

A negative association between DRB1*11/DQB1*07 haplotype and MS was observed. The haplotype DRB1*07/DQB1*02 was positively associated with EM.

We did not confirm the presence of the most common antigens observed in the Caucasian population (DR15). Our findings differ from other studies with White and Afro-brazilian patients. The DRB1*07/DQB1*02 association with the development of EM has not yet been previously reported.

**P650**

**Increased myoinositol in the NAWM of clinically isolated syndromes.** K. T. M. Fernando, C. M. Dalton, D. T. Chard, S. M. Leary, K. M. Miszkiel, D. Altmann, M. A. McLean, D. G. MacManus, G. T. Plant, A. J. Thompson, D. H. Miller, Institute of Neurology for the NMR Research Unit (London, UK)

Abnormalities detectable by proton magnetic resonance spectroscopy (MRS) occur in the white matter of patients with established Multiple Sclerosis (MS) in areas that appear normal on conventional T2 weighted MRI. The stage at which these abnormalities first occur is less clear. The aim of this study was to investigate this by looking at the normal appearing white matter (NAWM) of patients with clinically isolated syndromes (CIS) suggestive of MS using single voxel proton MRS.

Methods: Eighty nine CIS patients had a baseline MRI study of the brain and cord within 12 weeks of presentation (mean 5.5 weeks). They then underwent a combined MRI/MRS study of the brain 12 weeks after the baseline scan (mean 13.2 weeks). 44 healthy controls were also studied. A single voxel spectrum was acquired from NAWM in the posterior parietal/centrum semi-ovale region. The MRS acquisition was a PRESS sequence with TR 3000ms, TE 30ms and 192 averages. The spectra were fitted with the full set of metabolites thought to be present at short TE. Concentrations of the following were estimated using the LC Model; N-acetyl aspartate (NAA), total NAA, Creatine, Choline and Myoinositol (Ins). Differences between patients and controls were assessed by multiple regression of metabolite concentrations on a binary disease status variable and age and gender covariates to control for potential age/gender confounding.

Results: Seventy six of the CIS patients presented with optic neuritis, 8 with brainstem syndromes and 5 with spinal cord syndromes. The median EDSS was 1 (range 0–6). 26/89 CIS patients had normal T2 weighted MRI scans of the brain at baseline. Of the remaining 63 patients, 46 had 1–20 T2 lesions and 17 had more than 20 T2 brain lesions. 26 patients fulfilled McDonald criteria for MS at 3 months. Ins was significantly raised in the NAWM of all CIS patients compared with the control group (mean 4.26mM, SD1.28 vs mean 3.89, SD1.03, $p = 0.028$) and in those with abnormal MRI at baseline (mean 4.29 mM, $p = 0.026$). This increase was more strongly significant in those who satisfied McDonald criteria for MS at 3 months (mean 4.43mM, $p = 0.016$), or had more than 20 T2 lesions at baseline (mean 4.62mM, $p = 0.007$).

Conclusions: This study demonstrates an increase in NAWM Ins of patients shortly after presentation with a CIS. The increase was associated with other MRI measures of lesion extent and disease activity.

**P651**

**Semantic clustering impairment in relapsing-remitting multiple sclerosis.** G. Zappalà, D. Smirni, A. Falsaperla, D. Maimone, Neurology Division (Catania, I)

Background: Cognitive disorders are very common in multiple sclerosis (MS) with an overall estimated frequency of about 43% (Rao et al. 1991). Patients with cognitive impairments most commonly display disturbances of learning and memory, verbal fluency, executive functions as well as motor dexterity and problem solving, both for the relapsing-remitting (RRMS and progressive forms of MS. Recently, numerous test instruments have been implemented to quantify and qualify such cognitive disturbances, most of which relying upon the investigation of learning and memory performance of MS patients.

California Verbal Learning test (CVLT) is a newly developed neuropsychological tool, ecologically valid, which allows to collect information about "how" we acquire information and "how" we recall such information. We used such instrument as part of a longer test battery with a group of RR MS patients aiming at demonstrating more qualitative aspects of learning impairment in MS.

Method: Ninenteen RR MS patients (mean age 34.3 yrs; Females/Male ratio 4:1) were administered a neuropsychological battery including CVLT and their performance was later compared to that of normal subjects matched for sex, age and education, pooled from a larger sample of normative data. Their disease duration was 6.8 years (s. d. = 5.2), current average EDSS was 1.9 (s. d. 1.3; number of relapses was 3.6 (1.8) on the average.

Results: Memory performance of SM patients was equivalent to that of normal case-matched controls (number of total correct words recalled over five trials, number of words recalled after an interference list, and number of recognized words). However MS patients' performance was significantly impaired in all qualitative aspects of verbal learning, including semantic-strategical clustering and organizational ability of learned words. Total Clusters during learning trials were 15.2 for MS patients and 24.2 for normals ($p = 0.009$). Performance on number of clusters on short and long-term free recall were as well highly significantly different among the two groups (3.8 and 4.1 for MS patients; 7.0 and 6.9 for normal controls; $p = 0.003$ and 0.005 respectively).

Conclusions: Our study corroborates the findings of those who have found cognitive disturbances in MS patients, especially in learning and memory. Further we have found a dissociation betwen quantitative (number of words learned and recalled from a list) and qualitative aspects of memory performance (strategical approach to the learning test and clustering words ability) which is heavily affected in MS patients. CVLT is proved to be a very sensitive test instrument to differentiate between MS patients and normal controls and gives more information about the strategic aspects of learning and remembering of such patients. Qualitative aspects of memory performance could as well be more valid measures of investigation in the development of new drug trials and/or rehabilitative efforts in the field.

**P652**

**Multiple sclerosis after age 50 years.** B. Topcular, M. Kurtuncu, F. Bilgili, G. Akman Demir, M. Eraksoy, Istanbul University (Istanbul, TR)

Multiple sclerosis (MS) is primarily a disease of young adults with the mean age at onset 30 years. The onset of MS is also known to occur after age 50 years even into the eight decade.

The aim of this study is to reveal clinical, demographic, magnetic resonance imaging and cerebrospinal fluid findings of the late onset of MS (LOMS) (> 50 years).

The incidence of the LOMS was 1.2% in a clinic-based MS cohort (27/2286 patients in January 2003). All the patients had clinically definite multiple sclerosis according to Poser and co-worker's clinical criteria. The mean age at onset was 55.3 (range 50–65 years). There were 16 women and 11 men in this group (W/M ratio: 1.4:1).

The most frequent initial manifestations were sensory-motor ($n = 9$), brain stem ($n = 7$), optic neuritis ($n = 5$), motor ($n = 5$) and cerebellar ($n = 1$). The patients had relapsing-remitting ($n = 11$), relapsing progressive ($n = 9$) and primary progressive ($n = 7$) course. The mean EDSS score was 4.8 (range 1.0–6.5) at 2003 with the mean 8.2 years disease duration (range 2–22 years). The progression index was 0.7 in January 2003 (range 0.2–2.5).

Brain MRI examination was performed in all patients and the findings met the Paty's radiological criteria in 19 out of 27 patients. Oligoclonal bands were positive in 10 patients in whom cerebrospinal fluid was investigated.

The differential diagnosis of MS might be difficult in patients with LOMS. They tend to have a chronic progressive course from onset similar to many degenerative disorders seen in older age such as osteoarthritic myelopathy or system degenerations which can occasionally mimic MS. Sometimes cerebrovascular disease may be important in differential diagnosis.

We concluded that clinical, MRI and CSF findings of LOMS seemed to be similar to early and adult onset MS patients except for progressive-relapsing and primary progressive course were more common in LOMS group.

**P653**

**Evidence of association between paraoxonase 1 gene polymorphism (M55L) and multiple sclerosis.** K. Aggelakis, E. Dardiotis, D. Papadimitriou, A. Nikolaidou, K. Flabouriari, P. Kolia, A. Papadimitriou, G. Hadjigeorgiou, University of Larissa (Larissa, GR)

Multiple sclerosis results from an interplay between as yet unidentified environmental factors and susceptibility genes.

The human serum paraoxonase (PON1) is an esterase closely associated with HDL in the plasma that is involved in the detoxification of non-physiological substrates like pesticides. PON1 is coded by a gene located on chromosome 7q21.3–22.1 in a cluster with two similar genes, PON2 and PON3. A PON1 polymorphism at position 55 involves a methionine (M allele) and leucine (L allele) interchange and affects the enzyme-protein concentration. So far, the PON1–55M/L polymorphism has been associated with two disease multifactorial in etiologys, cardiovascular diseases and Parkinson's disease.

Using standard methods (PCR/RFLP) we studied, for the first time, the association of the PON1–55M/L polymorphism with MS. We analyzed DNA from 116 Greek patients (80 women and 36 men) with clinically definite MS and from 144 age- and sex-matched controls. Patients and controls were from Thessaly district (Central Greece), a mainly agricultural area. Thirty-five patients with relapsing remitting and 57 patients with secondary progressive MS were classified as bout-onset ($n = 92$) whilst 24 patients referred progressive-onset. The age-at-onset was $28.9 \pm 8.3$ (mean $\pm$ SD) years for the patients with bout-onset and $38.0 \pm 6.7$ years for the patients with progressive-onset whilst the disease duration was $10.5 \pm 8.6$ years and $7.8 \pm 4.6$ years respectively. The EDSS score was $4.6 \pm 2.1$ for patients with bout-onset and $4.2 \pm 1.4$ for patients with progressive-onset. The possible association of the PON1–55M/L polymorphism with MS was tested using the Fisher s exact test.

Results: The distribution of PON1–55 genotypes (MM, ML, and LL) and alleles (M and L) was statistically different between MS patients and healthy controls ($P = 0.03$ and 0.01 respectively). In particular the frequency of genotypes and alleles was as follow: a) Patients: MM = 17.2%, ML = 46.6%, LL = 36.2%, M = 40.5, and L = 59.5; Controls: MM = 27%, ML = 51.4%, LL = 21.6%, M = 52.7%, and L = 47.3%). Although the stratification for disease type of onset provides evidence that the association is limited to the patients with bout-onset (for genotypes, $p = 0.01$; for alleles, $p = 0.02$), this has to be interpreted with caution due to the limited number of patients with progressive onset.

Conclusions: We provide evidence that the PON1–55M/L polymorphism is associated with MS patients.

**P654**

**Effect of immunomodulators for multiple sclerosis therapies on cytokine production in healthy volunteers.** D. Reske, H. F. Petereit, University of Cologne (Cologne, D)

Background: Methylprednisolone (MP), interferon beta-1b (IFNB), glatiramer acetate (GLAT) and immunoglobulins (IGG) are well established therapies in multiple sclerosis (MS) at different stages of the disease. Beyond others, one mechanism seems to be the influence on cytokine production by these drugs. With this study we investigated the effect of these different therapies on cytokine production in a comparative study design.

Materials and methods: The in-vitro effect of MP, IFNB, GLAT and IGG on interferon gamma (IFNG) production in CD4 + and CD8 + cells as well as the interleukin 10 (IL10) and tumour necrosis factor alpha (TNF) production in CD3 + cells was measured by flowcytometry in 10 healthy individuals. Additionally, interleukin 12 production in CD14 + cells was measured. The cells were stimulated with phorbolmyristate acetate and ionomycine or LPS and interferon gamma in the presence or absence of the different therapies.

Results: IFNB leads to a significant reduction of IL12 producing monocytes ($p = 0.005$). The proportion of IL10 producing CD8 + lymphocytes and of IFNG in CD4 + cells showed a trend to enhanced production ($p = 0.016$ and $p = 0.059$). The other cytokines were unchanged.

The co-incubation with GLAT resulted in a significant reduction of CD8 + IFNG and CD14 + IL12 producing leucocytes ($p = 0.007$ and $p = 0.005$). The other cytokines demonstrated no significant changes beside the proportion of IL10 producing CD3 + cells, showing a trend to an enhanced production ($p = 0.047$).

IGG led to a significant reduction of CD4 + IFNG producing lymphocytes ($p = 0.005$). In addition, the proportion of IL12 producing monocytes was reduced ($p = 0.005$).

MP reduces the proportion of IL12 producing CD14 + monocytes ($p = 0.005$). The effect of MP on TNF production demonstrated a trend towards a reduced proportion of TNF producing cells ($p = 0.013$). The changes of IFNG in CD8 + cells showed a trend to a reduction ($p = 0.037$).

Conclusion: The presented study demonstrates the cytokine regulatory effect of different immunomodulatory agents used in the treatment of MS (MP, IFNB, GLAT, IGG). All drugs lead to a significant reduction of IL12 producing monocytes. Beside this unspecific treatment effect, the investigated agents demonstrate distinct patterns of cytokine production in subsets of peripheral mononuclear cells. In summary the investigated drugs are able to influence the immunoregulatory mechanisms at different stages of the cytokine cascade in healthy volunteers in-vitro.

**P655**

**Cerebrospinal fluid tau protein levels in multiple sclerosis.** S. Arda, A. Claus, M. Maier, H. Tumani, University of Ulm (Ulm, D)

Background: Axonal damage has been described in patients with multiple sclerosis (MS) by various histopathological and neuroimaging methods. Moreover axonal damage is the morphological correlate for nonreversible clinical deficits. Tau protein (tau) is a phosphorylated microtubule protein primarily located within neuronal structures. Elevated tau levels in cerebrospinal fluid (CSF) may be an additional tool to detect axonal damage in MS.

Goal: To investigate CSF tau levels in different disease stages.

Patients and Methods: CSF tau concentrations were determined using an ELISA, which recognizes normally phosphorylated and unphosphorylated tau. CSF samples were obtained from 30 non-neurological controls and age-matched 91 patients diagnosed according to the McDonald criteria. Subgroups were defined on the basis of the clinical course in CIS (clinically isolated syndrome; $n = 53$), relapsing-remitting (RR; $n = 29$) and secondary progressive MS (SP; $n = 9$).

Results: CSF tau ranged broadly in patients with MS. Highest levels could be detected in patients with first manifestation (CIS) of multiple sclerosis (median = 236 pg/ml), while patients with signs of secondary progression (SP) showed lower values (median = 190 pg/ml). No significant difference could be detected between RR vs. CIS and RR vs. SP, respectively. The lowest tau levels were detected in non-neurological controls with a significant difference between CIS patients and the controls ($P = 0.004$). There was a mild but no significant correlation between tau levels and disease duration (Spearman rank correlation, –0.96; $P < 0.184$).

Conclusion: Elevated CSF tau levels indicating axonal damage occur in early stages of MS before persistent clinical deficits become apparent. Follow up studies will show whether CSF tau levels are of use for prognostic evaluation of disease activity and progression.

**P656**

**Short-term evolution of white and gray matter brain damage in untreated relapsing-remitting multiple sclerosis patients: an in vivo study using diffusion tensor MRI.** C. Oreja-Guevara, M. Rovaris, D. Caputo, R. Cavarretta, M. Sormani, P. Ferrante, G. Comi, M. Filippi, Neuroimaging Research Unit, Scientific Institute Fondazione "Don Gnocchi", Department of Neurology, HSR (Milan, I)

Diffusion tensor (DT) MRI has the potential to elucidate in vivo many characteristics of tissue microstructure that are inaccessible to other MR-based techniques. In multiple sclerosis (MS) patients, the diffusion characteristics of the normal-appearing brain tissues are consistent with the presence of "occult" structural damage of varying severity. With the present study, we aimed at assessing whether DT MRI is sensitive to longitudinal changes of brain damage which may occur at a level beyond the resolution of T2-weighted images in untreated relapsing-remitting (RR) MS patients.

Twenty-six patients with RRMS were followed-up for 18 months with three-monthly MRI scans. None of the patients had had chronic immunosuppressive or immunomodulating treatments before or during the observation period. At each time-point, we obtained dual-echo, DT and post-contrast T1-weighted MRI scans of the brain. Mean diffusivity (MD) and fractional anisotropy (FA) histograms of normal-appearing gray (NAGM) and white matter (NAWM) were produced. Total T2-hyperintense and T1-hypointense lesion volumes (LV), normalized whole brain, gray matter and white matter volumes, average lesion MD and FA were also calculated. A random effect regression model was used to evaluate the time trend of histogram-derived metric and LV evolution. Bivariate correlations were assessed using the Spearman rank correlation coefficient.

Over the study period, a significant decrease of average lesion FA, NAGM FA and MD histogram peak heights and a significant increase of average NAGM MD and T2-hyperintense LV were observed. No mutual correlations were found between the observed percentage changes of the individual parameters. There were no significant differences as regards the percentage changes of LV and histogram-derived metrics between patients who did and those who did not experience clinical MS relapses during the study period.

DT MRI is sensitive to microstructural changes occurring in the NAGM of untreated RRMS patients. This sounds promising for its use to provide paraclinical markers reflecting the evolution of MS brain damage with greater pathological specificity than conventional MRI-derived measures.

**P657**

**Reduction dose study 1 year after.** P. Barbero, M. Bergui, E. Verdun, A. Pipieri, M. Clerico, A. Ricci, M. Iampaglia, C. Coppo, L. Durelli, University of Turin (Turin, I)

Objective: To evaluate clinical and MRI signs of disease activity 1 year after the increase of the IFN beta dose and frequency of administration.

Background: Patients with relapsing remitting (RR) multiple sclerosis (MS), that after over 3 years of every-other-day 250 mcg interferon (IFN) beta-1b with clinical and MRI absence of disease activity, were switched to once-weekly 30 mcg IFN beta-1a dose for 1-year. Most patients showed disease activity resumption.

Design and methods: Patients were switched to multiple-weekly administration IFN beta (6 to IFN beta-1b, 4 to 44 and 3 to 22 mcg IFN beta-1a). Clinical and laboratory assessments were repeated every 3 months, MRI after 1 year.

Results: One year after IFN beta dose and frequency of administration increase outcome measures were: relapse rate, $0.3 \pm 0.48$; and proportion of patients with exacerbation, 30.7 %, with MRI activity 53.8 %, with new PD/T2 lesions 53.8 %, with enhancing lesions 38.4 %. Mean number of new PD/T2 lesions was $1.2 \pm 1.5$; and of enhancing lesions, $1 \pm 2$. Relapse rate and proportion of patients with exacerbation, compared with the year before (that is the period of low dose/low frequency IFN beta) were the only two outcome measures that were decreased significantly ($p < 0.05$).

Conclusions: The reduction of IFN beta dose and frequency of administration is associated with increased disease activity that persisted even after returning to the high dose/high frequency of administration protocol. Resumption of disease activity and long disease duration may render patients less sensitive to IFN beta.

**P658**

**Interleukin-6 decreases in cerebrospinal fluid of multiple sclerosis patients during one-year glatiramer acetate treatment.** D. Obradovic, D. Vojvodic, E. Dincic, R. Raicevic, Military Medical Academy (Belgrade, YU)

The role of interleukin-6 (IL-6) in multiple sclerosis (MS) is rather controversial. It is considered either as inflammatory or down-regulatory cytokine. Recent studies favour its inflammatory properties and upregulation of IL-6 mRNA expression is found in cerebrospinal fluid (CSF) of MS patients. The role of glatiramer acetate (GA) in MS treatment is well established and shifting toward Th2 cytokine production is one of proven modes for action of GA. There are no data concerning possible changes of IL-6 during GA treatment, so it's been our aim to determine if possible changes of IL-6 are related to clinical outcome during one year treatment.

In 12 remitting-relapsing MS patients (8 females, 4 males; mean age 35 ± 2,6 years; mean EDSS 4), CSF and plasma were obtained prior to treatment and after 3, 6 and 12 months of GA therapy. At the same time interval EDSS was recorded in each patient. IL-6 were measured in CSF and plasma by ELISA method.

Pretreatment annual relapse rate was 0.95 and during the treatment it declined to 0.25 ($p < 0.01$). Clinical improvement was noted in 3 patients with mean EDSS reduction per 1.5 point, 7 patients were stable and 2 worsened with mean EDSS deterioration of 1.5 point. CSF concentrations of IL-6 were higher compared to plasma concentrations. During the treatment, IL-6 in CSF showed transitory, nonsignificant increase, after the first three months (pretreatment value 1.43 ± 0.6 pg/ml, after the 3 months 1.8 ± 0.6 pg/ml). Significant decrese was noted after 6 months (1.06 ± 0.3 pg/m, $p < 0.03$) and after twelve months (0.86 ± 0.3 pg/m, $p < 0.05$), with IL-6 concentration being almost 2-fold lower compared to pretreatment one. In plasma, IL-6 also decreased during the treatment – pretreatment value was 0.8 ± 0.3 pg/ml, while after 3, 6 and 12 months IL-6 concentrations were 0.6 ± 0.2 pg/ml, 0.4 ± 0.2 pg/ml and 0.3 ± 0.1 pg/ml respectively. IL-6 in CSF and plasma positively correlated with clinical outcome.

Local production of IL-6 in CNS by residential cells could explain higher concentrations of IL-6 in CSF compared to plasma. Our data have shown GA antagonizing effects on IL-6 in relation to favorable clinical outcome, apart from well known GA functions towards Th2 cytokine shift. Importance of IL-6 decrease in CSF during GA treatment is underlined by its higher concentrations in CNS compared to plasma.

**P659**

**Neutralizing antibodies to interferon beta in multiple sclerosis: impact of dosage on therapeutic strategy.** C. Giannesini, O. Heinzlef, D. Pez, P. Lebon, E. Roullet, Hôpital Tenon, Hôpital Saint-Vincent de Paul (Paris, F)

Background: Interferon beta (IFN-b) had proved its efficiency on relapse rate reduction and on magnetic resonance imaging (MR) activity in multiple sclerosis (MS). But this efficiency is only partial. It is impossible to know before starting the treatment whether the patient will be a good responder or not. Moreover, IFN-b induces neutralizing antibodies (NAB) which could be associated with reduced clinical and MR efficiency.

Objectives: the aim of this study is to evaluate how NAB development influences any treatment decision for non responder patients.

Methods: Between January 2002 and January 2003, patients treated with IFN-b and considered by their neurologist not to respond to the treatment, were evaluated for the presence of NAB. A blood sample was collected at least 7 days after the end of the treatment and tested in the same specialised laboratory by a viral cytopathic effect assay.

Results: 29 patients (22 women, 7 men), mean age 37.7 range 24–65) were evaluated for the presence of NAB: 23 of which with relapsing remitting MS (RR-MS) (19 women, 4 men) mean age 34.6 years (range 24–51), mean disease duration 8 years (range 3–25) and 6 with secondary progressive MS (SP-MS) (3 women, 3 men) mean age 50.7 years (range 38–65), mean disease duration 11 years (range 3–26)). The treatment started after a mean time of 60 months (range 9–276) for RR-MS patients and 103 months (range 36–246) for MS-SP patients. The reasons for NAB evaluation were:

1-relapse occurrence for 23/23 RR-MS and 5/6 SP-MS patients

2-disability progression for 1/23 RR-MS patients and 4/6 SP-MS patients. Among these 29 patients, 9 were positive for NAB (NAB +), 8 in RR-MS group and 1 in SP-MS group. In MS-RR group, treatment was modified for 7/8 NAB + patients and for 5/15 NAB– patients ($p < 0,05$). Because of cross reactivity of NAB between the three different IFN-b, another drug class was selected for NAB+ patients. 5 NAB– patients stopped INF-b because of adverse drug reaction. Moreover, the RR-MS NAB + group seems to be different from NAB– group considering disease activity the year before and during the first year of treatment. The test for occurrence of NAB was performed earlier in the RR-MS NAB + group: mean delay 27 months [12–34] vs 37 [3–76]. In the SP-MS group, the only one patient NAB + stopped IFN-b and 2/5 NAB– patients received immunosuppressive drugs because of significant disability progression.

Conclusion: Presence of NAB when patient is considered to be no responder to IFN-b is significant for treatment decision, so test for NAB should be performed systematically for no responder patients to IFN-b therapy, in order to evaluate impact on therapeutic strategy.

**P660**

**Symptoms of depression in patients with multiple sclerosis and spinal cord injuries.** D. Christidis, A. Delipalta, M. Paschalidou, I. Milonas, Aristotle University of Thessalonica, AHEPA Hospital (Thessalonica, GR)

The aim of this study was to compare depressive symptoms in patients with multiple sclerosis (MS), mainly with spinal cord involvement individuals with spinal cord injuries (SCI) and healthy individuals (control group (CG)).

Depressive symptoms were assessed using the Greek version of the Beck Depression Inventory-2nd edition (BDI-II, 1994), a well accepted instrument for investigating depression in various populations.

Thirty patients with MS (15 men, 15 women), twenty persons with SCI (16 men, 4 women) and thirty healthy controls (15 men, 15 women) participated in this study. These three groups did not differ statistically with respect to their age ($M = 39$, $SD = 9.29$) and education status ($M = 10$, $SD = 2.92$). Moreover, the two groups (MS and SCI) had the same level of independence, as it was evaluated with the Daily Activity Scale (Randall et al. 2000)

The results illustrated that the BDI-II scores for the MS and the SCI groups were significantly higher than the controls ($p < 0.05$), whilst amongst them (MS and SCI) they did not reach statistical significance ($p > 0.05$)

**P661**

**A survey on the pharmacodynamic profile of interferon beta-1a (Avonex®) in multiple sclerosis.** G. Kalski, P. Schicklmaier, A. Richter, C. Wernsdörfer, Biogen GmbH (Ismaning, D)

Objective: To investigate neopterin and beta2-microglobulin induction by interferon beta-1a (IFN beta-1a) in 74 patients with rr multiple sclerosis (MS).

Background: IFN beta is widely used for the treatment of multiple sclerosis. It induces the production of many molecules, including neopterin and beta2-microglobulin, which have been used to test the in vivo biological activity of beta-interferons. To date most of the analyses have been short term during periods of up to six weeks. We investigated the induction of neopterin and beta2-microglobulin in patients treated with IFN beta-1a for periods up to 48 months.

Design/Methods: Seventy-four patients with rrMS were examined. All patients received 30 mcg of IFN beta-1a intramuscularly (AVONEX(R); Biogen, Inc., Cambridge, MA). Blood levels were analysed once for each patient at baseline (predose) and approximately 48 hours after the injection. Serum neopterin and beta2-microglobulin were assayed using a commercially available kit according to the manufacturer's instructions.

Results: The mean baseline concentration of neopterin was 6.35 nmol/l (median 5.49 nmol/l). Serum neopterin concentrations were increased from baseline predose levels to 11.04 nmol/l (median 9.95 nmol/l). Accordingly the mean baseline concentration of beta2-microglobulin was analysed (1.25 mg/l; median 1.19 mg/l) and the increase at 48 hours postinjection was determined as 1.52 mg/l (mean; median 1.45 mg/l). 9 of the 74 patients did not show an increase of the examined interferon induced proteins. Subgroup analyses with regard to duration of treatment (treatment ≤ 2 years, treatment > 2 years) show that the mean induction rate of neopterin, and to a lesser extent of beta2-micoglobulin, seems to decrease with duration of treatment. (Further analyses and tables and charts of the existing data will be presented.)

Conclusions: Once-weekly administration of IFN beta-1a resulted in an increase of the serum levels of neopterin and beta2-microglobuline. Nevertheless, data regarding beta2-microglobuline as a biological marker seem to be less marked than data regarding neopterin. This increase can be regarded as an indirect measure for the absence of neutralizing antibodies. As patients were included with treatment up to 48 months, we can state that an increase of interferon-induced proteins was detectable for at least 4 years.

P662
**The relation of cellular CSF changes to MRI parameters.** B. Rosche, S. Cepok, J. Shiratori, S. Bien, N. Sommer, B. Hemmer, Neuroimmunology Group, Department of Neuroradiology (Marburg, D)

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) with as yet unknown etiology. Inflammation is also observed in the cerebrospinal fluid (CSF) of MS patients involving the occurrence of oligoclonal IgG bands and mild pleocytosis. We previously demonstrated heterogeneity of cellular CSF changes in MS patients with respect to the distribution of B cells and monocytes. In the current study we investigated the relation between CSF cells, immunoglobulin levels and MRI findings in 50 MS patients. Spinal taps were performed in timely relation to the cranial MRI examination. CSF cells and antibodies were analyzed by flow cytometry and nephelometry. We confirmed the heterogeneity of cellular CSF changes in MS patients with highly variable numbers of monocytes, B cells and plasma cells. The number of B cells and plasma cells strongly correlated with the extent of the intrathecal IgG synthesis. The relation of B cells and monocytes was stable over time, whereas the absolute cell numbers of each cell type varied significantly throughout the disease course. None of the cellular and humoral parameters showed a strong correlation with the MRI parameters. When we compared patients with and without Gadolinium-enhancing lesions we found that the absolute numbers of B cells, T cells and most notably plasma cells were increased in patients with active lesions whereas the absolute number of monocytes did not differ between both groups. Our findings support the concept of heterogeneity in the immunopathogenesis of MS and suggest that cellular CSF changes may reflect ongoing acute CNS inflammation.

P663
**Albumin ratio and IgG index changes in multiple sclerosis patients during glatiramer acetate treatment.** D. Obradovic, R. Raicevic, E. Dincic, S. Popovic, Military Medical Academy (Belgrade, YU)

Albumin ratio (AR) is the marker of blood-brain barrier integrity while IgG index is the marker of intrathecal IgG synthesis. Both parameters might be considered as useful markers of disease activity. The role of glatiramer acetate (GA) in multiple sclerosis (MS) treatment is well established, but since no data are available concerning changes of AR and IgG index during GA treatment, it was our aim to determine if possible changes of those parameters are related to clinical outcome.

In 12 remitting-relapsing MS patients (8 females, 4 males; mean age $35 \pm 2{,}6$ years; mean EDSS 4), cerebrospinal fluid (CSF) and plasma were obtained prior the treatment and after 3, 6 and 12 months of GA therapy. At the same time interval EDSS was registered in each patient. AR was calculated as ratio between CSF and serum albumin (normal value < 5,7) and IgG index was calculated by using formula IgG CSF/albumin CSF/IgG serum/albumin serum (normal value < 0.7).

Pretreatment annual relapse rate was 0,95 and during the treatment it declined to 0,25 ($p < 0{,}01$). Clinical improvement was noted in 3 patients with mean EDSS reduction per 1.5 point, 7 patients were stable and 2 worsened with mean EDSS deterioration of 1.5 point. Pretreatment mean value of AR was $5.5 \pm 1$, after 3 months $6.2 \pm 1.6$, after 6 months $3.8 \pm 0.7$ ($p < 0.05$) and after 12 months $6.8 \pm 1.8$. Pretreatment mean value of IgG index was $0.7 \pm 0.2$, and after 3, 6 and 12 months those values were $1.1 \pm 0.3$, $1.1 \pm 0.2$ and $1.1 \pm 0.2$ ($p < 0.05$) respectively. No correlation was found between AR and EDSS, while IgG index positively correlated to EDSS changes.

Observed increase of IgG intrathecal synthesis and positive correlation with clinical outcome might be explained by benefical effect of GA on reparative immunoglobulines production within the CNS. This is one of proposed, but still not confirmed beneficial functions of GA.On the other hand, GA seems to improve BBB integrity after the 6 months of treatment, but this effect hasn't been sustained after 12 months.

P664
**Follow-up study of multiple sclerosis patients treated with glatiramer acetate.** G. Jakab, Uzsoki Hospital on behalf of the Copaxone Follow-up Study Group

Goal: To evaluate the sustained therapeutic effect of glatiramer acetate (Copaxone) on the clinical activity of multiple sclerosis (MS).

Patients and methods: 193 patients with relapsing-remitting MS (RRMS) started Copaxone treatment in 2001. 6 of them dropped out due to various reasons (gravidity, progression, side effects). The data of remaining 187 patients (male: 45 (24%), female: 142 (76%)) were analyzed using non-parametric methods. The age of patients ranged between 18 and 54 years (41.1"b8.4 year), the EDSS was below 5.5 in all cases. Patients were controlled every three months for two years. The numbers of relapses, relapse-free patients, days of MS-related admissions in hospital, systemic and injection site adverse reactions were documented during the year before immuno-modulatory treatment, and for two years on Copaxone treatment.

Results: The advantageous changes of these clinical parameters developed in the first year of treatment and remained stable in the second year. A significant decrease in the number of total relapses was found already in the first year of treatment (69 versus 170, $p < 0.05$). The number of relapse-free patients increased from 81 to 154 (the difference is significant, $p < 0.05$). Patients spent significantly less days in neurological wards due to MS symptoms (mean 11.9 vs. 7.9 days, the difference is significant, $p < 0.05$). A continuous decline was seen in the number of systemic adverse reactions (data shown in 6 month periods: 32–13–8 and also in the frequency of injection-site reactions 53–46–23).

Conclusion: The glatiramer acetate treatment significantly reduced the clinical activity of MS already in the first year of treatment, and this tendency continued in the second year of follow-up. The injections were tolerable, the frequency of adverse reactions decreased gradually.

P665
**Predictive value of optic neuritis for future development of multiple sclerosis: report of 236 cases from Iran.** A. Soltanzadeh, A. Jahansooz, Tehran University of Medical Sciences (Tehran, IR)

Introduction: Optic neuritis (ON) could precede multiple sclerosis (MS) even years before the development of other manifestations of MS.

Methods: During a 12-year period [1988–2000], 236 cases with ON were followed in terms of the development of MS. All patients underwent clinical examination and brain magnetic resonance imaging (MRI) at the time of ON and after the development of MS. Those who had neurologic manifestations of MS in addition to ON during the first attack, were excluded from the study.

Results: Female to male ratio was 4. Fifty-four percent of the patients developed MS during the follow-up period, 88% of whom suffered more than one attack of ON. Left eye involvement was more common than right eye. Family history of MS was positive in 15.4% of patients. Twenty-seven percent of the subjects mentioned severe emotional stress before the onset or relapse of ON or MS. Patients whose brain MRI had shown demyelinating plaques at the time of ON attack, were more likely to develop MS than those without plaques.

Conclusion: Isolated optic neuritis could be a harbinger of multiple sclerosis in a significant proportion of patients.

P666
**Botulinum toxin: a new therapy for essential tremor in multiple sclerosis.** R. Clerici, A. Cappellari, M. De Riz, M. Ronzoni, E. Scarpini, P. Baron, G. Conti, N. Bresolin, IRCCS Ospedale Maggiore Policlinico (Milan, I)

Although Botulinum Toxin (BTX) has been in widespread clinical use for some thirty years, its effect on tremor cannot be yet established. The best outcome is observed treating tremor essential and related to dystonia. The effect on tremors of the extremities is substantially less clear.

We treated a 42 year-old woman affected by secondary progressive Multiple Sclerosis (MS) with BTX, who developed since 1997 (after a cerebellar attack) a progressive intentional tremor not responsive to standard therapies: she was treated with a Beta blocker, antiepileptic drugs (carbamazepine and gabapentin), a benzodiazepine (clonazepam) and a muscle relaxant (baclofen) without any effect.

For the intentional tremor, she was not able to walk, to eat by herself, to wash herself and to perform the other activities of daily living.

The treatment with injection of low doses (15–20 UI) of BTX in the proximal muscles of arms, repeated every three months, was started. After one year, she showed an improvement with variable periods (from two days to three weeks) in which she was able to eat by herself.

At least in this case, BTX seems to be a new effective and safe approach for treatment of MS intentional tremor not responsive to previous standard therapies.

## Muscle disorders

**P667**

**Single-fibre electromyography in Na/Ca exchanger type III deficient mice.** S. Sokolow, S. Schurmans, J. M. Vanderwinden, A. Herchuelz, M. Manto, Free University of Brussels (Gosselies, Brussels, B)

The Na/Ca exchanger (NCX) is a plasma membrane protein responsible for calcium extrusion from cells. Three isoforms have been cloned and although the role of NCX1 is well known (e. g. in the heart), the role of NCX2 and NCX3 remain to be elucidated. The expression of NCX3 gene seems to be restricted to brain and skeletal muscle. Using classical genes knock-out strategy, we have generated a mouse strain deficient for the NCX3 isoforms of the Na/Ca exchanger. We performed single-fiber electromyography (SFEMG) on NCX3 deficient mice (NCX3-/-) and control mice. The cathode was inserted close to the proximal sciatic nerve and a SFEMG electrode was introduced into the gastrocnemius muscle. EMG potentials (SFAPs) were selected on the basis of: [1] clear isolation from stimulation artefact and from other discharges; [2] all-or-none responses at low levels of stimulation; and [3] stable waveform morphology. For each fiber, one response corresponded to 50 consecutive SFAPs, and these measures were repeated one to four times to compute the mean consecutive difference (MCDs) of the potential latencies. Acquisitions with intermittent blocking were discarded to avoid false increase of jitter. A total of 67 responses were recorded from 23 single muscle fibers of NCX3-/- mice and a total of 88 responses were recorded from 22 single muscle fibers of control mice. MCDs ranged from 47 to 130 microsec (mean ± SEM = 82.34 ± 0.78) in NCX3-/- mice and from 30 to 84 microsec (mean ± SEM = 58.8 ± 0.64) in control mice (intergroup difference, $p < 0.001$). Twenty-two percent of NCX3-/- fibers presented abnormal neuromuscular blocking (% of bloc ranged from 6–25). In disorders of the neuromuscular junction, decreasing jitter abnormalities are observed with a higher discharge rate in many endplates. Therefore, in order to compare the MCD values in both groups, square-wave pulses of 50 microsec duration were delivered successively at 10, 15 and 20 Hz to the stimulating electrodes. For each fiber, 50 consecutive SFAPs were recorded for jitter calculations. Twenty-one single fibers from NCX3-/- mice and nine single fibers from control mice were stimulated successively at 10, 15 and 20 Hz. Our study showed improvement of mean MCDs from 77.19 ± 6.61 microsec to 64.38 ± 4.58 microsec at the higher discharge rate in NCX3-/- fibers. By contrast, the mean MCDs increased from 43.56 ± 5.57 microsec to 75.89 ± 7.19 microsec in control mice. Our results suggest that NCX3 exchanger is required for normal neuromuscular function.

**P668**

**Side effects of long-term immunosuppressive treatment in myasthenia gravis - follow-up of 163 patients.** C. Rozsa, L. Fornadi, G. Szabo, S. Komoly, Jahn Ferenc Teaching Hospital (Budapest, HUN)

Background: A randomized double-blind controlled trial proved the effectiveness of the combined treatment with azathioprine (AZA) and alternate day prednisolone (ST) in myasthenia gravis in 1998. We describe the long-term adverse reactions which we observed in a prospective follow-up of 163 consecutive patients who were treated with combined AZA + ST treatment at our institution during the last 5 years.

Patients and methods: 123 patients had severe generalized MG not controlled satisfactorily with anticholinesterase inhibitors alone, in 40 patients the treatment was initiated after a myasthenic crisis. The mean age of the patients was 58.5 years. The mean follow-up period was 33 months [6–70]. The treatment was begun with 1.5–2.5 mg/kg body weight azathioprine daily, combined with alternate day methylprednisolone, the latter at an initial dose of 4 mg, increasing by 4 mg per dose to 1–1.5 mg/kg body weight. The steroid was slowly tapered after 2 months of stable remission, and discontinued if possible (discontinuation of steroid was possible in 87 % of the patients).

Results: 61.34 % of the patients suffered from some side effect. 45 % of these patients showed only transitional cushingoid features and weight gain during the combined period of the treatment. The other observed side effects were hair loss (15 %), hematologic toxicity (15 %), diabetes mellitus (11 %), gastrointestinal side effects (10 %), arthralgia, muscle pain (9 %), hypertension (9 %), severe infections, chills, fever (5 %), severe osteoporosis (6 %), worsening of liver function (5 %), allergic skin reactions (1 %). Combination of different adverse reactions was observed in 31 % of patients. Discontinuation of treatment due to severe side effects was necessary in 6.1 % of patients.

Conclusions: Combined AZA + ST treatment in severe MG is a safe treatment with generally mild and transient side effects. The majority of side effects was attributable to steroid treatment. Discontinuation rate of treatment due to side effects was very low in our patient cohort. AZA has a steroid-sparing effect, and fewer side effects than the steroids, consequently the combined treatment is better tolerated.

**P669**

**Language and reading abilities in Duchenne muscular dystrophy.** F. Civati, M. G. D'Angelo, A. Tavano, F. Fabbro, M. Castelletti, G. Cerina, C. Gagliardi, A. C. Turconi, N. Bresolin, IRCCS E. Medea, La Nostra Famiglia (Bosisio Parini, San Vito-Pordenone, Milan, I)

Background: Duchenne muscular dystrophy (DMD) is an x-linked muscular disease characterized by the lack of muscle dystrophin. In most patients, the muscle disease is associated with variable degrees of cognitive impairment. Many studies documented a significant discrepancy between Verbal Intelligence Quotient (IQ) and Performance IQ, suggesting that language acquisition could be globally disordered. Impairment of cognitive abilities in some DMD patients may be related to deletion in the distal portion of the dystrophin gene, involving alteration in the Dp140 and Dp71dystrophin cerebral isoforms.

The aim is to find evidence for deficits to the language system as distinct from deficits to the more peripheral articulatory system. Second, we mean to find evidence for correlating the acquisition of reading skills with language and articulatory deficits.

Patients and methods: All DMD patients were diagnosed according to international standard criteria. They were aged from 4 to 12 years with intelligence quotient performance > 85.

General intelligence was assessed using Wechsler Intelligence Scales.

Language abilities were determined with following tests:

1. Battery of standardized tests to evaluate language development in children "Batteria 4–12"
2. "Test dello Sviluppo Morfosintattico"

Reading abilities were determined with following tests:

3. "Battery to evaluate Dyslexia and Dysorthography"
4. Tasks of correctness and rapidity MT.

Results: Analysis of language abilities and reading capacity of one DMD patient of 7 years with Verbal-Performance Intelligence Quotient discrepancy of 23 points (VIQ = 78; PIQ = 101) and borderline Full Intelligence Quotient (FIQ = 87) showed difficulties in syntax comprehension (Token test), grammatical comprehension, sentences repetition and derivational morphology. Another DMD patient, 7 years and 10 months old with Verbal-Performance Intelligence Quotient discrepancy of 14 points (VIQ = 118; PIQ = 104) and middle-high Full Intelligence Quotient (FIQ = 112) showed only difficulties in sentences repetition.

Discussion: Our results indicate that DMD children may show language and articulatory deficits (patient 1) as well as deficits limited to the articulatory system (patient 2). This may be taken as evidence for a causal relationship between DMD and language acquisition disorders.

**P670**

**Mycophenolate mofetil in the treatment of severe myasthenia gravis.** D. Parissis, M. Gelagoti, N. Taskos, I. Milonas, Ahepa Hospital (Thessaloniki, GR)

Background: Mycophenolate mofetil (MM) is a potent immunosuppressive agent, which has been shown to prevent and reverse acute rejection episodes in heart and kidney transplant patients. MM blocks de novo purine biosynthesis (by inhibiting inosine monophosphate dehydrogenase) in activated B and T lymphocytes and therefore impairs their proliferation. To our knowledge, there are only two open label studies, which have showed that MM is a safe and effective drug in patients with severe myasthenia gravis (MG). The aim of our study was to evaluate the efficacy, tolerability and steroid sparing effect of MM in selected MG patients.

Patients-Methods: We studied 5 patients (3 males and 2 females, 32–72 years old; mean 58 years) who had refractory generalized MG defined by QMG (quantified myasthenia gravis) score of at least 10 and a manual muscle test (MMT) score of at least 5. All patients (except one with recently diagnosed MG) were taking corticosteroids with side effects and required occasional treatment with i. v. immunoglobulin in the past. Patients received MM 25 mg/kg/day orally for an average of 5 months (range, 3 to 8 months). The efficacy of the treatment was assessed monthly by QMG, MMT and ADL (activities of daily living) scores. Moreover, frequent physical and laboratory examination evaluated the safety of the drug.

Results: All our patients improved by QMG and MMT during therapy. A reduction of at least 3 points in the QMG score was evident in 2 patients, while all the patients showed a reduction of at least 2 points in the MMT. Two [2] patients achieved a reduction of at least 50 % in steroid-dose for at

least two months without worsening of the disease. Moreover, no relapse of MG was observed and no immunoglobulin treatment was required during the clinical trial. Finally, the drug was well tolerated and no major side effects (including leucopenia and opportunistic infections) were found.

Conclusions: The results of this ongoing study indicate that MM appears to be useful as adjunctive therapy in the treatment of refractory MG. The safe side effect profile of the drug and its relative rapid onset of therapeutic action would make MM an alternative to other immunosuppressants for MG. However, larger and controlled trials are needed to confirm these preliminary results and to investigate the long-term efficacy and safety of MM in severe MG.

P671

**Amyloid myopathy: a mechanical disorder with disruption of muscle fibres seen in systemic amyloidosis.** M. Hauf, A. Lobrinus, A. Cairoli, D. Piguet, J. Bogousslavsky, T. Kuntzer, Centre Hospitalier Universitaire Vaudois (Lausanne, CH)

Background: Myopathy is an uncommon manifestation of systemic amyloidosis. We report on 3 patients seen recently with biopsy-proven amyloid myopathy and characterize clinical, pathological features and response to therapy.

Case reports and Results: A 58yo man presented a limb girdle syndrome with dysphagia and cardiomyopathy. Needle emg demonstrated fibrillation potentials in proximal muscles and polyphasic motor unit potentials. Nerve conduction study (NCS) showed evidence of a mild distal sensory-motor polyneuropathy. Further investigation showed normal levels of creatine phosphokinase (CK), a deltoid biopsy demonstrated changes that mimic those seen in polymyositis, but with deposition of amyloid in the endomysium and vessels walls, and a monoclonal IgG lambda gammopathy (29.4 g/L, normal < 16 g/L). He was diagnosed multiple myeloma. A dose-intensive melphalan therapy with blood stem cell support normalised the level of immunoglobulins, but no improvement in muscle weakness was observed after 32 months.

A 50yo lady recently diagnosed cardiomyopathy and nephrotic syndrome presented a limbgirdle syndrome with myalgia. Neurological examination and NCS were normal, but fibrillation potentials were seen at needle examination in proximal muscles. Deltoid biopsy confirmed results of a myocardial biopsy sample that were positive for light chain amyloid deposition in endomysium and in small arteries, but no inflammation was observed. CK were normal. The patient was known for a monoclonal IgG lambda gammopathy for 5 years, without evidence for recent increase of serum concentration. Melphalan therapy was administered and well tolerated so far with a 2-month follow-up.

A 72yo man known for rheumatoid arthritis presented a subacute mononeuritis multiplex and weight loss. NCS showed an asymmetric sensory-motor neuropathy of axonal type. Deltoid and peroneus brevis biopsies revealed amyloid plaques in the endomysium, scattered muscle fiber atrophy but no inflammation. A sural nerve biopsy showed atrophy of myelinated and non-myelinated axons without amyloid plaques or inflammation. CK were normal. A monoclonal IgM lambda gammopathy was found (13 g/L, normal < 2.4 g/L) and further investigation led to diagnose a B-cell lymphoma. An associate treatment with cortisone and rituximab was not efficient and death occurred 6 months after the diagnosis.

Conclusions: We provide evidence that amyloid myopathy may mimic polymyositis as well as vasculitis. Congo red staining could prevent misdiagnosis. Clinical deficits related to muscle amyloidosis appear to be rapidly progressive and irreversible, emphasise the importance to elucidate the pathophysiological mechanisms that remain poorly understood. We believe that one of the possible mechanisms may be mechanical disruption of the sarcolemma by the abutting amyloid fibrils. At this time, treatment is determined by the underlying pathology with the aim to stabilise the clinical course of muscle weakness.

P672

**LAMP-2 deficiency: two new Italian cases.** O. Musumeci, C. Rodolico, I. Nishino, A. Mazzeo, A. Migliorato, G. Di Guardo, M. Aguennouz, A. Toscano, G. Vita, University of Messina, National Institute of Neurosciences, Ospedale Garibaldi (Messina, I; Tokyo, JP; Catania, I)

Primary LAMP-2 deficiency is an X-linked glycogen storage disease characterised by the clinical triad of cardiomyopathy, vacuolar myopathy and mental retardation, known previously as Danon's disease. LAMP-2 is a lysosomal membrane structural protein and mutations in the lamp-2 gene have been reported in 11 patients, only one of them being an Italian case.

Here, we describe two new Italian cases of LAMP-2 deficiency. A 23 year-old man had received a diagnosis of Wolf-Parkinson-White syndrome at the age of 21 and one year later developed a hypertrophic cardiomyopathy. He was admitted to our department because of muscle fatigability and persistent increased serum CK (783 UI/L). Neurological examination was normal. EMG revealed increased percentage of polyphasic motor unit potentials. The second case, a 17 year-old boy, had had a floppy infant syndrome at birth and a slight delay in the development milestones. At the age of 4 a brain MRI showed a partial corpus callosum agenesia. Since childhood he complained of proximal muscle weakness and exercise intolerance. Clinical examination showed waddling gait, mild proximal weakness, brisk reflexes and bilateral Babinski sign. Mild mental retardation was also evident. Increased serum CK (500 UI/L) was found. EMG showed a myopathic pattern with presence of myotonic discharges. ECG and echocardiography revealed a hypertrophic cardiomyopathy. Muscle biopsy revealed in both patients a vacuolar myopathy with increased glycogen content. Biochemical analysis showed normal acid maltase activity. LAMP-2 immunoreactivity was absent but vacuoles strongly stained for limp-1. C5b-9 complement fraction immunodelineated vacuoles.

P673

**Dropped head syndrome as early sign in very-late onset myasthenia gravis.** R. García-Ramos, T. Moreno, V. Gonzalez, M. Penas, A. Villarejo, J. Gonzalez de la Aleja, A. Alonso, J. Porta Etessam, Hospital 12 de Octubre (Madrid, E)

Introduction: Head ptosis results from weakness of the neck extensor and it is characterised by marked anterior curvature or angulation of the cervical spine and is associated with various neuromuscular and extrapyramidal disorder. Various neuromuscular disorders are reported to cause head ptosis but in some cases no obvious aetiology for the head drop is apparent even after extensive evaluation and on prolonged follow up. The commonest causes are myasthenia gravis in elderly people (only six cases described) and amyotrophic lateral sclerosis. We present three new cases of dropped head syndrome as early sign of very-late onset myasthenia gravis.

Patients: Case 1: A 81 year old man with chronic bronchitis developed proximal limb weakness and a fluctuation head ptosis over a period of few weeks. Examinations showed ptosis, fatigability language and proximal limb weakness with the neck extensor as the weakest. Tensilon test showed improving of head ptosis. Serum creatine kinase was normal, antiacetylcholine receptor antibody was positive and repetitive nerve stimulation in biceps and abductor digiti minimi showed decremental response. A thoracic CT scan disclosed a mass suggesting thymoma. Treatment with prednisone and pyridostigmine was satisfactory. Case 2: A 72 year old man with a renal failure presented with a painless anterior curvature of the cervical spine. The deformity was not fixed and was worse in the standing position. Weakness was demonstrable in neck flexors and extensors and proximal limb muscle. Tensilon test failed. His creatine kinase was at the upper limit of normal and antiacetylcholine repector antibody was negative. Electrodiagnostic evaluation showed decremental response to repetitive nerve stimulation. A therapeutic trial with prednisone and pyridostigmine reported improvement in neck strength and weakness. Case 3: A 76 year old woman with progressive proximal limb weakness and head ptosis over a period of 4 months. Examinations showed mildly proximal weakness. Tensilon test was positive. Serum creatine kinase was elevated [345] and antiacetylcholine receptor antibody was positive. No electrodiagnostic study was realized. A beneficial response with pyridostigmine and prednisone was observed.

Discussion: In elderly people age related loss of tissue elasticity and mild kyphosis predispose to chronic stretch injury of paraspinal muscles and may contribute to the development of head ptosis. Dropped head syndrome with a fatigability response suggest disorder of neuromuscular transmission in elderly people and we advise a therapeutic trial with pyridostigmine and prednisone.

P674

**Familial late-onset progressive of shoulder and ankle dorsiflexors weakness related to 4q35 chromosome delection: unusual phenotype of FSHD.** A. Pou-Serradell, I. Royo, A. López de Munain, P. Camaño, Hospital del Mar, Hospital Donostia (Barcelona, San Sebastián, E)

Background: Classical diagnostic criteria for facioscapulohumeral dystrophy (FSHD) include onset of the disease, in the second decade, in facial or shoulder girdle muscles, autosomal dominant inheritance in familial cases and evidence of myopathic disease in EMG.

Objective: To describe two patients, a mother and her daughter, with an atypical presentation of FSHD causing diagnostic difficulties. By DNA analysis the diagnosis could be established.

Cases report: We report the case of a 70-year-old woman. She was referred to a neurologist in 1993, at 60 years of age, because of a difficulty walking since one year. The diagnostic considered was an sporadic case (none other familial member were affected at this time) of late-onset acquired myopathy, probably inflammatory. Since then a progressive weakness of ankle's dorsiflexors and shoulder muscles without clear weakness of facial muscles has been noticed. The familial evaluation revealed a daughter, a 48-year-old woman, who has shown in the last five years (since 1998) a progressive scapuloperoneal amyotrophy with a mild facial involvement. A relatively benign clinical course was considered in both patients. Analysis of the propositus' DNA, of her daughter's DNA and of the daughter's son (an asymptomatic 15-year-old-boy) showed a mutation at locus 4q35, characteristic of FSHD. The EcoRI fragment's size was of 22 kb (5 units, relatively large size).

Conclusion: These cases illustrate the wide clinical spectrum of FSHD and the difficulty to diagnose unusual facial-sparing forms and familial cases when the onset of the disease is very late. These cases also confirm the inverse correlation between the EcoRI fragment size and the clinical severity.

**P675**

**Major histocompatibility complex and matrix metalloproteinases immunoexpression in focal myositis.** C. Rodolico, A. Mazzeo, A. Toscano, M. Gaeta, G. D'Arrigo, C. Messina, G. Vita, University of Messina (Messina, I)

Background: it is well established that MHC class I and class II molecules are expressed in muscle fibers, independently of infiltrates, in inflammatory myopathies, but no difference in MHC I or MHC II staining patterns between polymyositis (PM) and dermatomyositis (DM) has been so far found. The study of various metalloproteinases (MMPs) in inflammatory myopathies has provided equivocal data: overexpression of MMP-2 and MMP-9 was initially reported as a peculiar pattern obtained in PM. Recently MMP-2 has been observed slightly elevated in DM and a strong expression of MMP-9 has been also found in atrophic fibers in DM. Moreover, MMP-7 invaded myofibers have only been seen in PM.

Objectives: the aim of our study is to investigate MMP-2, MMP-7, MMP-9 in relation to the expression of MHC I and II in muscle biopsies from patients with focal myositis, in order to elucidate immunopathogenesis of such a disease.

Materials and methods: an immunohistochemical study with antibodies against CD4, CD8, B cells subset, macrophages, C5b9, MHC class I and II, MMP2, MMP-7, MMP-9 was performed in limb muscles biopsies from 5 patients (aged 20–85 years) with a focal myositis, confirmed by clinical, muscle MR and histological findings.

Results: expression of MHC I antigen was found in some muscle fibers independently of inflammatory infiltrates. No immunostaining for MHC II and MMP-7 was observed. MMP-2 was slightly expressed in few muscle fibers. A strongly cytoplasmic and sarcolemmal expression of MMP-9 was revealed in those fibers which were MHC I positive.

Discussion: our results indicate that MHC class I molecules only are involved in the pathological mechanisms of focal myositis. Absent staining for MMP-7 clearly differentiates focal myositis from PM. Understanding the reason why the inflammatory process remains localized to a single muscle in focal myositis could help in elucidating the pathophysiology of generalized inflammatory myopathies.

**P676**

**Polymyositis induced by a chronic CD8 + T cell leukaemia.** S. Spuler, M. Hofbauer, C. Skulina, R. Hohlfeld, N. Goebels, University Hospital Charité, Institute for Clinical Neuroimmunology, University Hospital Zurich (Berlin, Munich, D; Zurich, CH)

Polymyositis is an inflammatory disease of the skeletal muscle characterized by expanded CD8 + T cell clones surrounding, invading and destroying MHC class I + myofibers. Recently we could identify the T cell receptor (TCR) beta chain sequences of individual autoinvasive T cells by laser microdissection and single cell PCR and track these clones in the peripheral blood by CDR3 spectratyping (a PCR based technique based on the natural length polymorphism of the third complementarity determining region (CDR3) of the TCR beta chain) (Hofbauer et al. (in press) PNAS).

Now we present the rare coincidence of a 57 year old woman suffering from a histologically demonstrated polymyositis that was refractory to immunosuppressive therapy. Subsequently, additional diagnostic tests revealed a chronic CD8 + T cell leukemia. We wondered, whether the leukemic CD8 + T cell clone could be involved in the pathogenesis of the muscle inflammation.

CDR3 spectratyping and FACS analysis revealed a massively expanded CD8 + T cell clone expressing the V-beta 8 TCR chain in the peripheral blood. By FACS analysis BV8 + CD8 + T cells made up more than 30 % of the total peripheral blood T cells of this patient. CDR3 spectratyping and immunohistochemistry with a monoclonal antibody specific for BV8 indicated that this particular T cell clone is not only strongly expanded in the muscle tissue as well but also constitutes the vast majority of the (presumably myocytotoxic) T cells surrounding and invading the myofibers. Currently ongoing single cell PCR analysis will determine the sequence identity of these autoinvasive BV8 + T cells.

To our knowledge this is the first report of a leukemia and a (presumably autoimmune mediated) inflammatory condition caused by the identical T cell clone. Although noteworthy as is, this unusual case may give us the unique chance to not only identify potential target antigens in polymyositis but also to establish TCR based principles of a selective immune therapy in a human disease.

**P677**

**Sudden death in proximal myotonic myopathy.** A. Panousopoulos, G. Papasalouros, T. Avramides, M. Tsamouri, V. Nicolaou, I. Anastasopoulos, R. Divari, Red Cross Hospital (Athens, GR)

Proximal Myotonic Myopathy (PROMM) or DM II is an autosomal dominant inherited multisystem disorder characterized by proximal muscle weakness, myotonia, myalgias and cataracts. The responsible mutation is a CCTG repeat expansion of the ZNF9 gene on chromosome 3q21.3. The disease is similar but distinct from Myotonic Dystrophy (DM I), clinically and genetically. In DM II, contrary to DM I, cardiac involvement is uncommon and only a few cases of PROMM with cardiac arrythmias have been reported.

We present a case of late onset Proximal Myotonic Myopathy with cardiac involvement and sudden death.

A 70 year old female presented with a four year history of muscle pains and increasing proximal muscle weakness. Her past medical history was almost unremarkable. She underwent bilateral cataracts operation 5 years ago and she complained of somnolence the last 6 months. She had two asymptomatic sons.

During her hospitalization she developed multiple atrial tachycardia and paroxysmal atrial fibrillation. The patient died from sudden death five months after discharge.

Neurological examination revealed moderate weakness of neck flexors, mild weakness of sternomastoids, severe proximal muscle weakness of upper and mainly lower limbs and absence of overt myotonia. She also had mild frontal balding.

Muscle enzymes were normal. Electromyography (EMG study) disclosed myotonic discharges to all proximal muscles tested. Nerve conduction velocities of upper and lower limbs were normal. Muscle biopsy revealed fiber size disproportion, type I predominance and central nuclei in 30 % of fibers. DNA study for DM I was negative and DNA analysis for DM II is pending. Repeated electrocardiograms (ECG) showed prolongation of PQ interval, multiple atrial tachycardia and paroxysmal atrial fibrillation. Cardiac echo revealed left ventricle systolic dysfunction (ejection fraction of 35 %) and total hypokinesia of the left ventricle.

EMG study of both sons detected myotonic discharges to all proximal muscles.

Concluding, the clinical and laboratory findings are highly consistent with PROMM. Moreover, genetic analysis ruled out the diagnosis of DM I. Although cardiac involvement and particularly cardiac conduction system dysfunction in patients with PROMM/DM II is rare, severe cardiac complications and sudden death may occur. This possibility imposes the need of a detailed cardiological protocol in such patients.

**P678**

**Congenital muscular dystrophy and muscle inflammation.** E. D'Adda. C. Lamperti, R. Cagliani, P. Ciscato, G. Fagiolari, A. Prelle, G. P. Comi, N. Bresolin, M. Moggio, E. D'Adda, Dept of Neurology, Istituto E. Medea (Milan, Bosisio Parini, I)

Congenital muscular dystrophy are a heterogeneous group of autosomal recessive disorders presenting in infancies with muscle weakness, contractures, and dystrophic changes at the skeletal muscle biopsy. Structural brain defects, with or without mental retardation are often present. Recently congenital muscular dystrophy associated with mutations in FKRP gene has been described. This form is characterized by onset in the first weeks of life, inability to walk, cardiomyopathy, muscle hypertrophy, marked elevations of serum CK, normal brain structure and functions. The muscle biopsy showed dystrophic changes, secondary deficiency of laminin alpha 2 and a reduction of alpha dystroglycan molecular weight.

We present a new case of congenital muscular dystrophy characterized by muscle weakness and psychomotor developmental delay with normal brain MRI, and no cardiomyopathy. The main pictures of muscle biopsy were a severe inflammation and a complete absence of alpha dystroglycan at the immunohistochemistry. A described heterozygous mutation in the FKRP gene was found.

P679
**Idiopathic orbital myositis complicated with retrobulbar optic neuritis.** A. Ariatti, F. Tavani, G. Galassi, University Hospital (Modena, I)

Idiopathic Orbital Myositis (IOM) is a subgroup of idiopathic orbital inflammatory syndromes, commonly causing extraocular muscle enlargement. A 49-year-old woman with unremarkable history presented with pain in left orbital region associated with redness, eyelid swelling, ipsilateral lacrimation, limited eye movements and diplopia on lateral gaze. The pain was severe, recurring several times during day and not preceded by aura. On first admission, physical examination was normal. Neurologically patient had paralysis of left lateral and medial rectus muscles. Erythrocyte sedimentation rate, thyroid function tests and extensive blood examinations, including antinuclear antibodies, antineutrophilic cytoplasmic antibodies, rheumatoid factor and C reactive protein didn't show abnormalities. She had negative serology for virus, Borrelia burgdorferi and Lues. Spinal fluid was normal as well as brain computed tomography (CT) and imaging (MRI) scans. The diagnosis of cluster headache was posed. Patient was treated with gabapentin. Two months later patient was newly admitted because of paroxysmal headache, painful eye movements and blurred vision. Visual evoked response (VER) revealed prolonged P100 latency (129 msec in the left and right side; n. v.below 115 msec). Repeated brain and orbital CT performed with slice thickness of 3 mm showed enlarged extraocular muscles, especially of the left medial rectus; the retroorbital fat had normal density. Treatment with high dose of steroids (1 gr of methylprednisolone e. v. daily for three days) was instituted with relief; the dose was tapered to an alternate oral regimen (50 mg of prednisone every second day). When two months later the dose was further lowered to 37.5 mg, clinical symptoms recurred. Repeated CT and MRI studies showed enlarged lateral and medial rectus muscles. VER were still abnormal (P100 latency equal to140 msec in the right, 139 msec in the left side). Prednisone (75 mg daily) was administered with complete resolution of pain, diplopia and eyelid swelling, which recurred when steroids were tapered.This case of IOM shows some interesting aspects: on first onset, when diagnosis of cluster headache was posed, brain and orbital CT were normal; correct diagnosis was made on account of enlarged muscles observed on CT and MRI scans; exhaustive laboratory data excluded a systemic disorder; there was an associated optic neuritis as indicated by the abnormal VER.

P680
**Tibialis anterior syndrome by sport training.** M. Coletti Moja, E. Milano, G. Rosso, F. Mondino, F. Perla, Ospedale S. Luigi, Ospedale S. Croce (Orbassano, Cuneo, I)

We report and discuss a case of a male patient 52 years old who progressively complaint of pain in the antero-lateral compartment of his legs and 24 hours later, he became unable to walk and to stand. The day before the onset of this symptomatology he was skiing the whole day long. On the hospital admittance muscle necrosis blood samples were very high (Creatinephosphokinase 6694 mg/ml, LDH, SGOT and SGPT much over levels) while all other parameters were in range. Clinically a bilateral prominent muscle tissue oedema was observed with a marked foot and toes dorsiflexion deficit (MRC scale 1) and hypoesthesia on the first interdigits territory, asymmetrically on both sides. Dorsalis pedis artery was not involved. On the EMG examination a myopathic pattern was observed in the muscles tibialis anterior and peroneus longus and brevis bilaterally, asymmetrical and more evident on the right side, associated with a neuropathy of the nerve peroneus profundus. A treatment by anti-inflammatory drugs and lymph drainage was performed with a mild slow clinical improvement. A 2 months later control still showed a marked myopathic pattern with fibrillation potentials, polyphasic motor units potentials, sometimes of increased amplitude associated with a very slow peroneus profundus nerve conduction velocity (16,8 m/s) and delayed distal motor latency. Clinically a partial recovery of muscle strength bilaterally (MRC 3 on the right and MRC 4 on the left side) was observed with spare of a secondary nerves damage. Tibialis Anterior Syndrome is seldom observed after long and excessive army or athletics and footing training: it consists in an acute diffuse bilateral muscles splitting; since muscles are positioned in this tight and closed anatomical compartment its prognosis is related to the entity of the mass volume compression's damage and a tissue fibrosis is strictly to be avoided because a compressive entrapment neuropathy or a complete and massive muscles necrosis make recovery very poor or absent.

## Neuro-biology

P681
**In vitro and in vivo fate potential of the oligodendrocyte precursor cell.** P. M. Gaughwin, S. Chandran, M. A. Caldwell, D. A. S. Compston, Cambridge Centre for Brain Repair (Cambridge, UK)

Background: Oligodendrocyte Precursor cells (OPCs) are proliferating, undifferentiated cells present throughout the adult brain that are involved in the endogenous response to injury. Several recent in vitro observations have suggested that the fate of the OPC is not intrinsically limited to the oligodendrocyte lineage, but can express an astrocyte or neuronal phenotype in response to defined signals. We are examining how the extracellular environment can regulate OPC fate potential in vitro and in vivo.

Method: Neonatal rodent cortex-derived OPCs were immunoselected, expanded in B104 neuroblastoma cell line conditioned medium and characterized. The phenotype and transcriptional profile of OPCs following the sequential application of bone morphogenetic proteins (BMP-2, 4) and basic fibroblast growth factor (FGF-2) was examined by light microscopy, immunocytochemistry and RTPCR. To assess the fate potential of OPCs in response to neurogenic cues in vivo, GFP-labeled OPCs were transplanted to the dentate gyrus of the adult rodent hippocampus and stained for neuronal and astrocyte markers.

Results: A subpopulation of cortical OPCs expressed neuron-specific proteins (beta III tubulin, neurofilaments, and microtubule-associated protein 2) and exhibited a neuron-like morphology in response to BMPs and FGF-2. Furthermore, OPCs transplanted to the hippocampus survived, integrated into the dentate gyrus, and adopted a polar morphology. The expression of genes known to be involved in neuronal and oligodendrocyte fate specification during development was also examined.

Conclusions: The potential of a subpopulation of cortex derived OPCs to adopt a non-oligodendrocyte fate in response to the extracellular environment was confirmed in vitro and examined in vivo. This supports the hypothesis that the environmental context regulates how OPCs are specified towards, or limited to, the oligodendrocyte lineage.

P682
**Wallerian degeneration in sciatic nerve causes induction of neuropilin-1 and -2 and their ligands, semaphorin 3A, semaphorin 3F and vascular endothelial growth factor.** M. Scarlato, P. Bannerman, S. Scherer, D. Pleasure, Ospedale Policlinico Milano, Children's Hospital of Philadelphia (Milan, I; Philadelphia; USA)

We used cDNA microarrays (Clontech) to screen for additional genes by which Schwann cells and fibroblast contribute to successful peripheral nervous system (PNS) axonal regeneration. Comparing the expression profile of multiple messenger RNAs (mRNA) in sciatic nerves distal to transection with their levels in normal sciatic nerves, we found a 14-fold increase in Neuropilin-2 (NP-2) mRNA in the axotomized nerve segments 4 days post-transection.

The Neuropilin family is comprised of two members. Neuropilin-1 (NP-1) was identified as a neuronal receptor that, binding to Semaphorin 3A (Sema 3A), mediates repulsive growth cone guidance and has been shown to function as an isoform-specific receptor for Vascular endothelial growth factor (VEGF). NP-2 binds to Sema 3F with high affinity and mediates repulsion of sympathetic neurons. The Neuropilins and their ligands are known to play a role in axonal pathfinding, fasciculation and blood vessel formation during PNS development. In contrast little has been displayed about the expression profile of these genes during functional alterations of adult peripheral nerves like wallerian degeneration.

By in situ hybridization we showed induction of the Neuropilins and their ligands at four days post-crushed, distally to the lesion of the nerve. By Northern blot we pointed out that the expression of the neuropilins mRNA is developmentally regulated and is induced in a biphasic fashion by sciatic nerve crush.

Our results suggest the possibility that the Neuropilins and the Semaphorins ligands serve to guide, rather than to impede, regenerating axons in the adult PNS and support previous reports of the VEGF mRNA level rising during wallerian degeneration as effect of neuroprotection and increasing angiogenesis.

P683

**Neuronal P2x7 receptors in rat brain after ischaemic damage – an immunocytochemical and electrophysiological study.** A. Guenther, R. Reinhardt, J. Grosche, H. Faber-Zuschratter, P. Illes, H. Franke, D. Schneider, University of Leipzig, University of Magdeburg (Leipzig, D)

Background: Upon agonist application P2X7 receptors act as nonselective cationic channels by forming a large membrane pore leading to cell lysis. Although current data suggest a widespread distribution of P2X7 receptors among CNS cells only very limited knowledge exists about its neuronal expression and function under physiological and pathological conditions. The present electrophysiological and immunohistochemical study was aimed to determine ischemia induced changes of neuronal P2X7 receptor characteristics.

Methods and Results: Untreated rat primary corticoencephalic cultures were compared with ischemically damaged cultures. In vitro-ischemia over 5 or 30 minutes was induced by incubating the cultured cells in glucose free medium gassed with 100% argon, followed by a reoxygenation period (1h–72h).

Firstly, double immunofluorescence staining with antibodies against the P2X7-receptor subtype, the neuronal marker beta-III-tubuline or glial fibrillary acidic protein, a specific marker of fibrous astrocytes, was performed and analyzed by using confocal laser scanning microscopy. Fluorescence intensity was quantified with an image analyzing software. In vitro ischemia resulted in a markedly increased neuronal P2X7 immunoreactivity with a receptor-staining localized cytoplasmatic rather than plasmalemmal. Additionally, to further specify the P2X7 immunoreactivity an electron microscopy study was performed using periinfarct tissue from spontaneously hypertensive rats 4 days after permanent middle cerebral artery occlusion. The P2X7 receptor was shown to be localized at the nuclear membrane.

Secondly, membrane currents were recorded in the whole-cell configuration of the patch-clamp method at room temperature. In untreated cultured corticoencephalic neurons at a holding potential of –70 mV no inward current responses to pressure applied ATP (100 micromolar–10 millimolar) and the P2X7 receptor preferential dibenzoyl-ATP (3–300 micromolar) were found. Moreover, ischemic incubation and different duration of reoxygenation did not influence the electrophysiological unresponsiveness to either agonist.

Conclusions: The present data document an upregulation of the P2X7 receptor subtype of corticoencephalic cultured neurons in the cytoplasm after in vitro-ischemia. The negative electrophysiological results could be explained in agreement with the findings of immunecytochemistry and electron microscopy. The study indicates an important role of CNS P2X7 receptors in the ischemic process, thus providing a possible target for new neuroprotective strategies.

P684

**Differential proteomics analysis of neuronal stress induced by homocysteic acid: phosphorylated and methylated isoforms of chaperones as molecular signature of homocysteic acid-induced excitotoxicity.** A. Schrattenholz, S. Sommer, M. Klemm, B. Biefang-Arndt, G. Schwall, K. Hoelzer, K. Schroer, C. Hunzinger, S. Pütter, W. Stegmann, ProteoSys AG, Medice (Mainz, Iserlohn, D)

Plasma homocysteine (HC), a risk factor for cardiovascular disease and thrombosis augmenting oxidative stress and reducing nitric oxide availability, is also a risk factor for cancer and nervous system dysfunction [1]. One of the oxidative metabolites of HC is homocysteic acid (HCA), an excitotoxic glutamatergic agonist possibly enhancing detrimental effects of increased homocysteine in particular in the central nervous system. We used neurons differentiated from murine embryonic stem cells to investigate excitotoxic effects of HCA, which induces massive calcium influx with an EC50 of 63.8 µM. A differential proteomic analysis of stimulated neurons versus controls, focusing on changes of respective phosphoproteomes, reveals the rapid posttranslational modification of a distinct set of stress-related proteins, including a number of heat shock and oxidative metabolism proteins. This homocysteic acid induced molecular stress signature can be attenuated by the co application of vitamins (B6, B12 and folate), which are essential cofactors of the remethylation- and transsulphuration-pathways. This apparent reversal of some of the excitotoxic effects of HCA coincides with the appearance of posttranslationally modified isoforms of heat shock protein 70 and protein disulfide isomerase and might be mediated by adenosine kinase.

Protein carboxymethylation is part of repair functions of aging proteins and stress response [2]. Apparently, a cascade of harmful molecular mechanisms emerging from S-adenosyl-homocysteine exists, leading to neuronal cellular damage by HC, reactive oxygen species, HCA, and adenosine, all together inhibiting rescue mechanisms involving protein methylation [3]. Co application of vitamins B6, B12 and folate obviously accelerates recycling of S-adenosyl-homocysteine to SAM, thus readjusting the cellular system to a more "desirable" state of methylation homeostasis.

P685

**Is oxidative stress pathway a physiologic modulator for neural plasticity?** A. Kudinov, T. Berezov, Weizmann Institute Neurobiology, Russian Academy of Medical Sciences (Rehovot, IL; Moscow, RUS)

Oxidative stress is implicated in a variety of neurological diseases [1]. Almost nothing is known about its modulatory role in neural cell function and plasticity. One of the intermediate products of the cascade of the reactions of oxidative stress is hydrogen peroxide ($H_2O_2$). Previous studies of peroxide were focused on understanding the role of its high toxic doses in cell dysfunction. Normal modulatory role was not systematically thought about. One recent study by others [2], however, shows that micromolar (microM) dose of peroxide impairs slow onset component of the long-term potentiation (LTP), an electrophysiological phenomenon believed to represent the cellular and circuitry mechanism of synaptic plasticity and memory formation. Present study aimed to further understand the role of peroxide in synaptic plasticity. We used adult albino Wistar rat hippocampal slices for the field extracellular recording of evoked postsynaptic potentials (fEPSP) in CA1 using the paradigm of one stimulating/two recording electrodes [3]. The recording was accompanied by administration to the perfusion medium peroxide at different concentrations. A high concentration of the chemical (4.4 mM) caused the reduction of the fEPSP size at both pre-potentiated channel (indicating the impairment of the LTP maintenance phase) and at the baseline-recording channel to the same depressed value of a half of the baseline recording. Washout of the 4.4 mM peroxide restored the prior recording at both channels. The slices, however, were no longer capable to generate LTP. Doubling of the concentration (8.8 mM) had stronger effect as the impairment of the potentiated and baseline fEPSP slopes become not reversible after the peroxide washout. Much lower (and thus closer to the physiological) concentration of the peroxide (44 microM) in our experimental condition did not affect baseline recording and the ability of slices to generate LTP after the chemical washout. Interestingly, five times lower concentration of the peroxide (8.8 microM) additionally triggered slow-onset potentiation in the absence of the tetanic (100Hz) stimulation.

Our data imply complex activity of the peroxide and/or its downstream products as a modulator for physiological synaptic plasticity.

P686

**Aberrant calcium extrusion mechanisms may contribute to secondary spinal cord injury.** S. Elkabes, A. Nicot, New Jersey Medical School (Newark, USA)

The primary traumatic insult during spinal cord injury initiates pathophysiological mechanisms leading to secondary damage which exacerbates the deleterious effects of the first impact. Emerging evidence indicates that glutamate-induced excitotoxicity may play an important role in secondary damage. It is believed that excess glutamate increases calcium influx leading to Ca2 + overload, a trigger well known to promote intracellular injury cascades. Abnormal increases in Ca2 + levels may also result from defects in extrusion mechanisms, an alternative possibility which we have been investigating.

Indeed, we have recently shown that exposure of spinal cord slice cultures to low concentrations of kainic acid (4 micromolar) for 6 hours suppresses the expression of plasma membrane calcium ATPase 2, an important ion pump localized to neurons and regulating Ca2 + homeostasis (Nicot et al, 2003, Brain 126, 398). To determine whether Ca2 + extrusion mechanisms are affected after trauma, we analyzed the expression of plasma membrane calcium ATPases (PMCAs) in the spinal cord 24 and 48 hours following contusion injury. No significant changes in the mRNA levels of PMCAs were observed at 24 hours. PMCA2 expression within 2–3 mm of the epicenter in both the proximal and distal segments was drastically reduced at 48 hours. In contrast, mRNA levels of other PMCAs including PMCA3, which is primarily localized to neurons, were not altered. These findings suggest a selective effect of injury on specific extrusion mechanisms. We are now investigating whether the decrease in PMCA2 is the result of neuronal loss due to the traumatic insult or a component of the excitotoxic cascade which promotes secondary neuronal injury.

Supported by grant 01–3008-SCR-S-0 from NJCSCR.

P687
**Reversible subacute ataxia and dysarthria due to D-lactate encephalopathy.** B. Bardiaux, M. Dupuis, P. Jacquerye, M. Wauthier, Clinique Saint-Pierre (Ottignies, B)

A 67 year old man is admitted to emergency room in February 2002 for progressive unsteadiness and tiredness for 2 days. Neurologic examination reveals a static and kinetic cerebellar syndrome, dysarthria, horizontal nystagmus and slowness to answer to the questions. In 1999 an extensive small intestine resection due to mesenteric ischemia was performed and in January 2002 he suffered from occlusive syndrome due to intestinal adhesion. Brain CT, ECG, carotid and vertebral Doppler Ultrasound were normal. Toxicology was negative and the only detected abnormality was an anion gap.

Neurologic symptoms disappeared in 2 days. Cerebral MRI was normal. Serum lactic acid dosage was normal but high D-Lactate titer was demonstrated in admission serum sample and normalized on the next day.

30 cases of D-Lactate Human encephalopathy have been reported with striking similarity: a past extensive intestinal resection, a recent occlusive syndrome, reversible subacute encephalopathy with cerebellar symptoms, anion gap due to D-Lactate synthesis by colic bacteria. This syndrome must be recognized to be treated by antibiotics.

# Poster Session 5

## Cerebrovascular disorders

P688
**Mortality trends of cerebrovascular diseases in Croatia: 1958–1997.** M. Kadojic, V. Babus, M. Dikanovic, Osijek University Hospital, School of Public Health 'Andrija Stampar', General Hospital (Osijek, Zagreb, Slavonski Brod, HR)

Neuroepidemiological studies of cerebrovascular diseases (CVD) are very important for correct programming and planning of health service and its specific organization. Especially significant are the studies that show secular changes in the mortality from a disease during a long period of years or decades. This research comprised all death from CVD in Croatia between 35 and 74 years of age over the period 1958–1997. The number of deaths from CVD for investigated population in that period increased by 40 %. At the same time, the rates standardized by age and sex increased by 62 %. Proportional mortality rate from this disease increased from 7.1 % in the year 1958 to 14.8 % in 1997 (increase of 39.48 %). The specific mortality rates over 5-year period have shown a trend of increase in all male age groups and stagnation or decrease in females. Standardized mortality rates for CVD in continental communities (Osijek, Varazdin) are much higher (twice or even threefold) than those in coastal communities (Split, Rijeka). A cohort data analysis has shown that although mortality trends of CVD stagnated or even declined in some communities during the recent years, the secular trend for the entire country had a tendency of constant rise over the whole period of research. Therefore, the short-term prognosis predicts further increase of both the number and rates of deaths from CVD in our country.

P689
**Cerebrovascular risk factors predicting influence in non-lacunar stroke.** R. Merino, E. Díez-Tejedor, B. Fuentes, J. Gracia, V. Mejías, S. Monteagudo, S. Escalante, La Paz University Hospital (Madrid, E)

Background: Recent studies have demonstrated that presence of many cerebrovascular risk factors is associated with a poor outcome for ischemic stroke. Our goal is to evaluate which of this have a real influence in non lacunar infarction (NLI) outcome.

Methods: Observational and sequential study from Stroke Unit Registry since 1994–2001. NLI (Cardioembolic (CI), atherothrombotic (AI) and undetermined etiology (UE) were included. Risk factors (age, gender, hypertension, diabetes mellitus, Hiperlipidemia, cigarette smoking, alcohol abuse, atrial fibrillation, history of myocardial infarction, previous stroke, peripheral vasculopathy) were collected. Outcome was evaluated by means of modified Rankin Scale at discharge (poor outcome was defined mRS 3–6). Statistic test: Fisher test, Chi square, t-student and multivariate logistic regression analysis.

Results: Of 3735 patients with stroke, 1840 had diagnosis of NLI (men 970, women 870, age 70 ± 11 years), AI 42.25 %, CI 36.4 %, UE 21.35 %. Previous stroke ($p < 0.001$), older age ($p = 0.001$) and female gender ($p < 0.001$) were associated with a poor outcome in NLI. In multivariate logistic regression model, only older age was an independent predictor factor ($p < 0.001$); OR = 1.024 (CI 95 %: 1.010–1.038)

Conclusions: Older age was the main predictor factor of poor outcome although previous stroke and female gender were shown to be associated with a poor outcome too.

P690
**Language disorders in right hemisphere stroke.** R. A. Piusińska-Macoch, J. K. Kotowicz, Department of Neurology MMI (Warszawa, PL)

Introduction: Aphasic disorders are common complications in the left-hemisphere stroke, but in neuropsychological investigation we can observe language disorders also in right-hemisphere stroke.

Aim of the study: Clinical, neuropsychological analysis of the post-stroke linguistic disorders in right-hemisphere lesions.

Material: In preliminary phase, study covered 67 (28 male, 25 female) stroke patients with lesions in right hemisphere (24 persons) - RH group, left-hemisphere post-stroke lesion group LH (23 persons) patients and a (C) control (21 persons) group without neurological deficit.

Method: The study of the phonologic, semantic, syntactic, discursive and prosodic language systems was performed in non-parametrical, clinical-experimental method.

Results: Statistical analysis (MANOVA) showed significant differences ($p < 0.05$) between RH and LH group and among RH and LH and C groups in all 5 language levels.

Conclusions:

1. The linguistic disorders in semantic, phonetic, discursive and emotional prosodic systems were connected with frontal, posterior or mixed localised damages of the right brain hemisphere in stroke.
2. These specific deficits can be involved by right hemisphere spatial and holistic date processing.
3. Clinical analysis of these results enables as to construct the specialistic, neuropsychological, therapeutic program.

P691
**Spontaneous intracranial haemorrhage: which patients need diagnostic cerebral angiography? Comparative study between plain computed tomography and cerebral angiography in HUSM.** J. Abdullah, I. Abu Bakar, I. Shuaib, N. Naing, University Sains Malaysia (Kubang Kerian, Kelantan, MY)

Fifty-four consecutive patients with intracranial haemorrhage with an age range of 8 to 79 years old (mean 46.8 and median 46 years old) over 19-month period (October 1998 to April 2001). Inclusion criteria include: all patients in whom both computed tomography examination and cerebral angiography were carried out. Exclusion criteria include: traumatic, tumoural or coagulopathy related haemorrhages. Cerebral angiographies were performed within 6 weeks of the onset of the illness.

Overall angiographic detection of vascular lesion is 52 % (48 %, aneurysm and 12 % arteriovenous malformation). In the subgroup of patients with ICH ($n = 32$) the angiographic yield was 41 % with 28 % aneurysm and 13 % arteriovenous malformation. Presence SAH in combination with other type of haemorrhage (within this ICH subgroup) significantly correlated with the likelihood of finding of vascular lesion ($p = 0.007$). Other factors that have significant correlation are age less than 50 years old ($p = 0.009$) and absence of pre-existing history of hypertension ($p = 0.05$). All patients with deep intracerebral hematoma did not have any vascular lesion detected. Presence of IVH did not have significant correlation ($p = 0.946$). Independent sample t-test revealed no significant difference in the mean age, between patients with vascular lesion and patients without vascular lesion ($p = 0.134$). A p value less than 0.05 is considered significant.

P692
**Eales disease. A rare cause of stroke.** C. B. De Cock, S. Blecic, M. Dagonnier, J. Jacquy, CHU de Charleroi, Erasme Hospital (Charleroi, Brussels, B)

Background: Eale's retinopathy is characterized by recurrent vitreous and retinal hemorrhages. Few cases with stroke have been described. We report a series of 3 patients with Eales disease who had acute stroke.

Patients: 3 caucasian men with confirmed Eales disease were admitted to the stroke Unit for acute stroke. The first was a 51-year-old man with left

medullary acute infarction. Extensive stroke work-up was negative, including CSF examination and catheter angiography. Recovery was complete within 48 hours. The second was a 43-year-old man with acute spinal infarction. He evolved toward a C6 Brown Sequard syndrome. Extensive stroke work-up disclosed only spinal artery occlusion after angiography. CSF examination was normal.

The third aged 25 had a complete left MCA stroke. Catheter angiography disclosed M1 middle cerebral artery occlusion and multiple narrowings on distal branches of the left anterior cerebral artery and on external carotid artery branches. CSF examination was normal. Meninges and brain biopsies disclosed mild non inflammatory lymphocytic infiltration.

Discussion: In our 3 patients, retinal infarction and vitreous hemorrages, the classical ocular manifestation of Eales disease always preceded stroke. In all 3, stroke work-up did not disclose any other cause of stroke than the initial disease itself. Brain biopsy confirmed in the last patient the presence of a non inflammatory vasculitis.

Conclusion: In patients with Eales disease, stroke due to a non inflammatory vasculitis can occur. We suggest a careful follow-up must be performed in patients with Eales disease to prevent stroke. Despite the fact that the clinical hallmarks are vitreous or retinal hemorrhages, a prevention with antiplatelet therapy could be considered.

**P693**

**Percutaneous transluminal angioplasty and stenting of intracranial stenosis in the posterior and anterior cerebral circulation.** V. Puetz, G. Gahn, U. Becker, D. Mucha, A. Mueller, H. Reichmann, R. von Kummer, University Hospital Dresden (Dresden, D)

Objective: To review our experience with percutaneous transluminal angioplasty (PTA) and stenting of intracranial atherosclerotic stenosis including mid-term follow-up.

Material and Methods: From 06/99 to 12/02 we treated 12 patients (mean age 55 years) with stenosis of intracranial arteries with either PTA [6] or stenting [4] alone or a combination of these procedures [3]. Stenosis was localized at the M1-segment of the middle cerebral artery [7], basilar artery [1] and intracranial internal carotid [4] or vertebral artery [1], respectively. Indications for intervention were symptomatic ischemic stroke with either perfusion deficit of the related territory [3] or simultaneous stenosis of the contralateral artery [1], failure of medical therapy with either progredient stenosis or recurrent ischemic stroke and transient ischemic attack (TIA) [7], or asymptomatic stenosis with perfusion problems secondary to occlusion of the contralateral artery or tandem stenosis [2]. Clinical assessment and transcranial doppler sonography (TCD) were performed before and after the procedure. Follow-up was done by clinical interview and TCD.

Results: Nine of altogether 13 procedures (69.2%) were technically successful (< 50% residual stenosis). The other 4 procedures (30.8%) were partially successful with a residual stenosis from 60 to 70%. Mean stenosis was reduced from 76.7% (57–99%) to 28% (0–70%). Stroke as a periprocedural complication occurred in 3 patients (23.1%) with one major stroke secondary to postprocedural intracranial hemorrhage, and 2 minor embolic strokes. Asymptomatic dissection was noticed in 4 patients. Follow-up could be performed after 12 procedures for a mean period of 20.3 [1–36] months. Restenosis was detected in 4 patients (25%) that could be successfully treated by renewed PTA in 1 patient. One patient experienced TIAs 30 months after the procedure. No further TIAs or recurrent ischemic strokes were observed.

Conclusion: In selected patients PTA and stenting of intracranial artery stenosis is a treatment option resulting in good clinical outcome.

**P694**

**Study on stroke epidemiology in open population of 25–74 year-old men and women in a large city, Republic of Buryatia.** O. A. Klochikhina, V. V. Shprakh, S. P. Vinogradov, T. E. Vinogradova, Irkutsk Post-Graduate Medical Institute, Novosibirsk Blood Circulation Pathology Institute (Ulan-Ude, Irkutsk, Novosibirsk, RUS)

Background and purpose: Stroke incidence rate in Russia is one of the highest in the world, and mortality rate from stroke goes third after heart diseases and tumours of all locations. Besides, the higher stroke epidemiology index is registered in eastern regions of Russia as compared to the western ones.

The aim of our work was to study rates of incidence, attack, mortality and case fatality from stroke in Ulan-Ude city – in order to organize treatment and prevention of acute stroke.

Material and methods: The study was executed with a Register method according to uniform criteria of Methodological Instruction of Russian National Association of Fight against Stroke (2001). Study of epidemiological indexes of stroke was conducted in open population of men and women older than 25 years, in a district of Ulan-Ude city with a number of residents 138,496, among them 65,905 men and 72,591 women. The number of stroke cases registered for 1 year was 331. The cohort study in age group 25–74 years included 203 cases of first and recurrent stroke: 106 – among men (52%) and 97 – among women (48%).

Results: Standardized according to the European standard, stroke rate in Ulan-Ude for 1-year period was 234 per 100,000 population: 298 per 100,000 for men and 170 per 100,000 for women. Non-fatal incidence (not finished with case fatality in a period within 28 days from the onset of the stroke) was 156 cases per 100,000 population: 194 – among men, 118 – among women.

Stroke attack rate was 349 cases per 100,000 population: 450 – among men, 248 – among women. Non-fatal attack rate was 217 cases per 100,000 population: 292 – among men, 143 – among women.

First stroke case fatality rate was 78 per 100,000 population: 104 – among men, 52 – among women. All stroke case mortality rate (first and recurrent stroke cases) within 28-days period from the onset of the stroke was 131 cases per 100,000 population: 158 – among men, 103 – among women.

Conclusions: Epidemiological study of stroke in the open population of Ulan-Ude city demonstrates the high rate of incidence, attack and case fatality and shows the necessity of further investigation of stroke risk factors.

**P695**

**Post-stroke depression: no special stroke location or aetiology.** S. Pires-Barata, I. Henriques, Hospital Espirito Santo (Évora, P)

Background and purpose: Depression is one of the neurobehavioral sequelae of stroke. The correlation between stroke location, stroke type and post stroke depression is still controversal. We studied the relation between stroke location and stroke type with the occurrence of depressive mood after stroke.

Materials and Methods: We interviewed 71 first ever ischemic stroke patients (40 men and 31 women), 2 to 48 months after stroke. Age varied between 19 and 81 years, with median of 63. We used the Hamilton 21-D scale for depression and the MMSE (Mini Mental State Exam, adapted for our population) for cognitive impairment. Patients with previous history of depression were excluded. We considered the Oxfordshire classification for ischemic stroke location and TOAST criteria for etiology classification. We considered anterior circulation infarcts (anterior or medial cerebral artery infarcts) and posterior infarcts (cerebral posterior artery territory). In this prospective, descriptive and correlational study we used the statistical programme SPSS 10.0 for Windows and Chi-square and the C-Pearson.

Results: In our 71 first ever ischemic stroke patients, 36 fulfilled criteria for depression. From 60 patients with anterior location, depression was observed in 33. Having an anterior lesion was not associated with depression ($c = 0.197$, $p = 0.091$) or any stroke etiology. From 37 patients with lacunar infarct, 20 were depressed. We observed a trend for that depression was related with cognitive impairment but that was not statistically significative ($c = 0.254$, $p = 0.027$). 17 patients were taking antidepressive medication after stroke.

Discussion: In our sample half of the patients presented depression after stroke. Anterior location was observed in 85% of them, but no relation was found between stroke location and depression occurrence. No specific stroke etiology was also related with depression after stroke. Comparing different stroke locations and subtypes in post-stroke depression patients might contribute to improve preventive and therapeutical measures for target patients.

**P696**

**Saddle emboli of the carotid bifurcation and detection by colour duplex ultrasonography – case report.** O. G. Tuncer, Y. Krespi, O. Coban, R. Tuncay, S. Bahar, Istanbul University (Istanbul, TR)

Background: Approximately 15–30% of all ischemic strokes are of cardioembolic nature. Emboli generally lead to occlusions of intracranial vessels and proximal embolic occlusion of cervico-cephalic arteries is a rare condition.

Case Report: A 48-year-old male patient with a past medical history of mitral stenosis due to rheumatismal heart disease and mitral valvuloplasty presented with the recent onset of fatigue and abdominal pain. During his follow-up in the emergency room, he suddenly developed a transcortical motor aphasia and right-sided hemiparesis. Cranial MRI showed an acute left striatocapsular infarct. Emergency color duplex ultrasonography of

the neck vessels displayed mobile saddle emboli in the left carotid artery bifurcation, extending to the external and internal carotid arteries. Transcranial color Doppler imaging revealed bilaterally normal middle cerebral arteries. Transesophageal echocardiography showed severe mitral stenosis, moderate insufficiency of the aortic and tricuspid valves, and thrombus in the left atrial appendice. The patient was immediately anticoagulated and emergency thromboembolectomy performed. A follow-up color duplex imaging revealed a normal carotid bifurcation with an intact intimal lining of the external and internal carotid arteries. The patient's neurological status recovered completely at the end of hospital stay.

Conclusion: Emboli originating from the heart may theoretically lodge in a healthy carotid bifurcation and give rise to saddle emboli, but such a condition is rarely, if ever, documented.

Color duplex imaging is a powerful, non-invasive tool that can detect mobile thrombi in the carotid arteries. Presence of a mobile thrombus in the carotid bifurcation with the characteristics of saddle emboli implies that the stroke is of embolic nature and may prompt to an urgent thromboembolectomy to prevent further neurological damage.

**P697**

**Stroke as an initial manifestation of intracranial vascular malformations.** D. Kozic, M. Lucic, N. Sternic, J. Zidverc-Trajkovic, A. Pavlovic, S. Popovic, N. Prvulovic, M. Spirovski, Institute of Oncology, Institute of Neurology (Sremska Kamenica, Belgrade, YU)

Purpose: The aim of the study was to evaluate the stroke appearance as an initial clinical presentation in patients with intracranial vascular malformations (IVM). IVM were divided into arteriovenous malformations (AVM), cavernous angiomas (CA) and venous angiomas (VA).

Methods: Medical records of 51 patients with neurologic diagnosis of AVM, 46 with CA and 15 with VA, revealed by head MRI, were reviewed for stroke history.

Results: Clinical signs of stroke were predominant in 18 patients (35%) with AVM. In 13 of them intracerebral or subarachnoid hemorrhage was revealed by MRI while in 5 patients clinical signs of hemiparesis with no MR evidence of hemorrhage were found. Seizures were the initial symptom in 21 patients with AVM. Headaches, pyramidal/extrapyramidal syndrome, vertigo and clinical signs of intracranial expansile process were revealed in 3, 1, 1 and 7 patients, respectively. Spastic bihemiparesis was present in 1 patient with intraventricular AVM while spastic triparesis was revealed in 1 patient with pontomedullar AVM. Stroke-like symptoms were evident in 8 patients (17%) with CA, in 5 of them because of intracranial hemorrhage. Seizures were initial manifestation in 67% of patients with CA, while vascular headaches, ataxia, transitory hemifacial spasms and ophthalmoplegia were revealed in minority of patients. One patient with CA located in medulla oblongata had been clinically misdiagnosed and mistreated for multiple sclerosis for several years. Multiple CA were evident in 15% of patients. VA were revealed as an incidental finding in 7 patients. Seizures were the reason for MR examination in 3 while hemiparesis with or without headaches was the main clinical complaint in 4 patients with VA.

Conclusion: Stroke is rather frequent initial manifestation in patients with intracranial AVM, mostly due to resultant intracerebral or subarachnoid hemorrhage. Patients with CA much more frequently present with seizures than with stroke. Our impression is that most VA are revealed incidentally, especially on postcontrast images.

**P698**

**Complications and ultrasound findings after percutaneous transluminal stent angioplasty of the internal carotid artery: a mid-term follow up.** U. Becker, G. Gahn, T. Goldhagen, A. Müller, D. Mucha, H. Reichmann, R. von Kummer, University of Technology (Dresden, D)

Objective: To assess complications after percutaneous transluminal stent angioplasty (PTSA) in the proximal internal carotid artery (ICA).

Material and Methods: Since 01/2000, we consecutively and prospectively investigated the clinical, neuroradiological and sonographical course of 72 PTSA in the ICA of 66 patients (mean age 67 ± 9 years, 12 women, 54 men). Indications for intervention were symptomatic stenosis ≥ 70% [46], asymptomatic progressive or critical stenosis ≥ 80% [23] and symptomatic stenosis < 70% [3]. We used self-expanding stents in conjunction with angioplasty without balloon dilatation before stent placement. All patients received a combination of aspirin and clopidogrel 3 days before and 4 weeks after PTSA. We performed in all patients neurological examination (NIH-stroke scale) and color-coded duplex sonography (CCDS) before and after the procedure. Periinterventional diffusion-weighted MRI (DWI) was available in 56 patients (77.8%). Follow up was performed by CCDS and clinical interview after 1, 4, 10, 22 and 34 months (mean follow up 9 ± 7 months).

Results: During PTSA, 1 patient suffered a major (1.4%) and 3 patients a minor stroke (4.2%). In all of these and in 8 other patients DWI showed new ischemic lesions (16.7%). CCDS detected 7 residual stenoses (9.7%). Six of them were due to narrowing of the stent, were not progressive and remained asymptomatic. One of them was due to an acute symptomatic stent-thrombosis, which was successfully locally treated with abciximab and urokinase.

During follow up, 2 patients (2.8%) had a recurrent stroke, one ipsi- and one contralateral to the stent. Four patients (5.6%) died due to another reason than stroke. CCDS detected 15 restenoses ≥ 50% (20.8%), of which 3 were high-graded (≥ 70%). In 2 out of these 3 patients, diagnostic angiography was performed and showed intimal hyperplasia. All restenoses remained clinically asymptomatic.

Conclusion: PTSA has an acceptable clinical risk in the treatment of symptomatic or critical ICA stenoses and seems to be effective, because in this mid-term follow up the recurrence rate of stroke is low. Based on CCDS restenosis occurred in about 21%, but its clinical relevance remains unclear.

**P699**

**Three and four dimension ultrasonographic surface morphology characterization of the atheromatic carotid plaque.** I. Heliopoulos, K. Vadikolias, A. Chatzisotiriou, D. Artemis, H. Piperidou, I. Mylonas, Democritus University of Thrace, General Army Hospital of Thessalonica, Aristotle University of Thessalonica (Alexandroupolis, Thessalonica, GR)

The development of the three dimensional (3D) ultrasound has resulted in new approaches to the imaging of the carotid arterial system. It is known that carotid artery atheromatic disease is a common cause of ischemic stroke. The aim of our study was the application of the method for the characterization of the surface of the carotid plaque.

Material and Method: We included patients with at least one significant stenosis (> 50%) of the carotid artery, as classified by 2D Color Coded Doppler Ultrasound criteria. The appearance of the plaque surface was determined according to the 2D examination as: a. smooth and regular (n = 15) b. irregular (n = 27) and c. ulcerated (n = 3). The volumetric data acquisition was made using a linear 7–4 MHz scan probe. Post processing data analysis and reconstruction of 3D images were performed using a 3D software (3D version 4.0-Kretztechik AG). The best representation of the plaque surface was obtained by analysis with 6 degrees of freedom – 6DOF – (4D imaging).

Results and conclusions: 3D ultrasound examination confirmed the 2D classification for each atheromatic plaque. The free vessel lumen of the surface of the plaque and the vessel wall were better portrayed throughout the segment of the carotid artery that was within the 3D data set. In conclusion, due to the fact that the vessel and the plaque, which it contains, represent a complex 3D structure, 3D and 4D techniques provide additional information about the surface contour and the morphology of the atheromatic plaque. These may have important clinical implications in serial follow-up studies and be helpful in the selection of the appropriate therapeutic strategy.

**P700**

**Lacunar stroke outcome. Influence of cerebrovascular risk factors.** V. Mejías, E. Díez Tejedor, B. Fuentes, S. Monteagudo, J. Gracia, S. Escalante, Hospital La Paz (Madrid, E)

Background: There are few studies about cerebrovascular risk factors related to poor functional outcome in patients with lacunar stroke, and besides these associations vary in different reported series. Our aim was to evaluate the real influence of these factors in functional outcome of patients with lacunar stroke.

Methods: Observational and sequential study from the Stroke Unit Registry during 1994–2001. We selected patients with diagnostic criteria of lacunar stroke. Cerebrovascular risk factors (sex, age, coronary heart disease, hypertension, diabetes mellitus, hyperlipidemia, smoking, alcohol consumption, atrial fibrillation, valvulopathy, peripheral vasculopathy) were collected; and functional outcome at discharge (death/dependent) were determined by the modified Rankin Scale. Statistical analysis: $\chi^2$ test, Student's t test exact Fisher's test, univariate and multivariate logistic regression analysis

Results: Of 3735 patients with stroke, 783 (21%) had lacunar stroke; men: 63.7% and women 36.3%, with a mean age of 69.5–11.8 years. The logistic regression analysis showed that death or dependency were independently associated with older age (OR: 1.047, $p < 0.001$), diabetes mellitus

(OR: 1.665, $p < 0.02$) and previous cerebral infarction (OR: 2.246, $p < 0.079$). The other variables studied didn't show significant differences.

Conclusions: Age, diabetes mellitus and previous cerebral infarction were independent predictors of poor functional outcome of lacunar stroke. Agree with some previous report and reinforce the relevance of vascular risks in this aspect. Further prospective studies are needed to give consistency to these associations.

P701

**Haemorrhagic transformation in acute ischaemic stroke. Cerebrovascular risk factors and antithrombotic treatment.** S. Monteagudo, B. Fuentes, E. Díez Tejedor, J. Gracia, V. Mejías, S. Escalante, Hospital La Paz (Madrid, E)

Background: Haemorrhagic transformation (HT) appears in 10–70% of cerebral infarcts (CI) and the frecuency is greater in large sized and mass effect cardioembolic cerebral infarction. Other factors for occurrence aren't well-known. Our goal is to evaluate the influence of cerebrovascular risk factors and previous antithrombotic treatment on HT considering both predictive value and outcome relevance.

Methods: Consecutive patients with non lacunar infarction (NLI) attended in Stroke Unit [1994–2001] were selected. Inclusion criteria were previous independent functional situation and evolution time < 48 h. Findings CT scans on admission, at 48 h and if clinical worsening were classified into asymptomatic or symptomatic HT (decrease at least 1 point in the Canadian Stroke Scale). We used the Modified Rankin Scale to quantify functional outcome. Risk factors: age, sex, hypertension, diabetes mellitus, atrial fibrillation (AF), valvulopathy, coronary heart disease, dyslipidemia, smoking, alcoholism, previous cerebral infartion or TIA were collected as well as previous antiplatelet and anticoagulant treatment. Statistic analysis: chi square, t-Student, U-Mann Whitney, multivariate logistic regression.

Results: Of 1836 patients with NLI, 113 developed HT. AF was significantly associated with HT. Conversely patients with dyslipidemia or previous CI developed less HT. In the multivariate model only AF appears as independent factor for HT. Age was significantly associated with a poor outcome both in asymptomatic and symptomatic HT. Analysing only symptomatic HT group did not find any relation between these factors and functional outcome.

Conclusions: AF is an independent risk factor for HT in NLI. We did not find association between other risk factors and previous antithrombotic treatment and HT. Age is the only independient predictor of poor functional outcome in patients with HT.

P702

**Some epidemiological characteristics of young adult patients with ischaemic stroke in a university hospital of Turkey.** E. Köseoglu, A.-Ö. Ersoy, Y. Karaman, Erciyes University Medicine Faculty (Kayseri, TR)

The study was conducted on patients with the ages of 15–45 years with a first ever arterial ischemic stroke (n = 146, 70 males, 76 females) or a cerebral venous trombosis (n = 7, all female) in a four year period to evaluate some epidemiological characteristics of the patient group. Fifty-two male patients (74.3% of males) were aged over 35 years, while 42 female patients (50.6% of females) were under 35 years. Most common cause of cerebral infarction was found to be atherosclerosis (n = 47, 32.2%), followed by cardiac (n = 35, 24%), hematologic (n = 29, 19.9%) causes and nonatherosclerotic vascular disease (n = 3, 2%). In 19 female patients, hematologic cause related to pregnancy or puerperium was present. In 32 patients (21.9%) the etiology could not be found. However in 18 of them, one of the risk factors for atherosclerotic disease existed. Of the relatively rare causes reported in the literature, anticardiolipin antikor, alcohol intake, malignancy, high fibrinogen level, deficiency of protein C or S, Factor V Leiden mutation, ovulation induction, vasculitis, carotid artery dissection and migraine were identified in total, 14 of the patients (9.6%). Five patients with cerebral venous occlusive disease were pregnant or in postpartum period. The remaining two cases were caused by infection (otitis media, mastoiditis and mumps). Pregnancy and puerperium period in women seems to be a vulnerable period for ischemic events because 29% of the cases (n = 24) in our study have occurred in this period. Ten of the patients in this period also had anemia.Thirteen patients with cerebral infarction (9%) died within seven days after the disease onset. In 7 of the deaths, the cerebral infarction was related to cardioembolic causes.

As a result, atherosclerosis seems to be the most common cause of arterial ischemic stroke as it is in older patients. Additionally, risk factors for atherosclerosis are frequent in the patient group with unidentified cause. Anemia has been found to be a common associative of ischemic events in women in pregnancy and puerperium period.

P703

**Predicting severe strokes using common carotid artery intima media thickness in association with other stroke risk factors.** P. Talelli, A. Chrisanthopoulou, G. Terzis, G. Gioldasis, S. Papapetropoulos, A. Argyriou, J. Ellul, University of Patras (Patras, GR)

Background: Common carotid artery intima media thickness (CCA-IMT) has been identified as an independent and possibly early marker of atherosclerosis. Increased values of CCA-IMT have been significantly associated with the presence of certain cardiovascular risk factors both in stroke patients and controls. The hypothesis that the brain affected by atherosclerosis might by more vulnerable to the "ischemic insult", and thus resulting in a more severe stroke, has not been studied so far. Therefore, we set out to investigate the association between CCA-IMT, other stroke risk factors (alone and in combination) and initial stroke severity.

Methods: 284 consecutive patients (mean age 68.9 (±12.9) years, 125 (44%) females) with an acute ischemic stroke, were investigated with carotid ultrasound examination. CCA-IMT measurements were carried out by a single operator. Demographic data, cardiovascular risk factors and neurological assessments were available for all patients. The Scandinavian Stroke Scale (SSS) score on admission was used to define two categories of stroke severity ("mild" (score > 44) and "severe strokes"). According to CCA-IMT values patients were divided into two subgroups (cut point was set at 0.8 mm).

Results: Of all stroke risk factors, including CCA-IMT, atrial fibrillation, only, was associated with stroke severity. The association remained statistically significant after adjusting for age, sex, hypertension, diabetes mellitus, smoking, ischemic heart disease and CCA-IMT [OR = 4.07 (95%CI 1.11–14.89)]. CCA-IMT was not associated with stroke severity even after looking at each stroke subtype separately. Similar were the findings in the subgroup of patients with low CCA-IMT values. In the subgroup with high CCA-IMT values, only history of arterial hypertension was significantly associated with stroke severity [adjusted OR = 4.07 (95%CI 1.22–13.59)].

Conclusion: Of the investigated stroke risk factors, including CCA-IMT, only atrial fibrillation was associated with initial stroke severity. However, history of arterial hypertension in patients with increased CCA-IMT was a predictor of more severe strokes on admission. These patients might be targeted for more intensive prevention measures.

P704

**Intracranial subdural empyema in children, burrhole evacuation and outcome in relation to the initial and post treatment CT scan of the brain parameters.** J. Abdullah, M. Md. Ralib, A. R. Mohd.Ariff, I. L. Shuaib, University Sains Malaysia (Kubang Kerian, Kelantan, MY)

This study was done to predict the outcome of patients with intracranial subdural empyema using the initial and post-treatment CT scan brain parameters. The other objectives include correlating the clinical features with the initial CT scan parameters and also the outcome. It was intended to use the clinical features and the initial CT scan parameters to prognosticate the patients' outcome before surgery.

There were 24 clinical and CT scan variables studied with two sets of outcomes. The first set measured the good and poor outcomes. The second set measured the survival of the patients.

A total of 24 patients were included in the study. There was only one significant scan variable to predict the patients' outcome. The presence of hypodense areas in the initial CT scan, which could be attributed to areas of cerebral infarction, had a significant influence ($p < 0.05$) on the outcome. Patients with hypodense areas had poor outcome compared to patients without hypodense areas. None of the post-treatment CT scan parameters had a significant bearing on the patients' outcome.

For clinical parameters, the level of consciousness at presentation had a significant influence ($p < 0.01$) on the patient's outcome. Patients with low level of consciousness tend to have a poor outcome. Two patients who showed response to stimulation only died. Patients who were 'alert and orientated' and 'drowsy and disorientated' were still alive.

Correlating the level of consciousness with the initial CT scan parameters, only the presence of parafalcine extension had a significant correlation with the level of consciousness. The three patients with parafalcine extension had a low level of consciousness at presentation. Correlating with other factors, all three had diffuse subdural empyema at presentation. This indirectly indicates that patients with diffuse subdural empyema will have a low level of consciousness at presentation.

From this study, we also observed that patients with an extensive subdural epyema had a good outcome if treated early and aggressively with antibiotics and burr evacuation. The important factors that contributed to the good outcome in patients subdural empyema were early treatment

with antibiotics and burr evacuation. This must be performed before the patient had deterioration in the level of consciousness. The patient had a low level of consciousness, an urgent burr hole evacuation wouldn't make any difference, which was apparent in this study.

P705
**Long-term prognosis of 77 patients with reversible ischaemic neurological deficit.** P. Atanassova, M Vukov, N. Tchalakova, Higher Medical Institute, National Health Information Centre (Plovdiv, Sofia, BG)

Background: Very few studies have addressed the long-term prognosis of patients suffering reversible ischaemic neurological deficit (RIND). The RIND syndromes are ischaemic strokes in which the neurological deficit lasts more than 24 hours and disappears completely for days or at most for 3–4 weeks.

The aim of the present study is to perform a three-year follow-up of patients with first-ever RIND and to determine the rate of recurrent cerebrovascular events (nondisabling and disabling strokes), and the rate of cardiovascular and the fatal accidents after the first ischaemic stroke.

Material and methods: The subjects of the study were 77 RIND patients (21 women, 56 men) with an average age 61 ± 4.6 years, referred to a University Hospital, Plovdiv. Neurological deficit was evaluated using the modified Rankin scale. Demographic data, medical history, vascular risk factors, laboratory findings and imaging data were recorded for each patient. The patients were followed prospectively for 36 months and were seen every 3–6 months. Kaplan-Meier estimates were used to determine cumulative period after the first ischaemic stroke.

Results: During the course of the study 4 patients died, and in 3 the cause was vascular death. A stroke occurred in 15 patients (1 TIA, 16 ischaemic strokes, 1 intracerebral haematoma, 1 fatal ischaemic stroke). The subsequent strokes were more often disabling. Five patients had a major cardiac event – fatal myocardial infarction in 2 patients, and nonfatal myocardial infarction in 3 patients. One patient died from nonvascular death (carcinoma). The cumulative incidence for three-year time-period was 0,17 for recurrent nonfatal ischaemic stroke.

Conclusion: The RIND syndromes are powerful predictors of major cerebrovascular accidents. This study highlights the heterogenity of recurrent strokes after first RIND.

P706
**Cerebral venous thrombosis: a review of 30 cases.** J. M. Callejo, C. Sánchez-Bueno, E. Riva, R. C. Ginestal, J. Masjuan, J. Martínez-Castrillo, Ramón y Cajal Hospital (Madrid, E)

Background and objectives: Cerebral sinus thrombosis is not an infrequent cerebrovascular disease. It has a wide spectrum of clinical presentations and etiologies. Cerebral venous thrombosis (CVT) is more frequently diagnosed in recent years due to the available neuroimaging techniques. The prognosis is variable, and outcome may range from complete recovery to death. The treatment with anticoagulant drugs leads to a benefit for patients, although there are few randomized trials upon which to base treatment recommendations. We have reviewed the patients with CVT of our hospital, to determine the etiologies, clinical patterns, neuroimaging findings and final outcome.

Patients/methods: Retrospective review of the clinical and radiographic records of patients diagnosed of CVT from 1990 to 2002. We analyzed clinical patterns, etiologies and neuroimaging findings of these patients.

Results: Thirty patients (18 women), mean age of 46.1 years (range 17–77) were analyzed.

The more frequent etiologies were hematological disorders (23.3%), oral contraceptives (16.7%), cancer (13.3%), infections (10%), others (13.3% – puerperium:2; ulcerative colitis:1; tamoxifen:1 –), and unknown (20%). The most frequent clinical pattern was headache (76.7%), followed by focal cerebral signs (46.6%), seizures (40%), papilledema (36.7%), visual alterations (36.7%), and decreased level of consciousness (13.3%). The diagnosis of CVT was made by neuroimaging techniques, CT or Magnetic Resonance Imaging (MRI)- Magnetic Resonance Angiography (MRA), in all of the cases. Cerebral angiography was only performed in seven patients, to confirm the diagnosis. The most frequent site of venous occlusion was superior sagittal sinus (63.3%) followed by transverse sinus (40%). Most of the patients (76.7%) received anticoagulant therapy (intravenous heparin during the acute phase and oral anticoagulation for six months). Clinical outcomes were as follows: 50% of the patients were asymptomatic, there were mild sequelae in 20%, moderate-severe in 23.3%, and 6.7% of the patients died.

Conclusions: In this series the diagnosis was made by CT or MRI/MRA in all cases. Cerebral angiography is only reserved for cases whose diagnosis remains uncertain with MR. Hematological disorders were the most frequent etiological factor. CVT is a severe cerebrovascular disorder, although one half of the patients were asymptomatic on follow-up, one third of them had moderate-severe sequelae or died.

P707
**Cerebral venous thrombosis diagnosis and management: a 5-year experience.** S. Erimaki, M. Tzagournissakis, M. Mavridis, K. Pagonidis, O. Papakonstantinou, A. Plaitakis, University Hospital (Heraklion, GR)

Background: Cerebral venous thrombosis (CVT) is a potentially serious, but treatable disorder. Its remarkable diversity makes it a diagnostic challenge. Moreover controversy about treatment still persists, particularly regarding the use of antithrombotic agents.

Aim: To describe 11 cases with CVT in order to identify risk factors, to review means for early diagnosis and to assess the safety and efficacy of acute anticoagulation.

Methods: All cases of CVT diagnosed or treated in the acute inpatient service between 1997 and 2002 were reviewed. Patients were assessed for age, sex, predisposing factors, presenting symptoms. Imaging studies were reviewed. Treatment modalities and outcomes were assessed.

Results: Eleven cases of CVT (9 women, 2 men), aged 20–70 years (mean 46 years) were studied. Symptoms in presentation included headache (10/11), seizures (9/11), hemiparesis (10/11), stupor (4/11), nausea or vomiting (3/11) and papilledema (1/11). Predisposing factors were identified in 8 cases and included malignancy (3/11), estrogen treatment (2/11), anorexia nervosa (1/11), head trauma (1/11) and protein S deficiency (1/11). Brain MRI showed venous infarcts with (6/11) or without (5/11) hemorrhage, and brain MRV revealed the presence of thrombus in the sinuses or the deep veins. Nine patients were diagnosed immediately after admission and received treatment with intravenous heparine followed by acenocoumarol. Eight of them showed stabilization and improvement immediately after treatment despite the absence of thrombus recanalization. One patient died because of multisystem organ failure due to ruptured aortic aneurysm. Two patients were transferred to our department several days after the onset of symptoms and although they received heparine they did poorly. Long-term outcome was assessed in five patients. Four of them showed no focal neurologic deficits and no CVT recurrence.

Discussion: CVT should be considered in cases of progressive headache, particularly when associated with seizures, neurological symptoms and signs or papilledema. Magnetic resonance imaging with magnetic resonance venography is the investigation of choice. Anticoagulation with heparine and acenocoumarol remains the first-line treatment for CVT because of its efficacy, safety and feasibility, even in the presence of hemorrhagic transformation. This treatment should be introduced as soon as the diagnosis is made since early treatment affects the final prognosis.

P708
**Thrombotic risk factors in Turkish ischaemic stroke.** G. Celiker, U. Can, F. B. Atac, M. Kilinc, U. S. Benli, N. Ozbek, H. Verdi, Y. Kaya, Baskent University (Ankara, TR)

Altered hemostasis has been reported to occur in different clinical conditions such as ischemic coronary disease, peripheral vascular insufficiency, acute ischemic stroke and reversible cerebral ischemia. In addition to biochemical markers, point mutations and single nucleotide polymorphisms in coagulation factors have been intensively studied in terms of their association with stroke. Therefore the precise role of Factor V Leiden (FVL), prothrombin (Pt) A20210G and methylene tetrahydrofolate reductase (MTHFR) C677T mutations and angiotensin I converting enzyme (ACE) I/D polymorphisms in the pathophysiology of ischemic stroke is investigated in Turkish ischemic stroke patients. We collected 150 patients with acute ischemic stroke. This is the preliminary report of our study concerning the probable role of the mentioned coagulation factors.

We found that the heterozygoty of FVL mutation was 12%, and among these patients only one person was homozygot mutant. Pt heterozygoty was 2% and MTHFR heterozygoty was 0.7%. ACE I/D genotype was 35.3%, D/D genotype was 52.7% and I/I genotype was 12%. When ischemic stroke patients were compared with normal population only the ACE D/D genotype, which is an enhancing factor for thrombosis, reached statistical significance (Chi Square test, $p = 0.011$).

P709
**Intracranial stenosis in ischaemic stroke patients: relation with traditional atherosclerotic risk factors?** I. Henriques, S. Pires-Barata, L. Rebocho, Hospital Espírito Santo Évora (Evora, P)

Background: Biological basis of intracranial atherosclerosis is supposed to be similar to that of atherosclerosis affecting other major arteries but no definitive data exist on such matter. We analyzed the characteristics of ischemic stroke patients including the relation with traditional risk factors for atherosclerosis and intracranial stenosis occurrence.

Methods: We studied a hospital sample of 347 consecutive first ever ischemic stroke patients (345 Caucasians) with median age 65 [19–88]. Patients were studied according to a protocol including at least one CTscan or MRI as well as Triplex scan, echocardiography, demographic and clinical data. We considered hypertension, diabetes, dislipidemia, and smoking as major risk factors. We excluded patients without or with technical unsatisfactory transcranial Doppler (n = 44). Location and presumed etiology were according to the Oxfordshire and TOAST criteria. Statistical methods included Chi-square test and logistic regression analysis.

Results: From 303 patients, 79 (26.1 %) had intracranial stenosis or occlusion. There was no relation between intracranial stenosis and the presence of any risk factor (hypertension $p = 0.1971$; diabetes $p = 0.6891$; dislipidemia $p = 0.6286$; smoking $p = 0.9305$) neither with stroke location, age ($p = 0.2571$) or sex ($p = 0.2571$). Intracranial stenosis was related with worst Rankin at discharge ($p = 0.0474$, O.R:0.59; 95 % CI: 0.35–0.99).

Discussion: In our sample no association between traditional risk factors for atherosclerosis was found in ischemic stroke patients with intracranial stenosis or occlusion. There was neither an association with age or sex as is the case for atherosclerotic disease. Since Rankin at discharge was worst in this group of patients, extra effort must be done to understand the pathophysiology including any genetic, race or life style susceptibility to intracranial artery lesion, in order to optimize patient's management.

P710
**Carotid angioplasty in the older patient.** K. Rabe, H. Gödel, R. Perron, C. Rubel, W. Pfeil, K.-F. Beykirch, R. Theis, H. Sievert, Cardiovascular Center Bethanien, Bethanien Hospital (Frankfurt, D)

Introduction: Carotid angioplasty and stenting to treat stenoses of the carotid artery in older patients is an alternative to surgery. We would like to show that it is a safe and efficient method in these high-risk cases.

Patients: Since September 1995 we treated 38 stenoses in 35 patients older than 80 years. Mean age was 84 years ± 2 years. 41 % suffered from CHD, 67 % from Hyertension and 31 % from Diabetes mellitus. 46 % were symptomatic. Mean diameter stenosis was 80 ± 8 %, mean length of lesion 11 ± 7 mm. One patient had a thrombus in the lesion. 31 had a contralateral stenosis and 3 patients underwent a contralateral angioplasty in a second session.

Methods: In 32 out of 37 lesions we used one of the following embolic protection devices: Parodi Anti-Emboli System in 6, MO.MA in 4, Percusurge in 4, Angioguard filter in 4, EPI filter in 8, MedNova filter in 2, TRAP filter in 3 and the Interceptor filter in 1 case. All treated lesions received a self-expandable stent.

Results: The procedure was technically successful in 37/38 lesions. In one patient it was necessary to use a brachial access in a second session because a femoral access was due to the elongated vessels not successful.

During or immediately after the intervention 1 patient developed a TIA and 2 patients a minor stroke. 1 patient developed few hours after the intervention retroperitoneal bleeding followed by circulatory collapse, reanimation and an ipsilateral stroke. The patient died 6 days later due to asystolia.

Conclusion: Carotid angioplasty is a successful method to treat stenoses of the carotid arteries even in patients older than 80. Neurological complications are rare.

P711
**Hyperhomocysteinaemia and ischaemic stroke: lack of guidelines.** I. Henriques, L. Rebocho, L. Martins, M. Graca, Hospital Espírito Santo Évora (Evora, P)

Introduction: Fasting hyperhomocysteinemia is an independent factor for ischemic stroke. Both fasting test and an abnormal methionine-loading test are used to measure homocysteine (Hcy) levels. We studied consecutive ischemic stroke patients aged less than 65 years and looked for the prevalence of high plasma Hcy levels and the results of specific treatment.

Methods: We studied prospectively 162 consecutive ischemic stroke patients aged less then 65 years or of any age when etiologic investigation was negative. We measured fasting Hcy concentration by ELISA (FPIA) ($n < 15$ µmol/L) and 4 hours after the load of 100 mg/Kg of oral methionine ($n < 40$ µmol/L). We treated hyperhomocysteinemia patients during 1 year with folic acid (5 mg/day), vit B6 (150 mg/day) and we associated vit B12 if the Hcy levels were still elevated after 2 months of treatment. Median age was 56 years [19–64] and 111 patients (68.51 %) were male.

Results: From 162 patients, 15 (9.25 %) had hyperhomocysteinemia. Four had fasting Hcy levels above normal and eleven were identified after methionine-loading test. All patients completed 1 year of treatment according to the protocol and 14 normalised their Hcy levels and stopped their therapy after one year. One hypertensive patient had a new ischemic stroke after stopping therapy. One patient never controled his Hcy levels and is still on treatment without any recurrence.

Discussion: In our patients aged less than 65 years or free of any other risk factor for ischemic stroke, 9 % had Hcy levels above normal. Follow-up showed efficacy in correcting levels except for one patient. Data are missing about long-term management of these patients.

P712
**Does acute haemodilution affect cerebral blood flow velocities?** U. Can, A. Donmez, N. Gungor, D. Sargin, S. Mercan, Baskent University (Ankara, TR)

Acute neurological morbidity following repair of congenital heart disease in infants and small children is well documented. Patients with cyanotic heart defects and elevated hematocrit (Htc) levels have significant risk of an adverse neurologic event. Blood viscosity and Htc levels are the main determinants of cerebral blood flow (CBF), and cerebral perfusion as well. It has been demonstrated that hemodilution results in a decrease in cerebral oxygenation.

The aim of our study was to determine the effects of acute hemodilution on CBF velocities obtained with transcranial Doppler (TCD). After Ethics Committee approval 12 cyanotic and 16 noncyanotic children without known neurological disease between 2–36 months-old were studied. Standard anesthesia, and hypothermia protocols were used in both groups. Simultaneous recordings of haemodynamic variables, rectal temperature, Htc and $PaCO_2$ were performed. Peak systolic (PSV), end diastolic (EDV) and mean (MV) middle cerebral artery (MCA) velocities and derived parameters such as pulsatility index (PI) and resistance index (RI) were recorded with TCD at same periods. Statistical analyses were performed with Mann Whitney-U and Wilcoxon tests and $p < 0.05$ was considered as significant. In the cyanotic group preoperative Htc levels were significantly higher than the non-cyanotic group (40.75 ± 8.3 gr/dl versus 29.75 ± 3.4 gr/dl). In contrast to our hypothesis there was a statistically significant decrease in the PSV and MV (but not in other parameters) of the non-ciyanotic group.

In conclusion, although in many studies it is demonstrated that blood flow velocities are inversely proportional to viscosity our results demonstrated that acute hemodilution did not effect MCA flow velocity measurements.

P713
**Treatment with recombinant human alpha-galactosidase A improves cerebral blood flow velocity in Fabry patients.** M. J. Hilz, H. Marthol, M. Brys, G. Welsch, T. Haendl, B. Stemper, New York University School of Medicine, University of Erlangen-Nuremberg (New York, USA; Erlangen, D)

Background: Fabry disease is an X-linked disorder with glycosphingolipid storage in various organs, particularly in vascular endothelial and smooth muscle cells. Vessel wall alterations with narrowing of cerebral resistance vessels are likely to compromise cerebral blood flow velocity (CBFV) and to contribute to the early and increased incidence of stroke. Recently, enzyme replacement therapy (ERT) has become available in many countries.

Objective: In this study, we evaluated CBFV in Fabry patients before and after ERT.

Methods: In 22 Fabry patients (28.6 ± 8.3 years) and 24 male, age-matched healthy volunteers (28.6 ± 5.0 years), we monitored CBFV of the middle cerebral artery at supine rest using 2 MHz transcranial Doppler sonography. 11 patients (28.8 ± 9.1 years) received 0.9–1.1 mg/kg agalsidase beta every two weeks for 18 months, the other 11 patients (28.4 ± 7.9 years) received 0.9–1.1 mg/kg agalsidase beta every two weeks for 23 months. We compared CBFV values of patients before and after ERT using the two-sided Wilcoxon test. Moreover, patient values were compared to those of the controls using the two-sided Mann-Whitney-Test. Significance was assumed for p-values below 0.05.

Results: Before agalsidase beta therapy, CBFV was lower in the patients (43.8 ± 19.3 cm/s) than the controls (54.3 ± 11.5 cm/s; $p < 0.05$). After 18 and 23 months of ERT, mean CBFV of the 22 patients (53.4 ± 11.6 cm/s) was

higher than before ERT (43.8 ± 19.3 cm/s; $p < 0.05$), and no longer differed from the mean CBFV of the controls ($p > 0.05$). Conclusions: Cerebral angiopathy due to glycolipid storage is likely to account for the CBFV reduction in untreated Fabry patients. Normal CBFV after 18 or 23 months of agalsidase beta therapy supports the assumption that ERT clears glycolipid deposits from vessels and thus normalizes cerebral blood flow. Normalized CBFV is likely to be a surrogate marker of reduced cerebrovascular risk in Fabry patients.

Acknowledgement: This study was funded by Genzyme Corporation, Cambridge, MA, USA.

P714

**Endovascular stent-assisted angioplasty for symptomatic intracranial vertebrobasilar artery stenosis and dissection.** T. H. Lee, D. H. Kim, B. H. Lee, H. J. Kim, D. S. Jung, C. H. Choi, Pusan National University Hospital, Chosun University Hospital, Metrohospital Anyang (Pusan, Kwangju, Anyang, KOR)

Purpose: Stent placement has been shown to increase the safety and effectiveness of balloon angioplasty in every circulatory bed in which it has been applied. We investigated the feasibility, safety, and effectiveness of stenting for symptomatic intracranial vertebrobasilar artery stenosis and dissection.

Subjects and Methods: Between April 1998 and December 2002, 18 patients with symptomatic intracranial vertebrobasilar artery stenosis and dissection that produced stenosis of more than 50% were treated with stent-assisted angioplasty. We retrospectively analyzed the technical success rate of stenting, the procedure-related complications, clinical and angiographic outcome for 4 to 57 (mean 27.8) months of follow-up.

Results: The most common location was the distal vertebral artery (n = 13) followed by the vertebrobasilar artery (n = 3) and basilar artery (n = 2). Stent-assisted angioplasty was technically successful in all (100%) patients. Two stents were implanted in 3 patients. In all patients, the post-procedural angiography showed restoration of normal luminal diameter with smooth inner margin. There was no procedure-related complication such as stent thrombosis or vessel rupture. All patients were neurologically stable at the clinical follow-up. Angiographic follow-up (n = 2) revealed minimal restenosis (5%) after 5 months in one asymptomatic patient.

Conclusion: Endovascular stent-assisted angioplasty for symptomatic intracranial vertebrobasilar artery stenosis and dissection was feasible, safe, and effective for alleviating symptoms and improving blood flow to posterior circulation.

## Dementia/Higher function disorders

P715

**Alzheimer's disease: pathophysiological implications of measurement of plasma cortisol, plasma dehydroepiandrosterone sulphate and lymphocytic corticosteroid receptors.** F. Vecchio, E. Menegazzo, D. Armanini, C. Fattorello S., University of Padua (Mirano, Padua, I)

Background: It is known that aging can be associated with a disregulation of the hypothalamic-pituitaric-adrenal axis. These abnormalities are more frequent and evident in Alzheimer's disease, but the exact mechanism for the increase of plasma cortisol is not completely understood.

Materials and methods: We have studied 23 subjects, mean age 68 ± 10 SD with Alzheimer's type dementia. Patients were studied by physical examination, blood measurements, neuropsychological tests and imaging techniques (Electroencephalogram, computerized tomography scanner and magnetic resonance imaging). Patients with other types of dementia were not considered. The mean length of the disease was 4.3 ± 3.8 years. The controls were a random group of healthy subjects of the same age (66 ± 9) and sex. All subjets underwent measuring of plasma cortisol and dehydroepiandrosterone sulphate (DHEAS, by enzyme-immunoassay), of the number of Type I and Type II corticosteroid receptors in mononuclear leukocytes (by radioreceptorassay) and of the lymphocyte subpopulations (by cytofluorimetry).

Results: In Alzheimer' s disease, plasma cortisol was higher than in controls (27 ± 8 vs. 18 ± 4 mg/dl, $p < 0.001$). Plasma DHEAS, the ratio DHEAS/cortisol and the number of Type II corticosteroid receptors were significantly lower than in controls (respectively 68 ± 39 vs. 144 ± 76 mg/dl, 2.8 ± 1.8 vs. 8.3 ± 5.2 mg/dl and 1347 ± 445 vs. 1905 ± 567 receptors per cell, $p < 0.001$).

Conclusion: These data support the hypothesis of a disregulation of the adrenal pituitaric axis in Alzheimer's disease, which is probably the consequence of a damage of target tissues for corticosteroids. The implications of these endocrine alterations in the disease seem to be really important, even if we can not say at this moment whether they are primarily involved or an indirect marker of the disease.

P716

**Aphasia caused by topographical infarct in the left internal capsule.** S. Kory Calomfirescu, A. M. Mos, S. Iancu Deme, M. Kory Mercea, Clinical Hospital of Neurology (Cluj-Napoca, RO)

The clinical forms of aphasia because of subcortical lesions have only recently become known due to the use of computer tomography. Aphasia caused by a topographical vascular lesion in the left internal capsule of the left hemisphere (such as an infarct) can be ranged within these forms of subcortical aphasia.

Our study has been carried out on 100 patients with cerebral vascular stroke accompanied by aphasia. Sixty-nine cases (69%) had cerebral infarction. Eighteen cases (18%) out of the former ones had subcortical infarction with elements of aphasia. They were confirmed clinically and neurolinguistically, as well as by computer tomography.

Only two cases had topographical infarction in the left internal capsule, representing 2% of the total number of cases and 11.11% of the number of subcortical infarctions.

The neurolinguistic assessment of these patients pointed out certain features of aphasia: speech disorder in the form of atypical nonfluent aphasia, which was caused by an anterior (infarction type) lesion in the left internal capsule.

P717

**Relationship of plasma homocysteine and the gene polymorphisms of its related metabolic enzymes to Alzheimer's disease.** Y.-D. Zhang, Nanjing Brain Hospital (Nanjing, CHN)

Objective: To investigate the relationship of plasma homocysteine (Hcy) levels and the gene polymorphisms of N5, N10-methylenetetrahydrofolate reductase (MTHFR) and cystathionine beta-synthase (CBS) to Alzheimer's disease (AD).

Methods: The plasma Hcy levels was measured by means of high voltage capillary electrophoresis (CE) with UV detection, the polymorphisms of C677T in exon 4 of MTHFR gene and 844ins68 in exon 8 of CBS gene were analyzed by a combination of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 105 patients with AD and 102 healthy controls.

Results: The plasma Hcy in AD patients (16.04 ± 3.84 mol/L) was significantly higher than that in the healthy controls (11.94 ± 3.87 mol/L) ($t = 7.66$ $P < 0.001$). There were no significant differences of the genotype and allele frequencies of MTHFR C677T mutation and CBS 844ins68 mutation between the patients and controls. However, the plasma Hcy level was markedly higher in the subjects with T allele of MTHFR than that in the subjects with C allele.

Conclusions: The elevated plasma Hcy level in AD patients is probably involved in the pathogenesis of AD, which may be due to the environmental factor rather than the genetic factors of the mutations of MTHFR and CBS. Supplement of VitB12 and folate is in favor of the treatment on AD.

P718

**Vascular risk factors for cognitive dysfunction in type 2 diabetes mellitus: study design and preliminary data.** G. J. Biessels, S. M. Manschot, University Medical Center Utrecht on behalf of the Utrecht Diabetic Encephalopathy Study Group

Background: Type 2 diabetes is associated with cognitive deficits and dementia, particularly in the elderly. These deficits develop despite current diabetes treatment strategies. Given the prevalence of type 2 diabetes among the elderly and the impact of cognitive impairment and dementia on both quality of life and health care resources, the development of additional therapeutic interventions is important. We hypothesise that vascular dysfunction is a major determinant for the development of cognitive dysfunction in type 2 diabetes, and provides a target for future therapeutic interventions.

Methods: Fall 2002 we initiated a study, which will involve 150 type 2 diabetic patients and 75 age, education and sex matched non-diabetic controls. Recruitment will be completed by the end of 2004. Patients are recruited through their general practitioner. Assessment includes a neurological and neuropsychological examination and magnetic resonance imaging (MRI) of the brain, focussing on cerebral atrophy and ischaemic

changes. Cognitive function and MRI findings will be compared between patients and controls and related to macro- and microvascular function, in order to identify potential vascular risk factors. Indices of macroangiopathy include blood pressure, complaints of dysbasia and intima and media thickness of the internal carotid arteries. Indices of microangiopathy include the presence of micro-albuminuria and microvascular changes in the retina.

Preliminary results: As of February 2003 30 patients (average age 70 years; diabetes duration 10 years) and 15 controls (66 years) have been examined. Moderate cognitive dysfunction was observed in diabetic patients, particularly affecting complex information processing, attention and memory (effect sizes 0.4 to 0.7). On MRI white matter lesions (chi square test: deep: $p < 0.01$ periventricular: not significant) and (silent) cerebral infarction ($p < 0.10$) were more common in patients than in controls. Given the limited number of subjects at this time, an analysis of vascular risk factors for cognitive dysfunction was not yet performed.

Preliminary conclusions: Type 2 diabetes is associated with impairment of cognitive function and structural cerebral changes, as shown on MRI. We expect these cognitive changes to be associated with intra- and extra-cranial vascular dysfunction in future analyses.

P719

**Large-scale, observational study demonstrates broad spectrum of clinical efficacy of galantamine for Alzheimer's disease in Switzerland.** S. Schwalen, Janssen-Cilag GmBH (Neuss, D)

Introduction: Controlled clinical trials have shown that galantamine (Reminyl(R)) has a broad spectrum of efficacy including cognition, function and behaviour. We performed a large-scale observational study to assess the effects of galantamine in a naturalistic setting.

Method: The survey was performed by Swiss general practitioners. The observational period was 3 months. Doctors provided details of patients with AD who were receiving galantamine including demographic details, concomitant diseases and treatments. The Mini-Mental State Examination (MMSE) was performed at baseline and the last visit. Physicians also gave a clinical global impression (CGI) in several domains eg cognition, behaviour, depression and activities of daily living (ADL) at baseline and the CGI of change (better, equal, worse) at the last visit. Treatment discontinuation and adverse effects (AEs) were recorded.

Results: Data were collected from 1077 patients ranging in age from 42 to 105 (mean 78) years with 48 % aged > 79. There were more women than men (63 %). By the end of the study 66 % of patients received 16 mg and 29 % received 24 mg galantamine. Doctors intended to continue treatment beyond the study in 72 % of cases. Most patients (79 %) had at least one concomitant disease and the same proportion (79 %) were taking concomitant medication.

During the observation period, 189/1077 (17.5 %) stopped treatment with galantamine, the most frequent reasons were AEs (96, 8.9 %) and insufficient efficacy (68, 6.3 %). The most common AEs leading to withdrawal were nausea, vomiting and diarrhoea. Most doctors rated the tolerability of galantamine as very good (48 %) or good (35 %).

The efficacy of galantamine was assessed in 846 cases. Most doctors considered efficacy to be good or very good (53 %) or satisfactory (24 %). Mean MMSE was 19.9 (± 4.8) at Visit 1 and 21.1 (± 5.2) after 3 months. MMSE score improved in 504 (60 %), was unchanged in 140 (17 %) and decreased in 202 (24 %). Improvements were seen in attention in 59 %, behavioural disturbances in 51 % (depression 58 %, aggression 63 %, apathy 52 %), ADL in 22 % (eating 48 %, dressing 45 %, washing 41 %) of the patients who had a disturbance at baseline.

Conclusions: This naturalistic observational study confirms controlled clinical trials that galantamine is effective and well tolerated for patients with AD. It shows that it is very likely that patients with either non-cognitive or functional impairment may benefit from galantamine.

P720

**Influence of HITS on neuropsychological outcome of patients with mechanical heart valves.** C. Cordonnier, H. Henon, J.-L. Lecroart, M. Rousselpieronne, C. Gautier, O. Godefroy, J.-P. Pruvo, F. Pasquier, D. Leys, G. Deklunder, Hopital R. Salengro, University Hospital (Lille, F)

Background: Patients with mechanical heart valves (either aortic or mitral) frequently have High Intensity Transient Signals (HITS). Their clinical relevance is not clear, and whether they are associated with the frequent cognitive alterations of these patients remains unsettled.

Aim: The aim of this study was to evaluate the possible association between HITS, memory complaints, and neuropsychological outcome in patients with mechanical heart valves.

Methods: Twenty-seven patients(20 men, median age 64 years ranging from 39 to 77), were included at least 16 months after surgery. None had coronary artery disease or carotid artery stenosis, history of atrial fibrillation, nor stroke. They all underwent clinical, echocardiographic, carotid artery duplex and neuropsychological examinations, cerebral MRI and transcranial doppler monitoring of middle cerebral arteries.

Preliminary results: HITS were found in all patients but 2: the median number of HITS was 16/hour (range: 0–216). Thirteen patients had memory complaints and 11 had at least one neuropsychological test disturbed. The number of HITS tended to be more frequent in patients with memory complaints and in patients with at least 1 neuropsychological test disturbed. We found no correlation between the number of HITS and lacunes or white matter changes on MRI.

Conclusion: Memory complaints and neuropsychological disturbances tended to be more frequent in patients with a high number of HITS.

P721

**Cognitive decline in thalamic infarctions.** D. Örken, G. Kenangil, I. Satilmis, H. Forta, Sisli Etfal Education Hospital (Istanbul, TR)

In this study, patients with pure thalamic infarctions were evaluated for cognitive dysfunction.

Twelve patients were included in this study, of who 6 were classified as thalamogeniculate artery, 3 as tuberothalamic and 3 as posterior choroidal artery infarction. Patients who had prior cerebrovascular accident and known dementia were excluded. All patients were evaluated by minimental state examination, three words-three shapes memory test, and clock drawing test.

Patients were between ages 42–72, 7 of them were women. We did find cognitive decline in 9 of 12 patients. None of them had aphasia. In tuberothalamic artery group all three patients had orientation and anterograde memory dysfunction and two of them had also attentional deficit. In thalamogeniculate artery group 1 of the patients had only anterograde memory deficit, 2 of the others had attentional and visuospatial dysfunction. In posterior choroidal artery group all 3 patients had visual memory deficit, 2 of them also had deficits in orientation, anterograde memory, visuospatial and executive dysfunction.

We concluded that patients with tuberothalamic and posterior choroidal artery infarctions had a higher rate of permanent cognitive dysfunction.

P722

**Depression and Alzheimer's disease: benefits of trazodone therapy associated with acetylcholinesterase inhibitors.** C. P. Trevisan, E. Pastorello, S. Lombardi, L. Zuliani, L. Pasqui, R. Squarza, R. Ceravolo, University of Padua, Monselice Hospital, Geriatric Hospital (Padua, I)

Noncognitive symptoms in patients with Alzheimer's disease (AD) are frequent: among them, depressive disorders have been reported at rates higher than 40 %. In this disease, the treatment of subclinical or very mild depression may be questionable. We report the preliminary results of a clinical investigation on the efficacy of trazodone therapy associated with acetylcholinesterase inhibitors in AD patients with subclinical depressive disorders.

Among our series of 127 patients, affected by mild to moderate AD, we considered 42 cases, who exhibited very mild depressive alterations on Geriatric Depression Scale (GDS), without evident depression on clinical observation. All patients had been in treatment with acetylcholinesterase inhibitors (rivastigmine 6 mg or donepezil 5 mg, daily) for at least 3 months. In 18 of them (7 males and 11 females, mean age 77 years, range 58–86) the anticholinergic drug was associated for 6 months with 50 mg/die (range 25–100 mg) of trazodone. After this period, all exhibited an improved score on GDS, that changed 5/15 (range 3–8) to 4/15 (range 1–10). Furthermore, they did not modify their mean MMSE score (19/30), differently from the 24 cases not receiving trazodone, whose mean score declined from 21/30 to 19/30. These findings in AD patients with very mild depression, demonstrated that trazodone improves not only the mood disorder, but also the cognitive benefit of anticholinesterasic therapy. Moreover, trazodone treatment determined no side-effects, except transitory mild drowsiness in the morning, in 2 cases.

On the whole, this preliminary study indicates that patients with mild to moderate AD and very mild depressive disorder may benefit from trazodone treatment, in association with the acetylcholinesterase inhibitors, both on cognitive and noncognitive grounds.

Research partly supported by a grant from ACRAF (Ancona-Italy)

P723
**Galantamine is neuroprotective in conditions relevant for Alzheimer's disease.** E. Arias, E. Alés, M. Lopez, Universidad Autonoma (Madrid, E)

A substantial body of biochemical and genetic evidence suggests that amyloid formation is causally related to Alzheimer's disease (AD). The amyloid beta-A1–40 and beta-A1–42 peptides (Ab) are suspected to be neurotoxic in the Alzheimer clinical situation. This form of cell death bears resemblance to apoptosis. Recent neurobiology data also suggest a neuroprotective role for alpha-7 nicotinic ACh (nAChR) receptors in Ab mediated neurotoxicity and on apoptosis in general. On the other hand, endoplasmic reticulum (RE) stress (i. e. Ca2 + depletion) seems to be an initial and crucial step to induce apoptosis and neuronal cell death and has also been involved in neuronal degeneration in AD.

We have studied galantamine, a modest AChE inhibitor with an allosteric potentiating effect at nicotinic ACh receptors, in cultures of human neuroblastoma and bovine chromaffin cells subjected to either thapsigargin or beta-amyloid-1–40-induced apoptosis. The dose-range of galantamine (100–1000 nM) used corresponded to the brain concentration of galantamine in clinically relevant conditions.

Thapsigargin at 3 microM, induced neuronal apoptosis almost fourfold by interfering with intracellular calcium homeostasis and causing RE stress. Galantamine at the clinically relevant concentration of 300 nM reduced apoptosis almost to control levels. This protective effect was mediated by alpha-7 nAChR as alpha-bungarotoxin (a-BTX), an alpha-7 specific nAChR antagonist, inhibited galantamine's neuroprotective action.

Incubation of neuroblastoma cells with beta-amyloid peptide at a concentration of 10 microM for 24h increased apoptosis from 4 ± 0.8 % (basal) to 24 ± 4 %. Galantamine at 300 nM reduced beta-amyloid induced neurotoxicity to almost control levels (7.5 ± 0.9 %). Again, the protective effect of galantamine was blocked by a-BTX (20 ± 3 %).

These effects could possibly be explained through modest activation of the alpha-7 nAChR leading to small Ca-increases and upregulation of internal Ca-buffers, providing enhanced protection against Ca-homeostasis disruption. Also, other experimental evidence has identified a physical association between beta-amyloid peptides and the alpha-7 nAChR.

Taken together, these results suggest that galantamine interferes with the neurotoxic effects of Alzheimer-associated neurotoxins through its interaction with alpha-7 nAChR and provide a pharmacological rationale for investigating disease modifying aspects in clinical situations.

P724
**Differences of EEG findings in dementia patients. Are there any and how reliable can they be?** P. Ioannidis, S. Tsounis, I. Poulios, D. Parissis, T. Afrandou, I. Milonas, AHEPA Hospital (Thessalonica, GR)

Background: Dementia with Lewy bodies (DLB) is considered as the second most common cause of cortical dementia in elderly individuals following Alzheimer disease (AD). Few studies demonstrated that EEG findings may have a supportive role in the diagnosis of DLB.

Objectives: To evaluate the significance of EEG in differential diagnosis of AD and DLB.

Patients-Methods: We examined with standard EEG recordings two groups of demented patients; a) twenty patients (mean age 67,5 years old) were diagnosed as having probable AD according to the NINCDS-ADRDA criteria and b) eight patients (mean age 73 years old) with probable DLB according to the Consensus criteria of the disease.

The EEG results were, for the purpose of the study, separately examined by three reviewers and compared with a normal group consisting of 30 matched for age individuals.

Results: Demented patients revealed abnormal EEGs both in AD and DLB group. DLB group basically revealed alterations of background activity in all EEG recordings accompanied by transient slow sharply contoured activity in most of them, over mostly the temporal lobe areas, unilaterally. The AD group tended to preserve better slow background activity, even normal activity, with a more diffuse pattern of slow wave abnormality.

Conclusions: There is a certain discrimination concerning EEG activity between demented and non demented age-matched individuals. There are EEG differences between the two demented groups which can be focused on background activity as well as on the distribution and the form of the slow wave abnormalitly.

P725
**Wernicke's encephalopathy severity correlates with MRI findings.** C. Sánchez Bueno, J. Martínez San Millán, J. Masjuán, J. Martínez Castrillo, J. Alvarez Cermeño, Hospital Ramón y Cajal (Madrid, E)

Introduction: Wernicke's encephalopathy is due to thiamine deficiency. There are several associated conditions like alcoholism, pregnancy, hyperemesis, systemic malignancy, gastrointestinal surgery, hemodialysis or peritoneal dialysis, prolonged intravenous feeding, refeeding after prolonged fasting or starvation, anorexia nervosa and acquired immunodeficiency syndrome.

Original description includes the clinical triad of confusion,ophthalmoplegia and ataxia, which is not present habitually, however remains a clinical diagnosis, as there is no distinctive laboratory abnormality.

Neuroradiological techniques like CT scan and MRi may show typical abnormalities which correlate with severity of clinical findings and sequelae.

The MRi is a useful technique for later follow up.

Patients: We describe six patients who were admitted in the department of Neurology with Wernicke's encephalopathy and treated with thiamine.

Patient 1) Male, 43 years old. Severe alcoholism.Chronic pancreatic disease.Alcoholic polyneuropathy.

Patient 2) Male, 37 years old. Chronic alcoholism with polyneuropathy.

Patient 3) Male, 60 years old. Chronic alcoholism.

Patient 4) Female, 36 years old. Morbid obesity, gastric by-pass and vomiting.

Patient 5) Male, 40 years old. Chronic etilism.

Patient 6) Female, 40 years old, mild alcohol abuse.

Results: We observed nystagmus in 100 %,ophthalmoparesis (66 %), ataxia (83 %), fixation amnesia (83 %). 50 % developed Korsakoff. 33 % were aymptomatic at discharge from hospital after thiamine treatment and one patient was asymptomatic three months later.

CT scan showed global atrophy in one patient [5], thalamic hypodensity in two (1 and 2) and were normal in three (3,4 and 6).

MRi showed subcortical atrophy in 33 %, T2 hyperintensity in 33 %, and T1 with gadolinium showed mamillary bodies contrast enhanced.

In two patients (4 and 6) MRI was useful for follow up.

Conclusions: Wernicke's encephalopathy remains a clinical diagnosis, however MRi findings correlate with severity and prognosis and are useful for later follow up of patients.

P726
**Short- and long-term benefits of galantamine over donepezil in the treatment of Alzheimer's disease: clinical evidence for the dual mode of action?** I. McKeith, G. Hammond, S. Schwalen, Newcastle General Hospital, Janssen Cilag, Janssen-Cilag GmBH on behalf of the GAL-GBR-2 Study Group

Introduction: Acetylcholinesterase-inhibitors (AChEIs) have been shown to combat the cognitive decline associated with Alzheimer's disease (AD). Unlike pure AChEIs, galantamine allosterically modulates ACh receptors of the nicotinic type in addition to its AChEI properties. Nicotinic receptor function is particularly important for attention. We therefore performed a comparative study of the pure AChEI, donepezil, with galantamine to determine the effects on cognition with particular focus on measures of attention in the short and long-term.

Methods: This was a multicentre, rater-blind, parallel group trial. Participants with AD (meeting NINCDS-ADRA criteria, MMSE 9–18) were randomized to receive either galantamine (escalating from 8 to 24 mg) or donepezil (5–10 mg) for 52 weeks. Cognition was measured with MMSE, ADAS-cog and a validated computerized test battery, the cognitive drug research assessment system (CDR). CDR was used to investigate attention performance from week 6 to week 52.

Results: A total of 182 patients entered the study (94 on galantamine, 88 on donepezil). Mean age was 73 years, 85 % had moderate and 14 % had severe AD (MMSE at baseline 12–18 and < 12 respectively). Galantamine was superior to donepezil for MMSE cognition at week 13, 26 and 52 with an even more pronounced difference in the moderate group. Donepezil-treated patients dropped significantly below baseline after 52 weeks (−1.4 ± 0.4 points, $p < 0.005$) whereas MMSE for patients on galantamine was not different from baseline level after 52 weeks. ADAS-cog showed no significant difference between the two groups. Performance on all attention tasks on the CDR improved more on galantamine than donepezil with most pronounced changes evident at week 6. This difference was seen in speed and accuracy measures. Both drugs were well tolerated: severe adverse events were seen in 18 galantamine-treated patients (18.6 %) and in 18 donepezil-treated patients (19.6 %).

Conclusion: This is the longest, rater-blinded randomized clinical trial ever performed comparing the efficacy and safety of two well-established

AD treatments. Galantamine demonstrated significant short- and long-term benefits in cognition and attention compared to donepezil. The benefits of galantamine over donepezil may be linked to its dual mode of action, in particular to the modulating effect of the nicotinic receptors.

P727
**Subacute dementia and psychic disturbances with severe bipallidal involvement in an anatomic case of Erdheim-Chester disease.** F. Le Doze, M. Bonnan, F. Chapon, R.-M. Marie, G.-L. Defer, CHU Cote de Nacre (Caen, F)

Background: Erdheim-Chester disease (ECD) is a rare, sporadic, non-langerhans cell histiocytosis that affects multiple organs. We present an anatomic case with CNS involvement.

Observation: 68 y.o male patient presenting with memory loss and subacute cerebellar syndrome. Medical history included systemic hypertension, gastric adenocarcinoma and unexplained transient VI paralysis. Mental state was characterized by global cognitive decline (MMS 18), bradypsychia, pharmacoresistant depression and fluctuations in vigilance. CSF protein was 0.66 g/L. MRI showed T2 and FLAIR hyperintensities in pons and middle cerebellar peduncles. Clinical extra neurological involvement comprised bone lacunes, pulmonary interstitial syndrome, retroperitoneal fibrosis with left renal arterial stenosis. Death occurred after unsuccessful corticosteroid treatment and progressive clinical worsening with profound dementia and stupor. Brain examination disclosed nodules of dura, cortical and subcortical atrophy and brownish coloration of both pallida. Positive CD68 immunostained dense histiocytic infiltrates (CD68HI) were particularly numerous in the pallidum, also disseminated in the whole brain associated with gliosis and perivascular calcifications. The CD68HI were concentrated in the cerebellum especially in dentate nuclei. Medulla, pons and brainstem nuclei were also severely affected. General autopsy showed CD68HI in multiple organs.

Discussion: The multiple organ involvement and the presence of CD68HI make the diagnosis of ECD undoubtful. The most frequent CNS manifestations are cerebellar syndromes, diabetes insipidus, orbital lesions, and extra axial masses. Dementia and psychic disturbances have not yet been described. No precise neuropsychological examination could be performed due to the lack of cooperation but bradypsychia, depression and vigilance fluctuations were in favor of a participation of the fronto-subcortical structures to the clinical presentation. In this respect the severe bipallidal involvement in our patient must be highlighted, as we know that basal ganglia play a crucial role in mood, behaviour and cognition via the fronto-cortico-subcortical loops. ECD should be considered as a new cause of subacute subcortical-like dementia.

## General neurology

P728
**Neuro-Behcet's disease manifesting as a neoplasm-like lesion: case report.** Y. Kaya, M. Kilinc, S. Benli, U. Can, N. Tuncel, E. Derle, B. Atalay, M. Teksam, G. Celiker, Baskent University Hospital (Ankara, TR)

Neurological involvement occurs in 5% to 50% of patients with Behcet's disease, and the manifestations can be secondary to direct central nervous system involvement or vascular angiitis.

We report a rare case of neuro-Behcet's disease presenting as a mass like lesion. A 65 years old woman was admitted to hospital with severe headache. She has been treated with oral prednisone for pan-uveitis and recurrent oral apthaes for 4 years. On the first admission the neurological examination was normal. However, MRI showed multiple contrast enhancing lesions with accompanying vasogenic oedema in bilateral parietal white matter. Also vasculitic findings were determined in bilateral anterior, middle and posterior cerebral arteries on cerebral magnetic resonance angiography. She was put on immunosuppressive therapy with intravenous cylophosphamide and steroid. Two months later, she was readmitted because of disarthria, visual disturbances, recurrent headaches and confusion. This time neurologic examination revealed ptosis, paralysis of adduction in the left eye and quadriparesis. Cranial MRI showed multiple enhancing lesions in the brain stem, basal ganglia and right temporal lobe. The lesions were found to be enlarged when compared with the previous MRI. Magnetic resonance spectroscopy revealed an increased choline to creatinin ratio in the lesion localized to the right temporal lobe. The patient received three courses of pulse steroid therapy followed by intravenous cyclophosphamide. However, as there was no improvement in her symptoms, moreover persistence of severe headache, cranial computerized tomography (CT) was performed. Brain CT revealed a homogeneously enhancing, nodular, mass-like lesion in the right frontotemporal region. As there was no regression in the size of the lesion in spite of an effective immunosuppressive therapy, a stereotactic biopsy was performed to rule out a neoplasm. Pathologic examination showed no malignant cells; but revealed invasion of inflammatory cells around the blood vessels in the white matter. Ruling out a neoplasm, we planned to keep the patient on immunosuppressive and steroid therapy.

In conclusion, we wanted to present this case to point out that the radiological presentations of neuro-Behcet's disease may mimic a cerebral tumor. In such cases, stereotactic biopsy would be useful to exclude suspicion of a cerebral tumor.

P729
**Reclassification of fine motor disturbances in case of Wilson's disease using artificial neural networks.** W. Hermann, P. Günther, T. Villmann, H.-J. Kühn, A. Wagner, Universitätsklinik und Poliklinik für Neurologie (Leipzig, D)

Clinical background: Patients suffering from Wilson's disease are divided into several types according clinical symptoms only at time of manifestation. Thereby two main subgroups exist: neurologic and non-neurologic types. After long-term therapy the neurological symptoms occurring in hepatolenticular degeneration may be improved. However, frequently fine-motoric disturbances remain and should be used for evaluation of the actual patient state.

Aim: Remaining fine-motoric disturbances should be measured by an objective manner. Based on the achieved values a proper classification of fine-motoric abilities is needed reflecting the internal data structure. The procedure should finally lead to a better differentiation of subgroups of fine-motor disturbances.

Method: We measured fine-motor passive (tremors) and active abilities (movement tasks) based on a standardized test set (resting and holding tremor, forefinger tapping, target tapping, spiral drawing) using the VS-COPE-system. From this test set 11 parameters were derived describing the fine-motoric abilities. The database contains values from 37 patients and 24 random probands as reference group. The parallel evaluation of all fine-motor data was done using an artificial neural network (Growing Self-Organizing Map – GSOM) which performs a non-linear principal component analysis. It was followed by a special clustering scheme based on GSOM-results.

Results: The outlined method leads to a new cluster solution which is completely different to the clinical classification at time of manifestation according to Konovalov. Each cluster is spanned by persons of similar fine-motor abilities. Moreover, due to the non-linear neural network approach we are able to extract two main essential parameters (non-linear principal components) describing the fine-motor disturbances. These two parameters can be identified as active and passive fine-motor control abilities.

Summary: The resulted cluster solution can be used as reclassification of patients after long-term therapy. Each cluster corresponds to a special functional disturbance of basalgangliar and cerebellar loop.

P730
**Neurological manifestations in Sjögren's syndrome [2]. MRI, CSF and outcome profiles in a cohort of 82 patients.** S. Delalande, J. De Seze, E. Michelin, J. Y. Gauvrit, A. L. Fauchais, E. Hachulla, P. Y. Hatron, J. P. Pruvo, P. Vermersch, CHU Lille (Lille, F)

Introduction: Neurological involvement occurs in approximately 20% of patients with primary Sjögren's syndrome (SS) and can affect the peripheral (PNS) and central (CNS) nervous systems.

Aim: To describe the radiological, neurophysiological and cerebrospinal fluid (CSF) analysis of patients with neurological manifestations occurring during SS, and to report their clinical outcome.

Methods: We studied 82 patients with neurological manifestations associated with primary SS and evaluated the MRI, CSF and visual evoked potentials (VEP) and response to various treatments. We used the MOHS (Modified Oxford Handicap Scale) score to evaluate neurological disability.

Results: Fifty-six patients had CNS disorders and 51 patients had PNS involvement. Thirty percent of patients (all with CNS involvement) had oligoclonal bands. The VEP were abnormal in 61% of the patients tested. Fifty-eight patients had a brain MRI. We observed white matter lesions in 70% of patients. MS radiological criteria were present in 40% of patients (44% fulfilled Paty's criteria; 39% fulfilled Barkhof and Fazekas' criteria). Lesions were found in 80% of patients with CNS involvement. We also observed gray matter lesions in the basal ganglia (17% of patients). Corpus callosum lesions were rarely observed (14%). Thirty-nine patients had a

spinal cord MRI. Abnormalities were observed only in patients with spinal cord involvement. Among the 29 patients with myelopathy, 75% had T2-weighted hyperintensities (cervical in 82%, dorsal in 47% and terminal in 17%), 35% had extended lesions and 40% had centromedullar lesions. The mean neurological follow-up was 7 years. Twenty-six patients had relapses. Fifty-two percent of patients had severe disability, and were more likely to have CNS involvement than PNS involvement ($p < 0.001$). Treatment by cyclophosphamide allowed a partial recovery or stabilization in patients with myelopathy (92%) or multiple mononeuropathy (100%).

Conclusion: White matter changes on MRI are frequent in SS but could be distinguished from MS because lesions in the corpus callosum are and the basal ganglia may be involved. Spinal cord MRI is also discriminant, showing more an extended and centromedullar hypersignal in SS than in MS. The outcome is frequently severe, especially in patients with CNS involvement. Our study underlines the potential efficacy of cyclophosphamide in myelopathy and multiple neuropathy occurring during SS.

P731

**Acute onset syringomyelia with Arnold-Chiari malformation. Report of five cases.** C. Delpirou-Nouh, L. Bauchet, J.-M. Privat, B. Carlander, M. Pages, Centre Gui de Chauliac (Montpellier, F)

Objective: To describe five cases of syringomyelia with Arnold-Chiari malformation revealed by acute symptoms.

Background: The usual symptoms of syringomyelia with Arnold-Chiari malformation are pain, gait disturbances, sensory loss and motor weakness, with a slow and progressive course. Acute onset of the disease is less common.

Material and methods: From a data base devoted to syringomyelia with Arnold-Chiari malformation, we searched for cases with an acute presentation.

Results: From 1990 to 2002, 18 patients fulfilled the clinical and radiological criteria of syringomyelia with Arnold-Chiari malformation. Five of them were included in the study. There were 3 male and 2 female, aged from 22 to 64 years. Clinical symptoms included pain (5 cases), dysphonia (2 cases), dysphagia (3 cases), vertigo (2 cases), hypoaesthesia (2 cases), and motor weakness (3 cases). One case presented with acute respiratory failure with sleep apnea. Maximum deficit was reached in a few days. MRI showed Arnold-Chiari malformation type I in 2 cases, type II in 3 cases. The syrinx was located respectively from C1 to C4, C2 to C4, C1 to D10, Medulla Oblongata to D1, and holocord. All patients were treated with posterior fossa decompression. In all cases, a nearly full recovery was obtained. Sleep apnea required mechanical ventilation during the night.

Conclusions: Acute onset of syringomyelia is not uncommon. Supraspinal symptoms often have a good prognosis after surgical treatment.

P732

**Benign intracranial hypertension. Clinical features and treatment.** F. Rodriguez de Rivera, P. Martinez, J. Ojeda, J. Arpa, P. Barreiro, Hospital La Paz (Madrid, E)

Benign intracranial hypertension (BIH) is characterized by raised cerebrospinal fluid (CSF) pressure with normal composition, in absence of any space occupying lesion. Risk factors, CSF pressure and response to treatment were analyzed.

41 patients were BIH diagnosed between 1999 and 2000. CSF pressure was measured at admittance. Usual risk factors and response to medical and surgery treatment was evaluated. Results were statistically analyzed.

72% of the patients were women, with a mean age 28,7. Smoking habits (70%), obesity (57.8%) and recent gain weight (36.8%) were the main records. Mean time of evolution were 106.3 days (Males 27.2 d, Females 127.4 d). Clinical features were headache (84.4%), reduction in visual acuity (46.7%) and nauseas and vomiting (40.6%). All patients presented bilateral papilloedema. CSF pressure was 35.31 cm $H_2O$. 94.7% were treated with acetazolamide (favourable 50%) and 42% with lumboperitoneal shunt (favourable 81.2%). Patients with an unfavourable treatment response had a more elevated CSF pressure.

Female sex, smoking habit, obesity and recent gain weight were the principle antecedents. Main clinical features were headache and reduction in visual acuity. Diagnosis was earlier in men. CSF pressure was a predictive factor for the successful treatment.

P733

**A case of ophthalmoplegic migraine with recurrent oculomotor nerve palsy.** M. Sommer, P. Nomikos, W. Paulus, University of Gottingen (Gottingen, D)

Purpose: To present a patient with oculomotor ophthalmoplegic migraine, a rare episodic condition in which a unilateral oculomotor palsy is preceded by headache. Only seventeen similar cases have been previously reported.

Case report: A 16-year-old female with a five year history of migraine developed a nearly complete internal and external oculomotor nerve paresis ipsilateral to her headache at the age of 13 and 16 years. Each time the onset of migraineous headache preceded the neurological symptoms. Magnetic resonance imaging (MRI) showed enhancement and enlargement of the cisternal portion of the oculomotor nerve. Angiography yielded no evidence for a vascular malformation. No surgical action was undertaken and the neurologic symptoms resolved spontaneously over the course of four weeks.

Conclusions: Ophthalmoplegic migraine should be considered in the differential diagnosis of ophthalmoplegia. The diagnosis is strongly suspected when MRI demonstrates swelling and enhancement of the oculomotor nerve, which may represent an inflammatory process occurring in the interpeduncular segment of the oculomotor nerve. Oculomotor nerve schwannoma and vascular malformation should be considered for differential diagnosis.

P734

**A comparison between isometric force and position tasks in evaluating movement incoordination.** G. Brichetto, V. Sanguineti, P. Morasso, G. L. Mancardi, C. Solaro, University of Genoa, Center for Bioengineering Hospital Colletta, Ospedale P. A. Micone (Genoa, Arenzano, I)

Objective: To evaluate two kinematic methods, isometric force and position task, in order to study upper limb function of asymptomatic multiple sclerosis (MS) patients and normal subjects.

Materials and Methods: We selected 9 healthy subjects, ages 22–56 (mean 27.9) and 4 clinically or laboratory definite MS subjects (Poser criteria), ages 24–38 (mean 32.7). Inclusion criteria for MS subjects: negative at the neurological examination, expanded disability status scale (EDSS) < 1.0, no involvement of pyramidal, cerebellar or sensory functional systems. Normal score for the 'arm' portion of the Scripps's Neurological Rating Scale (NRS) in both arms.

TasksForce control: Isometric force steps (amplitude 10 N, target size 1N) with dominant hand in eight directions: 0°, 45°, 90°, 135°, 180°, 225°, 270°, 315° on the horizontal plane. The targets were presented in random order and two conditions: with vision and without vision i. e. no visual feedback of the instantaneous force. 15 repetitions for each condition and direction (only last 10 used for analysis). Position control: planar arm movements from exactly the same starting configuration of the arm, with an identical experimental design. Hand trajectories sampled at 125 Hz by a digitizing tablet.

Results: Force control (normal subjects): curvature, jerk and aiming error are significantly larger in directions where the required torques are larger (principal directions of the manipulability matrix). Amplitude error is larger in directions in which the required joint torque is lower. The role of vision: significant effects on speed asymmetry (smaller in the non vision condition) and step amplitude (in the no vision condition, subjects tend to overshoot the target). Force vs Position control (normal subjects): effect of task: in position control, larger reaction time and aiming error, smaller asymmetry. Direction dependence: not significant. Role of vision: with no vision, asymmetry, path curvature and jerk integral decrease significantly (trajectories are smoother and almost straight, denoting little or no feed-back corrections). In contrast, reaction time increases significantly.

Force control (MS subjects): effect of disease: larger reaction time, curvature and jerk. Direction dependence: similar to controls (but significantly larger) in curvature, jerk and aiming error. Role of vision: no significant increase of step amplitude, but significant interaction between disease and direction. In absence of vision, curvature and jerk decrease whereas reaction time increases.

Discussion: The two tasks differ in the way they use visual information.

In Force control task vision is mainly used in final adjustments when near the target (only endpoint error is affected by lack of vision). In Position control task vision is used in on-line trajectory corrections (they almost disappear in absence of vision). Isometric force task seems very good in detecting subtle incoordination problems.

P735
**Fibrocartilaginous disc embolisation syndrome.** J. Sharp, D. B. Rowe, Royal North Shore Hospital (Sydney, AUS)

Acute non-traumatic spinal cord syndromes represent a diagnostic dilemma. Differentiating between inflammatory and vascular causes is difficult and the diagnosis of transverse myelitis carries with it management and prognostic implications. The diagnosis of Fibrocartilaginous Disc Embolisation Syndrome (FDES) is not well recognised and undoubtedly under-diagnosed. Conventionally the definitive diagnosis of FDES has required post-mortem evidence. Such cases have resulted from a fatal ischaemic insult to the spinal cord however we believe that this aetiology is also responsible for many non-fatal cases. In fact, FDES is recognised in the veterinary literature as the commonest type of spinal cord infarction.

In this study we review five living cases we consider to be accurately diagnosed as FDES. The patients were seen for follow-up in a neurology clinic and notes and investigations were complied. It would appear that the application of good clinical acumen is the best means of identifying cases of FDES and this study compiles case information to create a clinical picture and indicate pertinent investigations.

We found FDES to be most common in women in their third decade. There is a strong association with trauma. Symptoms are dominated by sudden onset of severe pain over the spine. There is progressive development of paraesthesia and paresis and in most cases disturbance to bladder and bowel function. On examination a flaccid paresis commonly in a pyramidal distribution is found. The sensory changes are predominated by loss of pain and temperature sensation with preservation of proprioception and vibration sense. Investigation of blood and CSF for metabolic, infectious and immunological changes is negative. Signal changes, specifically hyperintensity in a well defined region of the spinal cord, on T2 weighted imaging is the most commonly reported MRI finding.

The five cases in this study, seen over the course of 18 months at one institution, represent more than ten percent of the world literature. From this we conclude that FDES occurs more commonly than is currently acknowledged. This study has followed patients for longer than any cases in the literature and has shown that most can expect to return to full function with only minor residual neurological signs. We propose that after exclusion of a compressive lesion, the diagnosis of FDES should be entertained in any acute non-traumatic spinal cord syndrome.

P736
**Neurological involvement in inflammatory bowel disease.** A. Demirtas, M. Celik, C. A. Alkim, K. Barkut, H. M. Sökmen, Sisli Etfal Hospital (Istanbul, TR)

The aim of this study was to evaluate neurological involvement in inflammatory bowel disease.

All patients who were diagnosed as inflammatory bowel disease (Crohn disease or ulcerative colitis) in the Department of Gastroenterology were referred to the Department of Neurology of Sisli Etfal Hospital. Neurological examinations were performed in a total of 30 patients, whereas electrodiagnostic examination (EDX) and cranial magnetic resonance imaging (MRI) could be performed in 18 and 11 of them, respectively.

The duration of the inflammatory bowel disease was between 1 and 31 years (mean: 5.7; SD: 6.5). Neurological examination was unremarkable in 10 patients. In another 10 patients, the alteration of vibration sense in the distal portions of lower extremities was the only abnormal finding. Neurological examination revealed diminished deep tendon reflexes in addition to the impaired vibration sense in 3 and weakness in proximal muscles of upper and lower extremities in 1 patient. One patient had left sided hemiparesis. Postural tremor in 1 patient and unilateral or bilateral equivocal plantar responses and cataracts are detected in the remaining patients. EDX disclosed sensory neuropathy in 2 patients, sensory-motor polyneuropathy in 1 patient, mononeuropathy in 3 patients, neurogenic changes of motor unit potentials in the distal muscles of the upper extremities in 1 patient and myopathy in one patient with proximal muscular weakness. Cranial MRI revealed small vessel disease in 4 patients including the one with hemiparesis.

The most common neurological finding was mild peripheral neuropathy in our patients with inflammatory bowel disease. Neurological syndromes causing severe deficit were rare.

P737
**Relapsing encephalopathy associated with autoimmune thyroiditis occurring years before onset of thyroid dysfunction.** S. Galgani, F. M. Corsi, S. Carella, C. Gasperini, San Camillo-Forlanini Hospital (Rome, I)

Encephalopathy associated with autoimmune thyroiditis (Hashimoto's encephalopathy) is a rare, life threatening, corticosteroid-responsive and possibly autoimmune condition. We describe a patient with "relapsing" encephalopathy occurring years before onset of thyroid disease.

In August 1991 a 40-year-old woman presented a confusional state with jerking in both arms. She rapidly developed coma and tonic-clonic seizures. Brain CT showed a diffuse oedema, CSF was normal. Her condition improved in 2 weeks and she was discharged with a diagnosis of "viral encephalitis". After one month she developed a relapse. Brain MRI showed diffuse T2 hyperintense changes and EEG showed diffuse slow waves and epileptic discharges. Blood tests, CSF, thyroid function tests and levels of anti thyroid peroxidase (TPO) and anti thyreoglobulin (TG) were normal. Intravenous (IV) steroids, IG and antiepileptic drugs (AED) were administered with improvement. In the following months the patient experienced four relapses after every trial of steroid withdrawal. Azathioprine was started. The patient recovered and MRI and EEG findings normalized. Immunosuppression and AED were continued until 1995. EEG, MRI, thyroid function were normal in the follow-up controls as well as anti-TPO and anti-TG levels except for one occasion when high titre was detected (1600 U/ml e 420 U/ml respectively). She had been stable and free from therapy for seven years when, in October 2002, she again presented confusion and jerking in both arms. MRI showed diffuse T2 hyperintensity, EEG diffuse slowing; thyroid function was normal but a high titre of anti-TPO (153 U/ml; normal values (NV): < 35) and anti-TG (1162 U/ml; NV: < 225) was detected. IV high dose steroid therapy was started and her condition drammatically improved. MRI findings and the antibodies titre normalized in a couple of months. Our patient underwent a relapsing encephalopathy responsive to high dose steroids and immunosuppressive therapy with a long period of remission. Initially thyroid function and antibodies were normal. A relationship between the clinical status, antiTPO and antiTG levels and its response to steroid treatment was established only during the last relapse. In other cases reported in literature the neurologic manifestations are not specific although two patterns of clinical presentation have been described: the first is an illness with stroke like episodes and the second one a dementia-like pattern with progressive cognitive impairment, seizures and altered consciousness. The pathogenesis remains unclear; the role of antithyroid antibodies has never been demonstrated although they are the main indicators of disease activity. Autoimmune cerebral vasculitis is widely postulated and is supported by recent pathological findings. Encephalopathy associated to autoimmune thyroiditis may represent an association of an uncommon autoimmune encephalopathy with a common autoimmune thyroid disease.

P738
**Chronic diarrhoea as first manifestation of Ross syndrome.** B. Legros, A. Hittelet, L. Mathieu, S. Blecic, Erasme Hospital, SPRL Mathieu (Brussels, Leuze-en-Hainaut, B)

Background: Ross syndrome has been defined as segmental hyperhidrosis and Holmes-Adie syndrome. Various associated autonomic symptoms have been frequently described such as orthostatic hypotension, chronic cough, impaired digital vasoconstriction to cold, reduced heart rate response to the Valsalva maneuver. On the contrary, chronic diarrhea was reported only once.

Case report: We report a 40 year-old woman who complained with chronic diarrhea for the last 3 to 4 years. She is used to having 3 to 4 episodes of liquid feces a day, without any obvious cause. In April 2002, she had a sudden blurred vision of the right eye and she noticed a pupil asymmetry without any other sign. Physical examination was normal. Neurological testing disclosed a right Adie pupil and tendon areflexia. Exhaustive blood testing was normal. Feces analysis disclosed no evidence of an infectious origin. Small bowel series disclosed an extremely rapid transit time of less than 10 minutes. Upper gastro-intestinal endoscopy and colonoscopy were unremarkable as well as serial biopsies. Nerve conduction velocities were normal as well as the cutaneous sympathetic response. On dermatological investigations, a right axillary hyperhidrosis was found. Extensive autonomic cardiac investigations were normal.

Discussion: This patient has been diagnosed as having Ross syndrome with motor diarrhea due to dysautonomia. Up to now it is the first description of Ross syndrome presenting initially with diarrhea without any other typical syndrome features.

Conclusion: In a patient with chronic diarrhea in absence of any known or obvious causes, a dysautonomic dysfunction should be considered.

P739
**Speech disturbances in patients with aphasia in sonographic assessment.** R. Podemski, K. Slotwinski, K. Guranski, S. Budrewicz, Medical Academy (Wroclaw, PL)

Human speech consists of verbal and paralinguistic elements, which – as carriers of information – constitute a complex acoustic phenomenon. If the process of speech is to be correct, it requires, among others, precise performing of programmed voluntary movements in determined time framework. Damage to the dominant hemisphere of brain often causes dysfunction of mechanisms programming speech. Clinical symptom of such dysfunction is disorder of speech expression. Individual perception of acoustic phenomena which composes speech image is not possible by means of hearing.

Aim of the study: to carry out an acoustic analysis of motor activity of speech with sonographic documentation in patients with motor aphasia, in the first two weeks after cerebral stroke.

Material and Method: 14 patients (6 women and 8 men, whose mean age was 52.3) with diagnosed ischaemic cerebral stroke, documented with CT examination and with motor aphasia and 10 healthy controls (5 women and 5 men, mean age 55.1 years) were subjected to the clinical-acoustic studies. Their clinical condition was assessed on the basis of detailed neurological examination. Aphasia was defined on the basis of Token Test and Goodglass-Kaplan scale.

Time-frequency parameters of speech signal were assessed in the first two weeks after cerebral stroke. Obtained results were compared with control group. Acoustic analysis of speech was conducted in all patients using computer programs "Medfon", "Wavelab"; "IRIS". Acoustic computer analysis was conducted on samples of linguistic tests recorded on disc of PC, carried out in the same conditions in the group of patients with aphasia and in control group.

Results:

a) In all the patients statistically significant lengthening of duration of utterance was found in comparison with control group
b) In all the patients variation in frequency of utterance was found in comparison with control group
c) In all the patients deformation of particular formants of human speech was noted.

Conclusions:

1. Motor speech disorders in poststroke patients determine acoustic parameters of speech expression, which can be assessed on the basis of sonographic analysis.
2. Acoustic speech analysis seems to be an objective method of evaluation of disturbances of verbal expression in patients with aphasia during early poststroke period.

P740
**SPECT and PET findings in a patient with SCA 17 – a new form of spinocerebellar ataxia.** P. Günther, J. Schwarz, W. Hermann, S. Hesse, A. Wagner, G. B. Landwehrmeyer, University of Leipzig, University of Ulm (Leipzig, Ulm, D)

Introduction: Autosomal dominant cerebellar ataxias are a clinically heterogenous group of dominantly inherited neurodegenerative disorders characterized by progressive degeneration of the cerebellum, brain stem and spinal cord. CAG repeat expansions encoding polyglutamine tracts seem to cause the disorder and allow a genetic classification.

Case report: We present a 35 year old patient with spinocerebellar ataxia (SCA) 17, to our knowledge the first case in Germany. This neurodegenerative disorder manifests in the patient with saccadic eye movement disorder and dysarthria. A mild cognitive impairment was detected additionally. Family history is positive with dementia and movement disorders in two family members of the patient. Magnetic resonance imaging (MRI) demonstrated nonspecific cerebellar cortical atrophy. FDG-PET showed pronounced loss of glucose uptake within the striatum. A loss of dopamine D2 receptor binding was revealed by SPECT imaging on both sides. Genotyping revealed an abnormal expansion in the TATA-binding protein (TBP) gene with 50 CAG repeats consistent with spinocerebellar ataxia SCA 17.

Conclusions: SPECT and PET show unspecific disturbances in spinocerebellar ataxia (SCA) 17. A classification of the SCA type is only based on genotype analysis.

P741
**Posterior leukoencephalopathy improved by folate treatment.** S. Wiertlewski, E. Auffray-Calvier, H. Desal, B. Guillon, Hopital Laennec (Nantes, F)

Neurological complications of folate deficiency in adults are rare and similar to those observed in cobalamin deficiency. Peripheral neuropathy and combined degeneration of the spinal cord are the two complications usually described, but such deficiency may be responsible for leukoencephalopathy.

To evaluate the impact of folate supplementation in patients with posterior leukoencephalopathy and folate deficiency, we identified 3 patients with subacute encephalopathy associated with T2-weighted MRI bilateral white matter hyperintensity in posterior brain regions. The extensive work-up only detected a decreased plasmatic folate.

One man and two women, mean age 34.6 years (range 26–49), presented with rapid visual disturbances, headache and neuropsychological disorders. Clinical examination revealed a pyramidal syndrome and visual acuity and field impairment. None of them had digestive disease or therapy explaining folate deficiency except a moderate alcohol consumption. Mean plasmatic folate level was 0.4 ng/ml (normal > 3). Dramatic and rapid clinical and radiological improvement occurred after supplementation in folate (15 mg bid).

In our patients, folate deficiency can be explained by alcohol consumption. Association between the posterior leukoencephalopathy and folate deficiency is very likely, as demonstrated by the improvement after folate supplementation. Both cobalamin and folate deficiency may interfere with the synthesis of the central myelin and could be responsible for leukoencephalopathy. Vitamin supplementation can reverse the symptoms.

P742
**Lupus coagulation-inhibitor associated neuropathy following cardiac surgery.** R. Mazzoleni, M. Vokaer, N. Mavroudakis, E. J. Bartholome, J.-C. Bier, Erasme Hospital (Brussels, B)

Background: Sciatic nerve palsy is an uncommon complication of cardiac surgery and is thought to be induced by a combination of reduced femoral artery blood flow, small vessel vascular disease or prolonged hypoxia. Although transient elevation of lupus coagulation inhibitor (LCI) is known to occur frequently in patients treated in an intensive care unit (ICU), there are very few data about the possible role of LCI in the generation of such neuropathies.

Case report: A 43-year-old man was admitted in our rehabilitation department for weakness of the right leg. One month before, seven coronary bypasses were performed for unstable angina. An intra-aortic balloon pump was set up during 48 hours for hypotension. During his stay in the ICU (11days), the patient presented an acute paresis of the distal part of the right lower limb.

Clinical and electrophysiological examinations disclosed right sciatic nerve damage. Laboratory findings showed a prolonged activated partial thromboplastin time (APTT) and disclosed the presence of LCI and antiphospholipids antibodies (APA). The APA level rose to a maximum of 84 U seven weeks after the surgery (normal range: 0–12). Noteworthy, the APTT was normal before the operation. All other causes of neuropathy were excluded.

A treatment by acetylsalicylic acid, 160 mg/day and intensive physiotherapy was performed. Motor and sensory deficits progressively improved. Four months after the surgery, only a slight paresis of foot extension and toes hypoesthesia remained. Meantime, the APTT normalized, LCI became undetectable and the level of APA decreased to 28 U.

Discussion: Ischemia was probably the cause of our patient's neuropathy. The presence of APA was a major thrombotic risk factor. Neuropathy likely induced by APA has already been reported.

Transient elevation of LCI occurs frequently in patients treated in an ICU, whatever the reason leading to admission. However, patients with transient elevation of LCI do usually not present thromboembolic event.

In our case, the simultaneous occurrence of the neuropathy and the transient increase of LCI level argue for a relation between these two events.

Conclusion: This case suggests that APA occurrence, even transient, may be involved in the generation of ischemic neuropathies. Further investigations are necessary to assess the precise role of APA/LCI in thromboembolic phenomena following ICU stay and cardiac surgery.

## Infection

P743

**Infectious inflammation of the CNS involves activation of the mitogen-activated protein kinase cascades.** L. Pollak, R. Seger, Assaf Harofeh Medical Center, The Weizmann Institute of Science (Zerifin, IL)

Background: The Mitogen Activated Protein Kinases (MAPK) serve as transmitters of many extracellular signals to their intracellular targets. Several MAPK cascades are distinguished by the motif of their phosphorylation site at a given level of the cascade. The Extracellular Regulated Kinase (ERK) is critical to mitogenic response, cellular differentiation and induction of hypertrophy. The JNK and p38 are activated by stress, inflammatory cytokines, or vasoactive peptides.

Involvement of ERK in human malignancies, adaptation processes, and hormonal signal transduction has been extensively studied. However, the role of MAPK in inflammatory processes of the human nervous system has not yet been investigated.

Objectives: Presuming that MAPK might be involved in the pathogenesis of inflammatory processes in the central nervous system we investigated the CSF (cerebrospinal fluid) of patients suffering from acute nonbacterial inflammation of the central nervous system for the presence of MAPK isoenzymes.

Patients and methods: The CSF of 12 patients was investigated: 9 patients suffered from aseptic meningitis, 2 from aseptic meningoencephalitis and 1 patient presented with an acute meningoradiculitis.

The CSF samples were processed by SDS polyacrylamide gel electrophoresis and the MAPK enzymes were detected by using specific antibodies.

The CSF of 18 individuals who underwent a lumbar puncture for diagnostic purposes served as controls.

Results: Six patients revealed the presence of activated ERK. P38 was detected in 5 patients, in 3 of them in its activated form. None of the controls showed the presence of MAPK enzymes.

The mean CSF cellularity was higher in MAPK positive than in MAPK negative patients. There was no difference of the mean age or gender between the patients and controls and between the MAPK positive versus MAPK negative patients.

The finding of ERK in CSF of patients with meningitis is in keeping with the function of ERK in cellular proliferation. The detection of p38 might be due to presence of inflammatory cytokines which also act as activators of p38.

Conclusions: Our work demonstrates that the MAPK participate in inflammatory processes of the central nervous system. Since selective inhibitors of the MAPKs are today available, their application may reduce inappropriate inflammatory responses and brain damage in severe cases of meningoencephalitis.

P744

**Comparison of the efficacy of Fluconazole alone, and Amphotericin B alone or plus Flucytosine in the treatment of cryptococcal meningitis.** G. Kim, Ilsan Hospital (Koyang-Shi, Kyungki-do, KOR)

Admistration of amphotericin B (AmB) plus flucytosine (5-FC) was the standard regimen until the late 1980s, despite a relatively high failure rate, a high incidence of adverse reactions, and the inconvenience of prolonged intravenous treatment. Since then, fluconazole (FCZ) has been tested as treatment for cryptococcal meningitis with AIDS.

On the basis of results of treatment given to patients with AIDS and because of its good tolerability and availability in oral form, FCZ has been widely prescribed to HIV-negative patients with cryptococcal meningitis, but its efficacy has never been analyzed in Korea. We retrospectively analyzed clinical outcomes of cryptococcal meningitis in HIV-negative patients with FCZ alone (FCZ group) or AmB alone or plus 5-FC (AmB group).The treatment efficacy did not differ between the FCZ and AmB groups. Nine of the 19 patients treated with FCZ (47 %) and 12 of the 20 patients with AmB were considered successful at the end of therapy. The pretreatment factors as predictive of treatment failure did not differ between the FCZ and AmB groups. The side effects were more common in the AmB group than the FCZ (95 % vs 47 %). Chill and rigor, a rise of serum creatinine, hypokalemia, and a fall in hemoglobin more than 2 g/dl were more frequent in the AmB group. In conclusion, fluconazole is an effective alternative to amphotericin B alone or plus flucytosine as primary treatment regimen of cryptococcal meningitis in patients with non-AIDS.

P745

**Confusion hyponatremia and pain as presenting symptoms of Lyme disease.** M. Dupuis, I. Mathy, P. Jacquerye, J. Harmant, L. Monfort, V. Luyasu, Clinique Saint-Pierre, Clinique Saint-Luc on behalf of the RILY group

We report on 2 cases of Lyme disease with, as presenting symptoms, confusion and hyponatremia associated to pain of Garin-Bujadoux-Bannwarth syndrome (GBBS).

Background: hyponatremia has not been associated until now to Lyme disease.

Case reports: Both cases were women, 68 and 86 years old, admitted in 2 different hospitals in 1995 and 2000, with hyponatremia at 106 and 116 mEq/L, suffering from sciatalgia or abdominal pain, with on CSF 353 and 103 white cells. Serology for Bb by immunofluorescence in the first case was positive only for IgM in serum (1/64)and positive for IgG and IgM in CSF (1/40 and 1/20). In the second case, serology by ELISA was confirmed by Western Blot in serum and CSF. Recovery occurred after Ceftriaxone.

Results: Both cases have in common radicular pain and meningitis typical of GBBS, the most common presentation of Lyme Neuroborreliosis in Europe, are supported by serology and have as particular complications time-related hyponatremia with GBBS meningitis as the only possible explanation for Schwartz-Bartter syndrome.

Conclusions: Lyme disease has to be added to the long list of etiologies of hyponatremia and inversely hyponatremia to the long list of Lyme symptoms.

P746

**Rapidly progressive subacute sclerosing panencephalitis in a 3.5 year-old boy: correlation between MRIs and EEGs.** C. Gürses, F. Erdogan, S. Yentür, E. Önal, G. Yilmaz, G. Direskeneli, B. Baykan, A. Gökyigit, Istanbul University, Erciyes University (Istanbul, Kayseri, TR)

Subacute Sclerosing Panencephalitis (SSPE), a rare and late complication of measles, is a neurodegenerative and inflammatory disease of central nervous system (CNS) that occurs in children and adolescents and progresses slowly. The frequent clinical findings are personality changes, mental difficulties, stereotyped attacks (myoclonia/atonia). A case with an unusual rapidly progressive SSPE will be presented.

A 3,5 year-old boy with sudden falling episodes was admitted to our clinic. Previously there was no remarkable history of prenatal, perinatal and postnatal periods and he was healthy. There was no history of trauma and contagious illness. He had several falling episodes and/or head drops lasting a few seconds unaccompanied with other complaints for 1.5 months. He had measles at the age of six months during an endemic. No measles vaccination had been done prior to measles.

In the first two EEGs of the patient, one of which was done with video-EEG monitoring, bilateral, synchronous, symmetric and generalized slow waves in delta frequency were seen mostly in cerebral hemispheres predominantly in the anterior regions lasting 1.5–2 seconds and recurring at 6–10 seconds intervals. His background activity showed 4.5–6 Hz in theta frequency. Clinically, his head was dropping and simultaneously he was closing his eyes every 6 to 10 seconds. His head dropped occasionally forward but predominantly toward the left side. High measles antibodies in his CSF and serum were seen. He was diagnosed to have SSPE. The third and last MRI revealed severe triventricular hydrocephalus, subcortical white matter changes such as diffuse hypointensity in T1W and hyperintensity in T2W images and severe cortical atrophy, significant atrophy of corpus callosum, cerebellar atrophy were also seen while the first MRI was normal. In his last EEGs, PCs were not seen and clinically no myoclonia was detected. He died in 4 months after being diagnosed.

Although the measles vaccination is administered without charge in our country, SSPE is still seen but with a decreasing trend.

Rapidly progressive cases are not seen often. There is no laboratory marker to make us understand this earlier however. In very short period in this case both clinical and neuroimaging findings correlate well although it is not easy to find the same correlation in usual SSPE cases. The answers to the questions whether having measles at an early age or the early onset of SSPE caused the rapid progression of the disease, should be well researched.

P747

**Demographic features and clinical characteristics of viral encephalitis in southwestern Greece: a 12-year experience.** S. Papapetropoulos, A. Argyriou, I. Nilas, I. Ellul, P. Polychronopoulos, Medical School of Patras-Neurology Department (Patras, GR)

Objectives: Encephalitis is an unusual manifestation of human viral infection with a wide variety of neurological manifestations. In the present

work we set out to study the clinical and epidemiological characteristics of viral CNS infection.

Methods: In our study we included 49 consecutive patients with the diagnosis of viral encephalitis that were treated and followed up in the department of Neurology of the regional University Hospital of Patras, Greece during a twelve-year period between 1990–2002. Demographic, clinical and laboratory findings were carefully collected and analyzed.

Results: Our patients had a mean age of 41.9 years (SD 22.7). 53.1 % were male. Their mean hospitalization period was 16.7 days (SD 7.5). Their main symptoms during admission were headache in 32.7 % (16 patients), seizures in 20.4 % (10 patients), fever in 16.3 % (8 patients) and decreased level of consciousness in 16.3 % (8 patients). Other symptoms included double vision, unsteadiness, acute psychosis and speech problems. Neurological examination revealed decreased level of consciousness in 13 patients (26.5 %), cranial nerve palsies in 8 patients (16.3 %), pyramidal signs in 6 patients (12.2 %) and mixed aphasia in 1 patient. CT (when performed) revealed lesions related to the encephalitis in 2 patients (4.1 %), MRI (when performed) in 7 patients (14.3 %) and EEG was abnormal in 14 patients (28.6 %). There was a clear seasonal distribution. Most cases were diagnosed during spring and winter months (41.7 % and 31.3 % respectively). There was a striking increase in the incidence of viral encephalitis during the last 5 years of the study. 12 patients (25 %) were diagnosed between 1990 and 1996 whereas 36 (75 %) between 1997–2002.

Discussion and Conclusions: Apart from the expected seasonal variation in the appearance of new cases of viral encephalitis there was a striking increase in the incidence of the disease between 1997–2002. A possible explanation is the population redistribution in the region during the last 5 years.

### P748

**Peripheral facial nerve palsy with positive varicella PCR in the CSF without cutaneous manifestations. A case report.** A. Nouh, C. Delpirou-Nouh, A. Landais, M. Pages, CHU Montpellier (Montpellier, F)

Introduction: In immunocompetent adult patients, the complications of Varicella zoster virus (VZV) are not common. Often, the peripheral facial nerve palsy (PFNP) secondary to VZV infection is accompanied with cutaneous eruption in the Ramsay-Hunt region. We report a case of PFNP in an immunocompetent adult, with no facial cutaneous lesions, but with widespread eruptions similar to those encountered in children chicken pox, on the trunk and on the limbs.

Observation: A 54 year old, immunocompetent woman was admitted into hospital with the symptoms of asthenia, followed by vesicular, pruritic cutaneous eruptions, distributed on the limbs and then on the trunk. One week later, a new onset of right PFNP was installed progressively and became complete 48 hours later. Ear, Nose and Throat (ENT) examination showed no cutaneous vesicles in the Ramsay-Hunt region, or in the external auditive canal. The cerebro-spinal fluid (CSF) analysis for polymyrase chain reaction (PCR) was positive for VZV. The patient was treated immediately with intravenous acyclovir. The course was good with complete recuperation after two months.

Discussion: Often, in immunocompetent adult, the PFNP secondary to infection with VZV, is seen in zona, accompanied with lesions in the Ramsay-Hunt region. Our report is interesting because the PFNP was encountered in chicken pox manifestations and immunocompetent adult.

Conclusion: In front of a PFNP, even in immunocompetent adult, and even in the absence of any ENT or facial skin lesions, we should check for the PCR of the VZV in the blood and the CSF.

### P749

**Clinical report of medical approach to an intramedullary abscess.** I. Guidi, R. Clerici, F. Cogiamanian, P. Baron, E. Scarpini, G. Conti, IRCCS Ospedale Maggiore Policlinico (Milan, I)

Intramedullary abscess of spinal cord (IASC) is a rare infection of CNS, traditionally associated with significant mortality and neurological morbidity.

We report a 59 year old woman with a congenital interatrial defect, who developed an acute progressive paraparesis and urinary retention. Two weeks before the onset of clinical signs, she complained intense dorsal and lumbar back pain, followed within few days by fever. At the neurological examination she was bedridden, and presented a Brown-Sequard syndrome on the right side with D4 level. The MRI images showed a segmental widening of spinal cord from C7 to D10, and T2-hyperintense irregular signal, and a dishomogeneous lesion at D3-D4 level with pathological enhancement. An increase in CSF protein (195 mg%) and polymorphonucleate cells (> 580/mm$^3$) was observed, as well as low glucose level. Chest x-ray scan showed several parailar lesions on the right side, resembling Staphylococcus epidermidis abscesses, and hemoculture was positive for the same bacteria. We started treatment with Mannitol, Vancomycin and Ceftriaxone, with sudden regression of fever and back pain, and dramatic reduction of CSF protein (84 mg%) and cells (73/mm$^3$). After four weeks, neurological deficits improved and the patient was able to stand-up with crutches help. Concomitantly was observed resolution of pulmonary lesions and improvement of spinal-cord MRI pattern. Even though the most suitable management for IASC is considered to be a combination of medical and surgical therapies, we had a good recovery with the use of combined antimicrobial and antioedemigen therapy.

### P750

**Intracerebral haematoma in herpes simplex virus meningoencephalitis: a case report.** A. Argyriou, P. Talelli, M. Marangos, I. Tsota, T. Petsas, E. Chroni, University of Patras (Patras, GR)

Purpose: We present the case of a patient, in whom herpes simplex virus meningoencephalitis was complicated with a large intracerebral haematoma.

Materials and methods: A 22 year-old man presented with frontal headache, fever 38-5 °C and a generalized epileptic seizure. On neurological examination he was confused, with cervical stiffness and extensor plantar reflex on the left. There was no evidence of intoxication, the patient's past medical history as well as the routine blood tests, including coagulation factors, were unremarkable. CSF analysis showed 425 WBC/mm$^3$ (99 % lymphocytes), protein 75 mg/dl and glucose 75 mg/dl. A diagnosis of viral meningoencephalitis was made and the patient was given intravenously Acyclovir (500 mg three times a day). On admission, brain computed tomography was negative. Two days later MR T2 weighted images showed unilateral increased signal intensity in the left temporal lobe. The electroencephalogram showed frequent slow and slow wave activity over the fronto-temporal regions bilaterally with a left temporal emphasis. CSF and blood cultures for ordinary bacteria and mycobacteria were negative. The assay for detection of antibodies against common viruses (HSV, HZV, VZV and CMV) was also negative. PCR assay for HSV-1 was positive and a diagnosis of HSV-1 Meningoencephalitis was established; therefore the administration of Acyclovir for a total duration of 21 days was decided. The patient was fully alert from the day after admission, while the fever and the headache subsided after the 6th day of hospitalization.

On the 11th day of hospitalization due to reappearance of intense headache with no amelioration to common painkillers, a new CT was obtained, which revealed a large intracerebral haematoma in the uncus of the left temporal lobe extending to the ipsilateral basal ganglia. The location of the haematoma corresponded to the previous MR abnormal signal. A selective angiography of the left common carotid artery was performed for the exclusion of an intracerebral aneurysm or AVM, which was negative.

Results: At discharge on the 22nd day of hospitalization, the patient had fully recovered and a follow up CT showed a nearly complete absorption of the haematoma.

Conclusion: Development of intracerebral haematoma is an unusual finding in HSV-1 Meningoencephalitis. To our knowledge there are only two reports in the literature describing haematoma complicating HSV-1 Meningoencephalitis bilateral or unilateral as in our case.

### P751

**Brain stem tuberculoma mimicking any space-occupying lesion: the role of antituberculous drugs as a diagnostic tool.** N. Sharafadin Zadeh, Ahwaz Medical University (Ahwaz, IR)

Brainstem tuberculomas are uncommon. The major diagnostic problem with these lesions is the lack of specificity of their clinical and neuroimaging findings.

In developing countries, 5–8 % of space-occupying lesions of the central nervous system are caused by tuberculomas. Among these, brainstem localization is rare and if extracranial tuberculosis is not found diagnosis can be extremely difficult. Failure to recognize brainstem tuberculoma may result in an unnecessary biopsy of the lesion and this approach may result in meningeal spreading of a focalized tuberculous infection.

Case report: A 30-year-old previously healthy woman complained of a one-month history of headache and diplopia with a history of pulmonary tuberculosis in her grandfather. Neurological examination showed only left lateral rectus palsy. Many investigations were done and according to her family history, MRI findings (solitary round enhancing lesion), living in endemic area for tuberculosis and after excluding other simulated diseases this patient received anti tuberculosis drugs and after regular follow-up all of the complaints and MRI findings were cleared.

Conclusion: This report demonstrates the usefulness of a trial with antituberculous drugs in patients with enhancing space-occupying brainstem lesions particularly in those living in endemic area.

P752
**Spinal neurocysticercosis: an untreatable case (How to treat?).** N. Canas, S. Calado, J. Vale, Hospital de Egas Moniz (Lisbon, P)

Introduction: Neurocysticercosis (NCC) remains a common parasitic infection in Portugal, mostly due to immigrants from ex-Portuguese African colonies. Spinal NCC is rare occurring only in 1–5 % of all NCC cases, and its usually associated with cerebral involvement. The management of spinal leptomeningeal NCC (slNCC) is problematic particularly if associated with extensive arachnoiditis (AR).

Case report: The patient was a 37-year-old man from Cape Verde with a 3-month progressive cauda-equina syndrome. He had a history of generalized seizures for 15 years, controlled with phenobarbital, and headaches of increasing intensity in the preceding weeks. Spinal magnetic resonance imaging (sMRI) showed multiple cystic lesions in the dural sac and diffuse spinal AR. Cerebral MRI revealed a compensated obstructive hydrocephalus; the cerebrospinal fluid (CSF) had an increase in mononuclear cells and proteins. A definitive diagnosis of NCC was confirmed by positive Immunoblot for NCC in serum and CSF. He was submitted to a ventriculoperitoneal shunt and treated for 2 weeks with albendazole and steroids with mild clinical improvement. One year later the patient developed an acute tetraparesis, and sMRI revealed cervical compressive cystic lesions associated with intense AR. Despite decompressive cervical laminectomy and cyst removal, plus treatment with praziquantel and steroids, he showed no further improvement.

Conclusion: This case highlights some aspects regarding slNCC pathogenesis and treatment: 1) the evidence of a previous NCC cerebral involvement (hydrocephalus, epilepsy), associated with the involvement of different spinal segments in different stages of the disease, favors CSF as the main route of parasite dissemination through the central nervous system; 2) The induction of an intense spinal AR by the presence of leptomeningeal cysts, led to several factors associated with bad prognosis, such as a reduced CSF penetration of anticysticercal drugs, difficulty in surgical removal and probable vascular insufficiency on the spinal cord. Cases like this, in which all the available therapeutic modalities are ineffective, justify the investigation of better treatment options for slNCC associated with AR.

## Multiple sclerosis

P753
**Evaluation of normal-appearing Corpus callosum and capsula interna in conventional MRI, with diffusion MRI in patients with relapsing-remitting multiple sclerosis.** N. Subutay-Oztekin, F. Oztekin, B. Renkliyildiz, B. Diren, SSK Ankara Education Hospital (Ankara, TR)

Background: Diffusion MRI (DW-MRI) which is a continuous and quantitative measurement can reflect the neuronal and axonal loss by apparent diffusion coefficient (ADC) measurements in cerebral gray and white matter. Previous studies with DW-MRI in Multiple Sclerosis (MS) have revealed that, both in lesions and in normal appearing white matter in T2 weighted images ADC values are increased indicating a hidden pathology. On the other hand there is a significant correlation between DW-MRI findings with clinical manifestations and disability of MS.

Objective: The aim of the study is to compare normal appearing capsula interna and corpus callosum with conventional MRI in patients with relapsing-remitting (R-R) MS with DW-MRI and relate these measurements with the patients EDSS in a 6 month follow up period, and also to compare these findings with age related normal control subjects.

Patients and method: 20 patients aged between 18–50 years old, with clinically definite R-R MS with normal appearing corpus callosum and capsula interna with conventional MRI are enrolled to the study. All the patients had EDSS measurements before entering the study. DW-MRI measurements are obtained at the same time with EDSS evaluations and after a 6 month follow up period DW-MRI measurements and EDSS values are reexamined. Control group consisted of 15 age matched healthy subjects. For statistical analysis Mann-Whitney U test, Spearman rank correlation test and variance analysis are used. MRI's are obtained with a 1.5 T (Siemens) device and are evaluated by a radiologist who is blind to the patients and their clinical condition.

Results: DW-MRI results of the patients revealed a significant increase in ADC values in the patient group with normal appearing corpus callosum and capsula interna in conventional MRI, whereas there was no difference in ADC values of the control group compared to conventional MRI. After the 6 month follow up period 4 patients had an increase in EDSS score, which is also in conjuction with the increased ADC values in DW-MRI.

Conclusion: The results of this study revealed that, although obtained in a high magnetic field, conventional MRI may not be able to reveal all the pathology in patients with MS, and other techniques such as DW-MRI and MR Spectroscopy can be helpful to detect hidden pathology in normal appearing white and gray matter. These results also support the previous findings that DW-MRI values have a significant relationship with disability and clinical findings in MS.

P754
**The effectiveness of the exercise programme to the isokinetic evaluation results on multiple sclerosis patients.** E. Tarakci, A. Yaliman, A. Baskent, Istanbul University (Istanbul, TR)

Objective: Multiple Sclerosis (MS) is a chronic, progressive disease characterized by demyelination of the central nervous system. It seems the weakness of the muscle and the lost of endurance at the lower extremities. The aim of this study is to determine the difference before and after exercises with isokinetic testing quadriceps and hamstring muscles groups.

Methods: Thirty MS patients with Expanded Disability Status Scale (EDSS) were 2–6.5 included in this study. The patients were selected randomly and two groups were done. Exercise group was 15 patients and worked three times a week an exercise programme at the hospital. The other 15 patients are the control group and they did their exercises like home programme. There were 12 F and 3 M at the exercise group. The mean of age were 41.0 ± 11.09. There were 11 F and 4 M at the control group. The mean of age were 39.46 ± 10.59. The muscle performance measured at the 30, 90 and 180 deg/sec. angular velocities with the Biodex System 3PRO isokinetic dynamometer. Peak torque parameter included at the evaluation. This measurements which were done before exercises were repeated 12 weeks after the exercise programme and the difference was measured between them.

Results: For the exercise group; at the right extremities for quadriceps muscles the peak torque values after the exercise programme were statistically significant at the 30 deg/sec ($p < 0.05$); but at the 90 and 180 deg/sec. at the left lower extremities for all the angular velocities they were not statistically significant ($p > 0.05$). For the hamstring muscles, both extremities at the 30 and 90 deg/sec. were statistically significant ($p < 0.01$); at the 180 deg/sec. for both extremities they were not statistically significant ($p > 0.05$).

For the control group; quadriceps muscle, at the right extremities, after the exercise programme the peak torque values at the 30 and 180 deg/sec. were statistically significant ($p < 0.05$). At the 90 deg/sec. for all angular velocities for the left extremities were not statistically significant ($p > 0.05$). For hamstring muscle, at the right extremities 30 and 90 deg/sec. were statistically significant ($p < 0.05$); at the 180 deg/sec. and left extremities for all angular velocities they were not statistically significant ($p > 0.05$).For both muscles groups; 30, 90, 180 deg/sec. angular velocities, differences of the peak torque values before and after exercise programme, comparing exercise and control group, there were not statistically significant differences between them ($p > 0.05$).

Conclusions: For MS patients which can occur the weakness of the lower extremities, for hamstring and quadriceps muscles groups the evaluation results with the isokinetic dynamometer for both groups there were improvements and these improvements were not different between two groups. The exercises are very important for the treatment of MS patients. It will be positive improvement for the muscle strength after working at hospital or at home.

P755
**The prognosis of Devic's neuromyelitis optica.** A. Ghezzi, R. Bergamaschi, M. Filippi, V. Martinelli, G. Mancardi, R. Tola, M. Troiano, M. Zaffaroni, G. Comi – MS Study Centre Italian Devic Study Group (IDESG)

Background: Devic's neuromyelitis optica (DNO) is a mono/multiphasic disease characterised by simultaneous/subsequent involvement of the optic nerves and of the spinal cord, and with an unclear relationship with MS. As data are not available on the long term evolution of DNO, our study was designed in order to evaluate its course and prognosis.

Material and methods: 46 patients affected by DNO (36 females) were collected by 15 Italian neurological departments. Patients were included if presenting optic nerve and/or spinal cord involvement in a mono/multi-

phasic pattern. Patients were excluded if: a) presenting CNS involvement beyond the optic nerve/spinal cord, b) presenting intrathecal IgG synthesis and > 2 brain MRI lesions at the baseline scan. Neurological examination was scored by using Kurtzke's scales. Prognostic factors were analysed by means of survival curves. Mean age was 40.1 ± 16.3, mean follow up duration: 8.8 ± 3.5 years.

Results:

- Disability (Kurtzke's EDSS) during the acute phase of the first 6 attacks was respectively: 3.9 ± 1.5, 4.4 ± 1.5, 4.9 ± 2.1, 5.4 ± 1.8, 5.6 ± 2.0, 6.3 ± 1.8. The residual EDSS in the same stages of the disease was (in brackets the % of reduction compared to the score during the acute phase): 2.1 ± 1.9 (-46%), 3.0 ± 2.0 (-32%), 3.9 ± 2.3 (-20%), 4.3 ± 2.0 (-20%), 4.6 ± 2.5 (-18%), 5.4 ± 2.3 (-14%). Death occurred in 5 cases after a mean follow up of 6.4 years (range1.1-13).
- The EDSS = 3 was reached by 65% of cases after 5 years, by 82% of cases after 10 years, and by 86% of cases after 15 years,
- The EDSS 6 was reached respectively by 42%, 53%, 69% of cases.
- The probability of reaching EDSS 3 was statistically correlated to age at onset, interval between the first and 2nd attack, and relapse rate. The probability of reaching EDSS 6.0 was correlated to the residual EDSS at onset and to relapse rate.

Conclusions: DNO has a poor prognosis in most cases. Compared to MS, DNO patients have a higher age at onset, females are more frequently affected, the course is more severe.

P756

**MR lesion load as a predictor of attention deficit and visuomotor tracking in patients with multiple sclerosis.** M. Semnic, R. Semnic, G. Ocic, Z. Todorovski, S. Banic-Horvat, N. Delibasic, M. Krkljes, Institute of Neurology, Institute of Oncology (Novi Sad, Sremska Kamenica, Belgrade, YU)

Attention deficit is a cognitive dysfunction often reported in patients with multiple sclerosis (MS). Little documentation of visuomotor tracking deficit in such patients is currently available.

The objective of the study was to explore correlation between total lesion load (TLL) obtained by magnetic resonance imaging (MRI) and cognitive scores, which represent the functions of attention.

Methods: 40 patients with relapsing-remitting MS were compared to 30 healthy volunteers matched for age, sex and education. Expanded Disability Status Scale (EDSS) score was determined. We administered the following battery of neuropsychological tests: Trail Making Test (TMT), Digit Symbol Subtest of the Wechsler Adult Intelligence Scale - Revised (WAIS-R), Mental Control Subtest of the Wechsler Memory Scale-Revised (WMS-R), and Visual Span Backward and Forward of the WMS-R. Outlines of MS lesions were traced on the MRI computer console and TLL in supratentorial compartment was calculated and expressed as volume ($mm^3$). The statistical difference between MS patients and healthy controls was determined using multivariate analysis (MANOVA). Cognitive test scores and TLL were analysed using canonical correlation.

Results: The mean EDSS was 2.34. The MS group showed significantly worse score on all administered cognitive tests except the Visual Span Forward test. Canonical correlation revealed strong relationship between TMT scores of the A form (reaction time and number of errors) and TLL ($p = 0.009$). For the Digit Symbol and Visual Span Forward tests a moderate but not statistically significant correlation with TMT was found. No correlation was found between the Visual Span Backward test and the TLL.

Conclusion: Our results showed that TLL is a good predictor of attention and visuomotor tracking deficit in MS patients. Thus, we can conclude that attention deficit is a consequence of the functional disconnection between different brain areas and/or the cumulative lesion effect.

P757

**Interferon beta mRNA expression in interferon beta treated and untreated multiple sclerosis patients.** K. Retzlaff, B. S. Kühne, P. Frielingshaus, J. Kraus, S. Mannes-Keil, G. Foerster, M. Kaps, P. Oschmann, University Giessen (Giessen, Munster, D)

Objective: To investigate the effect of interferon (IFN) beta-therapy on the gene expression of IFN beta in patients with relapsing-remitting Multiple Sclerosis (RR-MS) in relation to clinical activity.

Background: Mechanism of action of Interferon-therapy in MS is unknown. One possibility might be a regulatory effect on the transcription of the IFN beta gene. Therefore we measured IFN beta on the mRNA level.

Materials and Methods: We conducted a prospective, non-randomized study with two parellel groups (30 untreated, 40 IFN beta treated patients) over 12 months. Every three months clinical examination and blood sampling was done. Additionally blood samples of 20 matched healthy individuals were obtained once. The quantification of the mRNA expression of IFN beta was provided by quantitative "real time" and "online" RT-PCR.

Results: In comparison to healthy individuals MS-patients showed at baseline highly significant elevated expression levels of Interferon beta ($p = 0.0001$). Over the period of one year IFN beta treated and untreated patients showed an opposing trend regarding IFN beta mRNA expression-stabilization under treatment and significant decrease without treatment ($p < 0.0001$). Furthermore IFN beta mRNA expression correlated negatively with the EDSS-score in all patients - high values accounted for low scores ($p = 0.0109$).

Conclusions: Our results suggest a mechanism of action of IFN beta treatment by influencing the expression levels of IFN beta mRNA.

P758

**Prospective follow-up of acute partial transverse myelitis.** C. Cordonnier, J. de Seze, G. Breteau, D. Ferriby, E. Michelin, T. Stojkovic, J.-P. Pruvo, P. Vermersch, Hopital R. Salengro (Lille, F)

Background and objective: Clinical and radiological characteristics of myelopathy in multiple sclerosis (MS) are relatively well known. Nevertheless, it remains difficult for the clinician to ascertain an evolution to MS after a first episode of acute partial transverse myelitis (APTM). Furthermore, there is a need for predictive factors of bad evolution in order to decide which patients should be treated by immunomodulatory drugs early in the course of the disease.

Aim: The aims of this study were to define predictive criteria of conversion into clinically definite MS after an APTM and to define predictive factors of severe disability progression.

Patients and method: Between 1994 and 2001, we prospectively included 55 APTM (28.85% men, 71.15% women, mean age of 35 years). Three patients were lost during the follow-up. We evaluated clinical, spinal cord and brain MRI, cerebrospinal fluid (CSF) and visual evoked potentials (VEP) at admission. The mean duration of follow-up was 35 months (range 12-86). At endpoint, we evaluated diagnosis and among the MS subgroup, severity of the disease.

Results: Among the 52 APTM who completed the study, 30 became definite MS (23.33% men, 76.67% women, mean age of 33 years, mean follow-up of 37 months) and 22 remained of unknown aetiology (36.36% men, 63.64% women, mean age of 37.8 years, mean follow-up of 31.5 months). All the MS patients remained in the relapsing remitting form of MS. The discriminating factors for the evolution in MS were: initial sensorial symptoms ($p = 0.009$), latero-posterior spinal cord lesion ($p = 0.01$), abnormal brain MRI ($p = 0.002$) and oligoclonal bands in CSF ($p = 0.003$), but not VEP. In the clinically definite MS subgroup, we did not find any predictive factors of severe disability progression.

Conclusion: Our study demonstrates that clinical symptoms, CSF, spinal cord and brain MRI are highly predictive of an evolution of APTM into clinically definite MS. These patients should be selected for an early treatment by immunomodulatory drugs.

P759

**Survival, prognostic factors, magnetic resonance imaging and cerebrospinal fluid findings in early-onset multiple sclerosis.** M. Eraksoy, N. Turan, G. Akman-Demir, M. Kurtuncu, Z. Yapici, F. Bilgili, H. Ozcan, Istanbul University (Istanbul, TR)

The incidence of multiple sclerosis (MS) is age dependent being rare before age 10, unusual before age 16, and the peak age at onset is 25-30 years.

There has been some debate whether clinical, demographic, magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) findings of early-onset MS (EOMS) are similar to adult-onset MS (AOMS). On the other hand, the relationships between MS and acute disseminated encephalomyelitis (ADEM), childhood infections, immunizations are controversial.

This study presents 94 EOMS patients in whom 55 were seen at the onset (16 < age), together with clinical, demographic, MRI and CSF findings. These findings were compared to 150 patients with AOMS. Ninety-four patients with EOMS and 150 consecutive patients with AOMS were the members of our MS cohort (n = 2286 in January 2003). These patients were re-examined and the data were updated in January 2003.

The incidence of EOMS was 4% (94/2286). The mean age at onset was 12.48 ± 2.78 in EOMS and 28.62 ± 8.55 in AOMS. In 14 patients, initial manifestations began under 10 years of age. The mean age in 2003 was 27.2 ± 8.08 and 38.17 ± 10.01 in EOMS and AOMS respectively. There was no difference between the sex ratio of the EOMS and the AOMS groups. There was a difference between initial manifestations, brain stem findings were commonest in EOMS, however, sensory-motor findings were frequently encountered in AOMS. There was no statistically significant dif-

ference between two groups in terms of clinical course, but primary progressive course was not seen in the EOMS group.The first interattack interval was longer in EOMS, but there was no statistically significant difference in the number of relapses in the first five years of disease in both groups. Duration of follow-up was 12.65 ± 7.16 in EOMS and 7.11 ± 5.43 in AOMS. Duration of disease was longer (12.20 ± 6.79) in EOMS and was 9.28 ± 7.56 in AOMS. The EDSS scores were higher in EOMS in 2003, but there was no difference in the progression indexes in both groups. The median time taken for both EOMS and AOMS patients to reach a score of 6.0 on the EDSS was not different between the groups (Log Rank 0.31, p = 0.579).

Brain and spinal MRI and CSF findings were similar to those seen in AOMS group, however, EOMS group tended to have tumor-like, large lesions, particularly infratentorial regions.

In conclusion, this study reveals that EOMS and AOMS had similar clinical, prognostic, MRI and CSF findings.

**P760**

**The diagnostic value of VEMPs in multiple sclerosis.** C. Solaro, A. Beronio, E. Ghiglione, R. Parodi, L. Mazzella, F. Bandini, Ospedale P. A. Micone, University of Genoa (Genoa, I)

Objective: Aim of the study was to investigate whether the vestibular evoked myogenic potentials (VEMPs) have a role in identifying clinically silent brainstem lesions in patients with Multiple Sclerosis (MS) and to compare them with Magnetic Resonance Imaging (MRI) of the brain and pattern Visual Evoked Potentials (VEPs).

Methods: We recorded VEMPs and VEPs from 36 patients with definite MS and 21 age-matched healthy subjects. Latency and amplitude values of the P13 and N23 components of the VEMPs and latency values of the P100 peak of the VEPs were measured. The number of T2-weighted brainstem lesions of conventional MRI was counted in MS patients.

Results: P13 latency of the VEMPs proved to be a distinguishing feature of MS patients from controls. VEMPs were able to detect clinically silent lesions in 40 % of the patients, showing a lower sensitivity than VEPs (50 %) and brainstem MRI (55 %). In 20 patients (55.6 %) VEMPs and brainstem MRI provided discordant results. Fourteen of them (70 %) had an abnormal MRI in presence of normal VEMPs, while 6 patients (30 %) presented the opposite feature. Nine out of 20 patients without clinical evidence of brainstem dysfunction had an abnormal MRI in presence of normal VEMPs, while VEMPs were abnormal in 4 patients who had a normal MRI. A significant correlation was observed between VEMPs latency measures and clinical scores (EDSS and brainstem functional score), while no significant correlation was found between the number of brainstem MRI lesions and the brainstem functional score.

Conclusions: Our results suggest that VEMPs are a simple and reliable method in order to assess the vestibulo-spinal function in MS. On the other hand, they cannot be considered a test of sufficient discriminative diagnostic usefulness, as compared with brainstem MRI and VEPs. Rather, the good correlation of VEMPs latency measures with the clinical severity of the disease suggests a potential role for this technique in monitoring MS evolution.

**P761**

**Evolution of neutralizing antibodies against interferon-beta in patients with multiple sclerosis.** C. Gneiss, T. Berger, F. Deisenhammer, University of Innsbruck (Innsbruck, A)

Background: Neutralizing antibodies (NAB) against interferon-beta (IFN-beta) in patients with multiple sclerosis (MS) are reported to reduce the clinical efficacy of this treatment. NAB inhibit the biological activity of IFN-beta by blocking the receptor-binding of IFN-beta. NAB become detectable between 6 to 18 months after initiation of therapy and may disappear in the long term even though treatment is continued.

Objective: To determine whether the magnitude of NAB titers against IFN-beta influences the time to reversion to NAB-negative status.

Methods: Thirty-two NAB-positive patients with definite MS receiving IFN-beta-1a or IFN-beta-1b were included in this study. We chose 15 patients who reverted to NAB-negative status during treatment and 17 patients who were NAB-positive for a period of at least 48 months after IFN-beta therapy was initiated. The NAB titer was determined by the MxA induction assay. Titers of > 19 neutralizing units (NUs) were considered NAB-positive. A patient was defined as NAB-positive, if at least two consecutive NAB serum titers were positive. Reversion to NAB-negative status was confirmed by two consecutive negative samples after NAB positivity.

Results: There was a significant difference in NAB peak titers between patients who reverted to NAB-negative status (median peak titer = 61 NUs) during treatment and patients who remained NAB-positive (median peak titer = 1162 NUs) for at least 48 months after initiation of IFN-beta therapy ($p \leq 0.0001$). Additionally, NAB peak titers were observed significantly earlier in patients with reversion to the NAB-negative status (mean duration = 20 months) compared to the non-reverters (mean duration = 39 months) (p = 0.0002). The mean duration of NAB positivity in non-reverters was 57 months whereas the NAB-negative status was reached after a mean duration of 35 months. Although the observation period was too short to monitor all patients until NAB-negative status was reached, non-reverters had a tendency to a reduction of NAB titers over time (median NAB titer at last testing = 734 NUs).

Conclusions: The results show that the time to reversion to the NAB-negative status depends on the NAB peak titer. There is a tendency that patients with low NAB titers revert to the NAB-negative status after a relatively short period of time, which is not the case for patients with high titers. Nevertheless, patients with high NAB titers show the tendency to lose NAB over a longer period of time.

**P762**

**Cardiotoxicity secondary to mitoxantrone in multiple sclerosis.** I. Bosca, A. Pascual, I. Hervas, B. Casanova, F. Coret, P. Bello, Hospital La Fe, Hospital Clinico (Valencia, E)

Background: Mitoxantrone is a synthetic antineoplastic agent that has been proved effective to arrest the progression and relapses in multiple sclerosis (MS), but its use is limitated by cardiotoxicity associated with high cumulative doses, preexisting cardiovascular diseases, prior treatment with anthracyclines and prior mediastinal radiotherapy. In several reported open-label studies on MS, the incidence of clinically significant cardiac disfunction has been low. Isotopic ventriculography (IV) or echocardiography were used indistinctly to measure left ventricular ejection fraction (LVEF). Several studies show superior reproducibility in IV, which makes this test more sensitive to detect clinically significant changes.

Objective: To review symptoms and signs of cardiotoxicity in a series of 20 patients treated with mitoxantrone as a single agent for MS, using IV.

Patients and methods: We record serial (basal and every six months the first two years and before each dosage the third year) LVEF measured by IV. According to a previously established protocol, we consider to stop treatment if LVEF decreases to < 50 % or by > 10 % of the baseline LVEF. To date, 20 clinically definitive multiple sclerosis patients according to the Poser criteria, diagnosed of worsening RR, SP or PR MS according to Lublin criteria, have been treated with mitoxantrone: 10 mg/m$^2$ every three months for three years (maximum cumulative dose 120 mg/m$^2$) except for one patient who was administrated loading dose of 10 mg/m$^2$ monthly for three months and three-monthly dose afterwards. Duration of follow-up is a mean of 16.6 months.

Results: 20 patients, 60 % females, mean age 40.95 [26–58], mean Kurzke Expanded Disability Status Score (EDSS) 6.15 [4.0–7.5], mean disease duration 13.4 years [5–25]. No patients had preexisting cardiovascular disease before treatment. Cumulative mitoxantrone doses ranged from 10 to 70 mg/m$^2$ (mean 50 mg/dl). No patients experimented congestive heart failure. Baseline LVEF was > 50 % in all patients. In total, 6 patients (30 %) experienced a significant reduction of the LVEF according to our previously defined security range; in 4 patients the LVEF decreased to < 50 % (20 %), and in 2 patients > 10 % of the baseline LVEF (10 %). No patients experimented congestive heart failure. These reductions were confirmed repeating IV 6 months afterwards, and they were not significantly related to age, gender, disease duration, EDSS nor cumulative dose of mitoxantrone.

Conclusions: IV is more reproducible than echocardiography for measuring LVEF, as has been demonstrated in several reports, this could be the reason for finding more incidence of decreasing in LVEF in our MS patients treated with mitoxantrone than reported in previous open-label studies. We are concerned about the high incidence of cardiotoxicity we find, but prolonged follow-up is needed to determine whether the incidence of congestive heart failure increases.

**P763**

**Characterization of white matter damage in juvenile multiple sclerosis using magnetization transfer and diffusion tensor MRI.** D. Mezzapesa, A. Ghezzi, B. Colombo, M. Rodegher, M. Rocca, A. Falini, G. Comi, M. Filippi, Neuroimaging Research Unit, Ospedale di Gallarate, Department of Neurology, Department of Neuroradiology (Milan, Gallarate, I)

We obtained magnetization transfer (MT) and diffusion tensor (DT) magnetic resonance imaging (MRI) to quantify the extent of "occult" damage

of the brain and the cervical cord in patients with juvenile MS. Conventional, MT and DT MRI scans of the brain were obtained from 13 individuals with juvenile MS (mean age = 14.1 years, SD = 2.7, range 7–18), all with a relapsing-remitting form of MS, and from 10 age- and sex-matched controls. The mean number of relapses per patient was 2.7 (SD = 0.9; range 2–4) and the mean EDSS score was 1.4 (SD = 0.5; range 1–2.5).

Lesion volume was calculated using a local thresholding segmentation technique. MT ratio (MTR), mean diffusivity (MD) and fractional anisotropy (FA) maps were obtained and average lesion MTR, MD and FA calculated. Using SPM99, and maximum image inhomogeneity correction, gray matter (GM), white matter (WM), and cerebro-spinal fluid were automatically segmented from T2- and PD-weighted images. The resulting masks were superimposed onto the MTR, MD and FA maps on which hyperintense lesions were masked out and the corresponding histograms of the normal appearing WM (NAWM) and GM were produced. For each histogram, the average MTR, MD and FA values, the peak height, and the peak position were measured. Cervical cord lesions were identified on the fast-STIR scans. MTR maps and histograms were derived from the entire cervical cord, measuring the same histogram metrics computed for the brain. MTR histogram-derived metrics of the NAWM, GM and cervical cord from MS patients were not significantly different from the corresponding quantities from controls. MS patients had average FA and FA peak site lower than in controls, both for NAWM ($p = 0.005$ and $p = 0.002$ respectively) and GM ($p = 0.001$ and $p = 0.004$ respectively); FA peak height was higher than in controls both for NAWM ($p = 0.02$) and GM ($p = 0.01$); MD peak height was lower than in controls both in WM ($p = 0.025$) and in GM ($p = 0.047$). No correlation was found between MTR, MD, and FA either of lesions and of NABT, and the EDSS score. There was a positive correlation between number of cervical cord lesions and EDSS score ($p = 0.018$, $r = 0.723$). A net loss of structural barriers occurs in the GM of patients with juvenile MS, as it has been shown in adult MS. The lack of MTR detectable damage suggests a less severe amount of tissue damage in juvenile than adult MS. This fits with the notion that an early onset of the disease is associated with a favourable evolution.

**P764**

**Inter-nuclear, nuclear and fascicular eye movement disorders in multiple sclerosis patients: review and correlation with brain MRI and disease course.** P. Hradilek, O. Zapletalova, M. Häringova, University Hospital (Ostrava, CZ)

Background: Inter-nuclear ophthalmoplegia (INO) being one of the neuro-ophthalmological hallmarks of multiple sclerosis (MS) occurs due to damage to the medial longitudinal fasciculus, a primary fibre tract that connects the abducens nucleus to the medial rectus subnucleus on the contra lateral side of the brainstem. Oculomotor palsies with consequent diplopia could also be observed in MS with the highest frequency of sixth nerve palsy.

Objectives: To review frequency of inter-nuclear, nuclear and fascicular eye movement disorders in our group of MS patients in correlation with brain MRI findings and try to assess prognosis of the disease course in the patients with oculomotor disorder as first clinical symptom of MS.

Material and methods: Clinical and MRI data of our group of 584 MS patients (158 men and 426 women) were reviewed in our retrospective study.

Results: Totally we found 115 MS patients, who presented with oculomotor dysfunction during disease course (14 with the third nerve palsy, 52 with the sixth nerve palsy, 47 with INO, one with combination of the third and the forth and one with the third and the sixth nerves palsies). In 26 patients the eye movement dysfunction occurred as first clinical symptom of MS (III – 4 patients, VI – 16 patients, III + IV – 1 patient, III + VI – 1 patient and INO – 4 patients). We found corresponding lesions in the brainstem on MRI in 8 patients with the IIIrd nerve palsy (57%), 36 patients with the VIth nerve palsy (69%), 37 patients with INO (78%) and 1 patient with combination of the IIIrd and the VIth nerve palsy. The average expanded disability status scale score (EDSS) of the patients with oculomotor disorder in the beginning of the disease was 1.8 after 5 years from the disease onset [0–4.5].

Conclusions: Eye movement disorders are not as rare as believed. In most patients we could find corresponding lesions in the brainstem on MRI in the site of nuclear origin of affected nerve. Our patients with VIth and the IIIrd nerve lesions in disease onset tend to more benign disease course, while the course of these with INO is often more complicated.

**P765**

**The role of the interleukin 4-receptor in multiple sclerosis patients under interferon-beta therapy.** K. Retzlaff, B. S. Kühne, S. Mannes-Keil, P. Frielingshaus, J. Kraus, G. Foerster, M. Kaps, P. Oschmann, University Giessen (Giessen, Munster, D)

Objective: To investigate the effect of interferon (IFN) beta-1b on the gene expression and protein levels of the Interleukin 4 (IL4)-receptor in patients with relapsing-remitting Multiple Sclerosis (RR-MS) in relation to clinical activity.

Backround: IFN beta influence various cytokine and cytokine receptors e. g. TNF-receptor I and II show a strong increase under treatment. Probably this is due to the effect of IFN beta on cell growth and differentiation. Therefore we examined the IL4-receptor on the transcriptional and protein level to gain further insight in the regulatory mechanism.

Materials and Methods: We included 50 untreated patients, 27 patients with Rebif(R)-therapy and 18 patients with Betaferon(R)-therapy. All patients underwent frequent neurological examinations with scoring on EDSS and blood sampling (every 3 months) over a 12-month observation period. Additionally blood samples of 20 healthy individuals were obtained once a time. Serum levels of IL4-receptor were determined by ELISA, the quantification of the mRNA-expression was provided by quantitative "real-time" and "online" RT-PCR.

Results: MS-patients showed elevated IL4-receptor protein and mRNA levels in comparison to healthy individuals. An opposing trend regarding mRNA levels was found in dependence on treatment-therapy either with IFN beta 1a or IFN beta 1b stabilized mRNA levels in comparison to a decrease in the untreated group.

Furthermore mRNA levels correlated negatively with the EDSS-score in all patients, that means high values account for a low score ($p = 0.0029$), probably reflecting disease progression. In contrast soluble IL4-receptor correlated positively with the high relapse rate, probably reflecting short term disease activity ($p = 0.0064$).

Conclusions: Our results suggest an immuneregulatory effect of IFN beta on the IL4-receptor gene.

**P766**

**Aphasia in multiple sclerosis: MRI findings and follow-up.** E. Vidry, G. Chopard, E. Revenco, C. Clerc, L. Tatu, T. Moulin, L. Rumbach, Hôpital Jean-Minjoz (Besançon, F)

Goals: To describe five MS patients with aphasia and to evaluate prognosis

Background: Clinical presentation of MS is highly heterogeneous. Cognitive impairments appear in 40 to 60% of patients. While MS can impair speech production, aphasia is considered to be rare and prognosis has seldom been evaluated.

Design/Methods: The history, clinical presentation, diagnostic workup and evolution of 5 patients who presented aphasia are compiled and summarized.

Results: Five patients with MS presented to our Department with a history of an abrupt onset of aphasia. All had relapsing-remitting MS, evolving for from 1 to 10 years. All but one had had previous relapses. EDSS range was from 0 to 3 before aphasia. Aphasia occurred suddenly either alone (1 case) or associated with right hemiparesis. In 2 patients comprehension was also perturbed. MRI demonstrated in all cases large lesions, confluent in 3 cases (located in the frontal lobe) and non confluent in 2 cases (located beneath the inferior frontal gyrus). In all cases, there were multiple white matter lesions. Patients were followed for 1 to 8 years. Only the patient with no previous relapse recovered totally.

Conclusion: Acute aphasia is rare in MS. In our cases it only occurred in the relapsing-remitting forms; MRI revealed it was always associated with large inflammatory lesions. Prognosis was poor as only one patient had no language related sequelae.

**P767**

**Quality assessment in multiple sclerosis therapy: the QUASIMS Survey.** V. Limmroth, G. Kalski, A. Richter, P. Posel, C. Wernsdörfer, Neurologische Universitätsklinik Essen, Biogen for the QUASIMS-study group

Introduction: Multiple sclerosis (MS) is a chronic disease of the CNS and the most frequently occurring neuroimmunological disorder in the northern hemisphere. During the past decade substantial progress has been made in the development of disease modifying drugs. Immunomodulatory agents such as interferon beta became the standard for the treatment of remitting relapsing MS (rrMS). In most European countries four different interferon beta preparations are presently available for the treatment of rrMS: Interferon beta-1a (1x30µg im/week (A), AVONEX®), Interferon

beta-1b (3,5x250µg sc/week (B), BETAFERON®) and Interferon beta-1a (3x22µg sc/week (R22) or 3x44µg sc/week (R44) REBIF®). A prospective comparator trial between these four preparations, however, is not available. These retrospective data survey aims to compare the efficacy and tolerability of all available preparations in a four-arm, open, international multicentre study.

Patients and Methods: MS-patients with rrMS, who received interferon beta therapy for at least 2 years with continuous documentation of relapses, disease progression (EDSS) and adverse events were eligible for the study. The survey included demographic data, medical history and data about the reason of therapy discontinuation as well. By Feb 10th 1.789 patients from approximately 200 centres in Germany, Austria and Switzerland were included in the survey.

Results: Results presented here are based on an interim analysis of data from the first 1.000 patients. N of IFN preparation: A = 374, B = 406, R22 = 169, R44 = 51; average patient age: 36,9 (SD 10.1) years; mean disease duration by start of therapy 4.8 (SD5.5) years; mean duration of therapy 42.3 (SD16.1) months. No relevant differences in other patient characteristics were observed across treatment groups. Baseline EDSS (defined as EDSS at therapy start) in the four treatment arms: A 2.8 (SD 1.4), B 2.9 (SD 1.5), R22 2.5 (SD 1.5) and R44 2.7 (SD 1.6). Progression of disability (defined as changes in points on the EDSS over 2 years): under treatment with A, B, R22 and R44 = 0.2 (SD 0.9), 0.2 (SD 1.1), 0.1 (SD 0.8) and 0.3 (SD 0.9), respectively.

Conclusion: Demographics and patient characteristics were in line with data reported in previous phase III trials. Disease progression over 2 years appeared to be comparable among treatment groups.

## P768

**The prognostic value of sequential evoked potentials in multiple sclerosis – a retrospective 10-year follow-up study.** B. Kallmann, S. Büttner, M. Eulitz, M. Pette, K. Toyka, P. Rieckmann, K. Reiners, University of Wurzburg, University of Dresden (Wurzburg, Dresden, D)

Background: Evoked potentials (EP) have a long tradition as diagnostic tool in multiple sclerosis (MS) reflecting the pathogenic process of demyelination and axonal damage. Due to the sensitivity of this method visual evoked potentials (VEP) have also been included in the recently updated diagnostic criteria for MS (McDonald et al. (2002) Neurol). While for brain MRI several studies suggest that the number of lesions at clinical disease onset provide important information for the disease course the prognostic value of evoked potentials is not yet defined.

Objective: To determine the prognostic value of EP in MS we performed a retrospective study.

Methods: Patients referred to our MS clinic within a 2-year period after first presentation of clinical symptoms were included in this study. All patients (n = 50) were examined clinically including the expanded disability status scale (EDSS). Evoked potentials (EP) (visual (VEP), motor (MEP) and/or somatosensory evoked potentials (SEP) of upper and/or lower limbs) were performed at first presentation and at three additional time points during a 10 year follow-up period. All data were collected with the computer-based multiple sclerosis documentation system (MSDS).

Results: EDSS values at study entry ranged from 0–3 with a median of 2.0. Pathologically altered MEPs (either by central motor latency prolongation or amplitude reduction or both) of the lower limbs at baseline were significantly associated with those patients who progressed to an EDSS > 3.5 within 5 years ($\chi^2$-test, p = 0.008). Similarly, the combination of abnormal VEPs and SEPs at baseline (either by latency or amplitude or both) pointed to a significant disability (EDSS > 3.5) after 5 years ($\chi^2$-test, p = 0.007) and after 10 years ($\chi^2$-test, p = 0.05). Neither pathological baseline VEPs nor SEPs alone were of prognostic value.

Conclusion: These results indicate that functional lesions as evidenced by abnormal EPs at early stages of MS correlate with the degree of long-term disability. Therefore we suggest that sequential EP studies are a valuable addition for following MS patients beyond its initial diagnostic impact.

## P769

**Reliability and practice effect of the multiple sclerosis functional composite.** A. Solari, D. Radice, L. Manneschi, E. Motti, E. Montanari, Istituto Nazionale Neurologico C. Besta (Milan, Fidenza, Reggio Emilia, I)

Background: The Multiple Sclerosis Functional Composite (MSFC) is a multidimensional, multiple sclerosis (MS) specific outcome measure to be used in clinical trials.

Objective: To assess practice effect, inter- and intrarater reliability of the MSFC.

Methods: Thirty two MS outpatients (19 women, mean age 43 years, mean EDSS score 4.5) were tested by two neurologists on the MSFC, after a one-session formal training. Patient testing was performed using a standardised protocol (Fisher 1999). Each patient was independently assessed with the MSFC four times by one examiner, and two times by the other. The six sessions were completed in a single day, with at least 20 minutes rest between tests. The examiners were blinded to the results of previous tests. Inter-rater testing order was randomly determined. Practice effects in the individual MSFC tests were assessed graphically and by means of repeated measure analysis of variance. Reliability was assessed by means of the intraclass correlation coefficient (ICC).

Results: Out of the first session, we found no practice effect for the Timed 25-Foot Walk test. On the opposite both the Paced Auditory Serial Addition Test (PASAT) and the 9-Hole Peg test were characterised by marked practice effect, particularly in the first three sessions. Inter-rater reliability was excellent, with ICC ranging from 0.93 for the 9-Hole Peg test (95% confidence interval [CI] 0.84–0.96) to 0.99 for the Timed 25 Foot Walk test (95% CI 0.97–0.99). As far as intra-rater reliability is concerned, ICC ranged from 0.93 for the PASAT (95% CI 0.82–0.97) to 0.98 for the Timed 25 Foot Walk test (95% CI 0.93–1).

Conclusions: The MSFC is characterised by excellent intrarater and interrater reliability. Two MSFC tests (PASAT and 9-Hole Peg) demonstrated noticeable practice effect, and we recommend the administration of the MSFC three times, and results from the third evaluation be considered as baseline values.

## P770

**Application of different criteria for clinical response to beta-interferon in relapsing-remitting multiple sclerosis.** C. Giannesini, D. Pez, P. Le Canuet, J.-C. Ouallet, E. Roullet, O. Heinzlef, Hôpital Tenon (Paris, F)

Objective: To evaluate the rate of responders after one year of interferon beta; and to search for clinical predictors of response.

There is a growing need for definition of clinical response to interferon beta therapy in RRMS. Several sets of criteria have been proposed, but their sensitivity has not been evaluated.

Design/Methods: Inclusion criteria were: RRMS, EDSS: 0 to 5.5; initiation of interferon beta as first-ever disease-modifying drug in our MS clinic; ≥ 2 relapses during the last 2 years; follow-up ≥ 18 months.

Response to therapy was analysed at one year according to different criteria for treatment failure. They were either based on relapses (A: at least one relapse [similar to the on-going Antegren(R) add-on trial inclusion criteria]; B: same or higher relapse-rate than during the 1 or 2 years preceding treatment [Waubant E, et al. (2002) Neurology 58]), or on a combination of relapse and disability (C: recent National MS Society criteria; D: cyclophosphamide rescue trial inclusion criteria [Smith DR, et al. (2001) Neurology]). We also defined what we considered as an unequivocal response at one year; responders (UR): no relapse and stable EDSS; non-responders (NR): 1 point increment in EDSS (0.5 if initial EDSS ≥ 5); other patients were classified as partial responders (PR).

Results: Patients (n = 116) received IM (n = 59) or SC (n = 33) interferon beta 1-a, or SC interferon beta 1-b (n = 24). Their characteristics were similar to those of phase III studies of interferon beta. Interferon beta was stopped before one year because of patient's choice (n = 2) side-effects (n = 4), inefficacy (n = 8) or both (n = 2). The rate of responders varied widely, with some consistency, however, between the most stringent criteria: A: 33%; B: 67.3%; C: 73.3%; D: 66.6%. The pre-treatment characteristics of responders and non responders were not different, except for relapse rate, which was lower (criteria A and C) or higher (B) in responders than in non responders (B: not different). When using "unequivocal" criteria, NR (n = 20) tended to have more active MS than UR (n = 34), with higer pre-treatment relapse rate (2.3 ± 1.4 vs 1.6 ± 0.7, p = 0.05) and higher EDSS (3.4 ± 1.4 vs 2.8 ± 1.1, p = 0.07). The application of relase-only criteria (A and B) to the PR group (stable EDSS at one year but still relapsing, n = 53) gave results similar to those of the whole group.

Conclusions: The rate of responders, and the pre-treatment factors indicative of response, to interferon beta in RRMS are dependent of the choice of criteria for assessment of response. The inconsistency of results obtained by using available criteria based on the response/no response paradigm supports a 2-step approach leading to an operational definition of partial responders.

P771

**Interferon beta-1b (Betaferon(R)/Betaseron(R)) in early treatment of multiple sclerosis: the BENEFIT study.** C. H. Polman, G. Edan, M. Freedman, H. P. Hartung, L. Kappos, D. Miller, X. Montalban, F. Barkhof, L. Bauer, M. Ghazi, R. Sandbrink, VU Medical Centre, CHU, The Ottawa Hospital, Heinrich-Heine-University, Basel University Hospital, National Hospital for Neurology & Neurosurgery, Hospital Vall d'Hebron, Schering AG (Amsterdam, NL; Rennes, F; Ottawa, CAN; Dusseldorf, D; Basel, CH; London, UK; Barcelona, E; Berlin, D)

Evidence from clinical studies indicates that treatment of patients with a first demyelinating event suggestive of MS and an abnormal MRI with once weekly interferon (IFN) beta produces effects on clinical and MRI parameters. However, the long-term impact of IFN beta treatment at the first episode and the benefits of high dose/high frequency treatment have yet to be evaluated. High dose/high frequency treatment was recently shown to be more efficacious than a once weekly treatment in established Relapsing-Remitting MS (INCOMIN, EVIDENCE).

The BENEFIT (Betaferon(R)/Betaseron(R) in Newly Emerging MS for Initial Treatment) study has been designed to: (i) investigate the efficacy of high dose/high frequency IFN beta-1b initiated after the first clinical event; (ii) explore long-term effects beyond those on the second event and MRI lesion development; (iii) reveal the relationship of the two primary endpoints (time to diagnosis of MS [McDonald criteria], and time to clinically definite MS (CDMS) [Poser criteria]; and, (iv) compare treatments initiated before and after conversion to CDMS and evaluate the prognostic relevance of new lesions on follow-up MRI scans as well as molecular prognostic factors.

BENEFIT is a randomised, double-blind, placebo-controlled, parallel group, multicenter, phase III study in patients with a first demyelinating event suggestive of MS and an abnormal MRI. Ca. 400 patients from Europe, Israel, and Canada will be enrolled, 150 to receive placebo and 250 to receive 250 mcg (8 MIU) IFN beta-1b every other day. The maximum duration of the double-blind phase will be 2 years, or until both primary endpoints are reached (CDMS). Patients with CDMS, and those completing the 2-year study, will enter a long-term follow-up study and be offered open-label IFN beta-1b for at least 3 years. The aim is to maintain all patients in the extension study regardless of therapy.

A range of measurements has been defined to allow assessment of the objectives outlined above. Full recruitment is expected by early 2003 with the first results being available by mid-2005. Baseline characteristics of the patients so far enrolled will be presented.

BENEFIT is the first study providing data on the effects of higher dose, more frequently administered IFN beta on conversion to CDMS, also incorporating a long-term follow-up.

P772

**Decision making, risk taking and emotional experience in patients with multiple sclerosis.** L. Bruggimann, J. M. Annoni, L. Urbano, J. Bogousslavsky, M. Schluep, CHUV, Department of Neurology (Lausanne, CH)

Background and Purpose: Impaired emotional reaction, as sometimes described in multiple sclerosis (MS) patients, might alter decision making. According to the somatic marker hypothesis, wrong decisions are associated to poorer skin conductance responses (SCRs), considered to be a psychophysical marker for emotional reaction. The aim of this study is to determine the feasibility of the Gambling Task, a well-known paradigm that models real life decisions, to study decision making abilities in MS patients.

Methods: MS patients and healthy controls were administered the Gambling Task. MS patients were required to bet, using theoretical money, by choosing in each trial one of four presented decks of cards. There are disadvantageous decks (much profit, but even more loss) and advantageous decks (smaller profit, but more profit than loss), and there are 100 trials. SCRs are measured during the task. Number of disadvantageous choices was compared between MS patients and controls using Wilcoxon Signed Rank test. A two-sided two-sample t-test was applied to calculate the needed number of subjects to be included in the definitive study, with the purpose of having a power of 80 % with an alpha error of 5 %. Punishment, reward and anticipatory SCRs will be compared between MS and control subjects in the further analysis.

Results: 20 MS patients (18 females; median age 34.5) and 15 healthy controls (13 females; median age 37.5) were tested. Among the 20 MS subjects, 9 had relapsing-remitting MS, 4 presented possible MS (with a single relapse), and 7 had secondary progressive MS. Thirteen patients were treated with interferon-b, two with glatiramer acetate, and five had no immunomodulatory treatment. The median choice of "disadvantageous" decks was 52 (25–75 quartiles, 44–60) in MS patients, and 46 (25–75 quartiles, 37.5–48) in controls ($p = 0.15$; Wilcoxon Signed Rank test). Using a two-sided two-sample t-test, the needed number of subjects to include in our study was 250 MS patients and 60 controls in order to have a power of 80 % with an alpha error of 5 %.

Discussion: Gambling Task is feasible to apply in MS population. MS subjects tend to show impairment in decision making in comparison to controls, but a more powerful and definitive study is necessary to confirm this difference. SCRs analysis may be a potent tool for studying emotional impairment in MS patients.

P773

**Influence of sex hormones on brain damage in multiple sclerosis: MRI evidence.** V. Tomassini, F. Marinelli, E. Onesti, E. Giugni, C. Mainero, A. Paolillo, M. Salvetti, C. Pozzilli, University La Sapienza (Rome, I)

Background: Gender-related differences in the disease course and response to therapy have been reported in Multiple Sclerosis (MS). The hypothesis of a pathogenetic role of sex hormones in MS is based on experimental and clinical data. Recent MRI evidence provides support for a gender difference in the MRI characteristics of the lesions occurring in the brain as a result of MS, thus suggesting a modulation of the MS pathological changes by gender.

Objective: In order to clarify the role of sex hormones in the "gender gap" typical of MS, we investigated the relationship between serum levels of sex hormones and the MRI characteristics of the lesions in patients with relapsing-remitting (RR) MS.

Methods: Serum sex hormone levels (FSH, LH, 17-beta-estradiol, progesterone, testosterone, DHEAs) were evaluated in 61 drug-free RRMS patients (35 women and 26 men). Women underwent the hormonal assessment in the follicular and luteal phases during a menstrual cycle. On the same day as the blood samples, brain Gd-enhanced MRI was performed. Thirty-six healthy age-matched subjects (18 women and 18 men) underwent the hormonal assessment.

Results: No difference between patients and controls in levels of any single hormone was found in men. Women with MS had lower testosterone and estradiol levels during the luteal phase when compared with controls (testosterone: 0.46 vs 0.63, $p < 0.01$; estradiol: 80.7 vs 150.9, $p = 0.02$).

In men, estradiol levels correlated with T2-hyperintense ($r = 0.47$, $p = 0.02$) and T1-hypointense lesion load ($r = 0.43$, $p = 0.04$), whereas in women testosterone levels were related to higher T1-hypointense lesion load ($r = 0.50$, $p = 0.02$). This difference also emerged by using testosterone/estradiol (T/E2) ratio: a greater brain damage was associated with a higher T/E2 ratio in women, but with a lower T/E2 ratio in men.

Conclusions: Our study suggests that sex hormones may exert a gender-related modulation of pathological changes in MS as detected by MRI. Both estradiol and testosterone seem to be implicated in the process leading to an irreversible tissue damage, but their role differs between the sexes. The effect of sex hormones on the dynamic of the disease process is also consistent with recent experimental data indicating glia as a target for both estrogens and testosterone, thus supporting the hypothesis of a pathogenetic role of sex hormones on brain injury and repair mechanisms typical of MS.

P774

**The epidemiology of multiple sclerosis in Iran.** H. Pakdaman, R. Pakdaman, Beheshtee University, Tehran University (Tehran, IR)

Introduction: Multiple Sclerosis (MS) is one of the most common causes of neurological disorders in young adults. We studied indices of MS in Iran from 6 years ago. In spite of view, it is noted that prevalence of MS is high in Iran.

Methods: This is prospective multicentric cross section study, which is cried during 1995 till now; we started the research with seven different MS research centers in major universities of Iran (Tehran, Isfahan, Tabriz, Mashad, Gom, Ahvaz). The patients with MS.

Announced to the above Ms research center with newspapers and Journals and some poster send to all neurological wards and archive centers of hospitals and private clinics.

Of neurology. After taking history and complete examination, computerized data analyzed with statistical method of EP16.

Results: In this study 42,597 patients were diagnosed as having MS as the poser criteria.

Mean age of the patients was 34.4, 70 % of patients were female (F/M = 2.4), 64 % were Educated and had diploma or more and 34 % had university education, 26 % were single and 71 % were married. Family history was positive in 7.2 %. Incidence of disease was 40.6 % and 26 % in third and forth decade respectively. Mean duration of disease was 5.7 years. Ini-

tial symptom of MS were paresthesia (50%), weakness (38%), sensory deficit (23%), diplopia (20%), ataxia (14%), vertigo (11%), and bladder dysfunction (11%). Onset of MS with involvement of sensory system, cranial nerve, brain system and pyramidal system were 62%, 54% and 50% respectively. The most clinical finding were weakness 57%, ataxia 27% bladder dysfunction 21% sensory dysfunction 17.4% and optic neuritis 14.5% respectively. The 54% were RRMS and 34% were PPMS.Mean EDSS was 4.2.

Discussion: According to this study, it seems that prevalence of MS is high in Iran. In spite of geographic area of Iran the cause of this problem is not justified. But we work hard on it. In spite of mean duration of disease 5.7, the EDSS was 4.2which is approximately equal with developed countries. There do not seem to be significant differences in MS prognosis in Iran and other countries.

P775

**Electronystagmography findings in 32 multiple sclerosis patients.** M. Kilinc, U. Can, S. Benli, L. Ozluoglu, B. Akkuzu, Baskent University (Ankara, TR)

Electronystagmography (ENG) records the changes in eye position indicated by the polarity of the cornea-retinal potential relative to each electrode placed beside the eye. Since the vestibular apparatus contributes significantly to the control of eye movements, these movements can be exploited to examine the activity of the peripheral vestibular end organs and their central vestibulo-ocular pathways. Therefore, ENG can be used to reveal small lesions located in the brain stem, and cerebellum. Since the extra ocular system is frequently involved in multiple sclerosis (MS), it was also anticipated that quantitative analysis of eye movements might uncover previously unsuspected or obvious eye movement disorders. Our objective was to present and correlate the ENG findings of a group of MS patients with clinical and magnetic resonance (MR) findings.

Thirty two clinically definite MS patients with or without vertigo were evaluated with complete physical, neurological, and neurotological examination along with ENG test battery and contrast enhanced MR. The ENG test battery included saccades, gaze, sinusoidal tracking, optokinetic nystagmus, positional test, Dix-Hallpike maneuver and calorics.

The most prominent abnormality was detected by optokinetic stimulus, 78.1% of the patients had abnormal or asymmetric optokinetic nystagmus. Sinusoidal tracking was abnormal in 53.1%, abnormalities in saccades were found in 62.5%, and calorics in 37.5%. Gaze was abnormal in 15.6%; and abnormalities in positional tests were found in 12.5%. Nystagmus could be elicited in 9.3% with manevour. Vertigo was a symptom in 59.3%.

In MRI 96.8% of the patients had supratentorial, 65.6% had infratentorial, and 46.8% had spinal cord lesions. The lesions were located only in supratentorial regions in 34.3%, only in infratentorial regions in 3.1%. Gd enhancement was present in 43.7%.

Eight of the patients had abnormal ENG findings although they did not have any MR documented infratentorial lesion. Four of these patients had cord lesions. Although seven of these did not complain of vertigo, four of them had abnormality either in oculomotor or cerebellar examinations. One patient had only infratentorial lesions, and he had severe ENG abnormalities along with gaze nystagmus and mild cerebellar signs, confirming the clinical and MRI findings.

In conclusion, in our MS patients' group ENG test provided useful information in determining MR negative infratentorial lesions. Eleven patients had no infratentorial lesions, however in 8 of them ENG results were abnormal. Six of these 11 patients had servical cord lesions, but 5 of them had only supratentorial ones. Three of these five patients with only supratentorial lesions had abnormal ENG findings (mostly as optokinetic asymmetry). So we suggest that ENG can be used as a laboratory support in diagnosis and/or follow up of probable or definite MS patients with clinically silent and/or MR negative lesions.

P776

**The effectiveness of physiotherapy and rehabilitation on daily activity and quality of life in multiple sclerosis.** E. Tarakci, A. Yaliman, M. Eraksoy, Istanbul University (Istanbul, TR)

Objective: Multiple Sclerosis (MS) is a disease characterized by demyelination of the central nervous system. It shows varied symptoms about the localisation, the greatness and the frequency of the illness, and it shows an important defect. The aim of this study is to investigate the effectiveness of the exercise programme on activity of daily living and quality of life to the people physically handicapped because of the multiple sclerosis.

Methods: Thirty MS patients whose Expanded Disability Status Scale (EDSS) was 2–6.5 included in this study. 23 females and 7 male-participated and the mean age were 40.23 ± 10.68. At the beginning of the exercises the activities of daily living were measured with Functional Independence Measurement (FIM) and Modified Barthel Index (BI); and quality of life was measured with Nottingham Health Profile (NHP) and Quality of Life Scale (QoL). After twelve week exercises period the measurements were repeated and the differences between them were determined.

Results: For the activities of daily livings; FIM values were statistically significant after exercises program ($p < 0.001$).These improvements appeared especially in the self care, the transfers and the movements of the subgroups. The values of BI were also statistically significant ($p < 0.001$). These improvemens were in the walking, in the stairs and dressing subgroups. Regarding the quality of life; the values of NHP were statistically significant. And the pain reduced, the physical activity increased, fatigue decreased and the emotional reactions improved. The results of QoL were statistically higher at the total and all of the subgroups ($p < 0.001$).

Conclusions: All of the evaluations parameters were shown positive improvements with exercises. For MS patients at the level of good use, with the exercises programmes, the independence of the daily living and the quality of life will increase.

P777

**Safety profile of different doses of interferon-beta in association with azathioprine in relapsing-remitting multiple sclerosis.** A. Repice, A. Barilaro, A. Parigi, A. Catapano, E. Tragni, L. Massacesi, University of Florence (Florence, I)

Interferon-beta 1a (IFN-beta) and Azathioprine (AZA) are drugs used in the therapy of relapsing remitting Multiple Sclerosis (RRMS). However in many patients these medications show little or no efficacy, indicating that treatment of this disease needs to be improved. In addition the different action mechanism and toxicity profile make these drugs ideal candidates for use in association.

The present study was carried out for evaluating optimal doses of IFN-beta, when administered in association with AZA at immunesuppressive doses in RRMS patients.

The patients included [14] have been divided in 4 treatment groups: 3 patients received IFN-beta 11 mcg x 3/week (group 1), 4 patients IFN-beta 22 mcg x 3/week (group 2), 4 patients 11 mcg x 3/week + Aza 3 mg/Kg/day (group 3) and 3 patients have been treated with 22 mcg x 3/week + Aza 3 mg/Kg/day (group 4). The patients have been followed clinically and blood cell count as well as serum chemistry have been evaluated during nine months.

The patients treated with IFN-beta alone presented already described adverse events (fever, flu like syndrome) that were milder or absent in group 1 patients that received lower doses. In addition group 2 patients showed 1 herpes virus reactivation and 3 urinary infections. The group 1 and 2 patients showed 2 and 1 neurological relapses respectively. Group 3 patients showed minimal adverse events as group 1, and 1 neurological relapse. In this group, adjusting Aza dose around 2.5–3.0 mg/kd/day blood cell counts never reached grade 3 of the Common Toxicity Criteria (CTC). Two neurological relapses were observed in this group. On the other hand two of the group 4 patients interrupted the treatment after four and six months for lymphocytopenia > grade 3 CTC. The third patient showed neutropenia, photosensitization a relapsing candida infections, that suggested dose reduction.

This study indicates that administration of Aza at 2.5–3.0 mg/kg/day in association with IFN beta at 22mcg x 3/week can not be tolerated, because lymphocytopenia or relapsing infections are induced in the majority of patients. On the other hand administration of the same Aza dose with 11 mcg x 3/week seems well tolerated.

P778

**Serum antibodies to sulfatide in patients with multiple sclerosis.** A. A. Ilyas, Z-W Chen, S. D. Cook, UMD-New Jersey Medical School (Newark, USA)

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. It is characterized by primary demyelination and axonal injury. Although the etiology of MS is still unknown, a large body of evidence suggests that autoimmune mechanisms are likely to be involved in the destruction of myelin. We tested sera from patients with MS, other neurological diseases (ONDs) and healthy controls for IgM and IgG antibodies to purified sulfatide, a major glycolipid of myelin, using enzyme-linked immunosorbent assay (ELISA) system and a thin-layer chromatogram (TLC)/overlay technique. Elevated anti-sulfatide IgG antibodies were significantly higher in patients with MS ($p < 0.005$) and patients with ONDs

($p < 0.005$). Binding of high titer antibodies to sulfatide was confirmed with TLC/overlay. Although elevated levels of anti-sulfatide IgM antibodies were also detected in MS patients, their frequency was not significantly higher than controls. In conclusion, our data demonstrate that MS patients have elevated levels of serum IgG antibodies against sulfatide. However, the significance of these antibodies in the pathogenesis of MS remains unknown. (Supported by NIH grant 5R01 NS30891)

P779

**CSF evaluation in Devic's neuromyelitis optica.** M. Zaffaroni, A. Ghezzi, R. Bergamaschi, V. Martinelli, G. Mancardi, E. Merelli, R. Tola, M. Troiano, G. Comi, MS Study Centre Italian Devic Study Group (IDESG)

Background: According to clinical course and MRI findings, Devic's neuromyelitis optica (DNO) seems to be distinct from MS. The diagnostic support of CSF is not defined.

Aims: To clarify the role of CSF analysis in DNO, we collected the laboratory data of a multicentre Italian survey of DNO cases.

Results: We report the findings 75 CSF samples obtained at disease onset (36%) or later from 44 patients. Abnormalities were found in 40 samples (53.3%), namely: pleocytosis in 22 (29.3%), oligoclonal bands (OB) in 17 (22.7%), increased proteins in 13 (17.3%), high CSF/serum albumin ratio in 12 (16%), high IgG Index in 6 (8%), OB mirror pattern in 6. Abnormal findings were confirmed by further lumbar punctures in 7 patients, namely: OB in 3, pleocytosis in 2, increased proteins in 2 and increased albumin ratio in 1. CSF findings changed during the course of the disease in 7 of 21 patients with repeated spinal taps. In these patients, CSF converted from normal to abnormal in 6 subjects because of the appearance of pleocytosis and increased protein or albumin content in 5 patients. Conversely, CSF turned normal in 1 subject previously showing pleocytosis, increased albumin ratio, elevated IgG Index and OBs. Eighteen (60%) of 30 samples obtained during an acute phase of the disease were abnormal. On opposite, 25 (55.5%) of 45 CSF samples obtained in remission were normal. In both conditions, CSF abnormalities reflected approximately the same figures of the whole series, pleocytosis being the most frequent finding: cells were $> 50/mm^3$ in 10 samples (13.3%) and ranged $6–50/mm^3$ in 13 (17.3%). Samples with $> 50$ cells/$mm^3$ were significantly more frequent ($p < 0.02$) during acute phases of the disease.

Comment: Our findings suggest that the main features of CSF in DNO are pleocytosis, IgG synthesis and blood-brain barrier damage. Pleocytosis is more frequent during acute relapses. The variability of CSF findings in DNO compared with MS stresses the indication for repeated spinal taps in different stages of the disease.

## Muscle disorders

P780

**Alveolar hypoventilation in myotonic dystrophy. A clinicopathological correlation.** S. Ono, F. Kanda, H. Shiraishi, T. Irie, K. Wakayama, M. Suzuki, N. Shimizu, Teikyo University School of Medicine, Kobe University School of Medicine (Ichihara, Kobe, JP)

Respiratory insufficiency has frequently been reported in patients with myotonic dystrophy (MyD). It is unclear whether this respiratory failure results from a primary dysfunction of the central nervous system or whether it is secondary to the involvement of respiratory muscles. Recent data support the hypothesis that the respiratory center could well be directly involved. However, there have been few studies in patients with MyD regarding possible relationships between the lesion of the medullary reticular formation which is the respiratory center and the presence of alveolar hypoventilation.

Nine patients with MyD (mean age, 64 years) and twelve control subjects without neurological diseases (mean age, 66 years) were studied. Alveolar hypoventilation was present in four MyD patients whose pulmonary function tests revealed ventilatory insufficiency of a central origin and was absent in the rest of MyD patients and controls. The brains were fixed in 10% formalin and transversely sliced tissues of the medulla oblongata were embedded in paraffin. Sections of arcuate nucleus (ARC) and central medullary nucleus (CMN) were cut at a level through hypoglossal nucleus, nucleus ambiguus, and dorsal vagal nucleus and were stained with Nissl and Klüver-Barrela's (K&B) methods. The areas of the ARC and the CMN were determined according to Olszewski and Baxter. For quantification of neurons in the ARC and the CMN, 20 serial 6-micrometer-thick sections from each individual were examined. All necleolus-containing neurons were counted under 400-fold magnification.

Severe neuronal loss was observed in the ARC and the CMN in MyD patients with hypoventilation but were observed in neither those without hypoventilation nor controls. The density of the ARC in MyD patients with hypoventilation ($51.8 \pm 11.7/mm^2$) was significantly decreased ($P < 0.001$ and $P < 0.001$, respectively) versus MyD patients without hypoventilation ($86.8 \pm 7.9$) and controls ($91.5 \pm 13.1$). The density of neurons of the CMN in MyD patients with hypoventilation ($7.1 \pm 0.5/mm^2$) was significantly lower ($P < 0.02$ and $P < 0.01$, respectively) than in MyD patients without hypoventilation ($10.1 \pm 0.7$) and controls ($11.7 \pm 1.0$). There was a significant positive relationship ($r = 0.84$, $P < 0.02$) between the ARC and the CMN in patients with MyD.

These data suggest that the neuronal loss of the ARC and the CMN is associated with the presence of hypoventilation in MyD and may be an important feature of MyD.

P781

**Modafinil for excessive daytime sleepiness in myotonic dystrophy: a randomized placebo-controlled double blind cross-over trial.** V. Ceschin, F. Gragnani, S. Miano, E. Bucci, S. Morino, O. Bruni, A. Barbato, A. De Vincentiis, G. Antonini, University of Rome, Dompé Biotec (Rome, Milan, I)

Objective: To evaluate, in a double blind cross-over trial, whether modafinil reduces excessive daytime sleepiness (EDS) and improves the quality of life in patients with myotonic dystrophy (DM).

Background: EDS, a common feature in patients with DM, is characterized both by a tendency to oversleep and by a disruption of circadian and ultradian rhythms. It is considered to seriously interfere with patients' social lives. Modafinil is a novel wake-promoting agent which, at a daily dosage of 200–400 mg, has been shown to reduce the number of sleep attacks in narcolepsy.

Methods: Ten patients with DM (7 men, 3 women) aged between 30 and 52 years (median age 39 years) were randomized to receive Modafinil (200 mg/day)-placebo or placebo-Modafinil (200 mg/day) in a double blind cross-over trial for 30 days each. The two treatment phases were divided by a 30-day washout period. EDS was evaluated in each patient before and after each trial phase by means of the Epworth Sleepiness Scale (ESS) and the Multiple Sleep Latency test (MSLT). An overnight polysomnography was performed in all patients at randomization to exclude the possible influence of sleep disturbances on EDS. Quality of life was evaluated using the MOS 36-item short form health survey questionnaire (SF-36). Statistical analysis was performed using t-test.

Results: Mean ESS score was lower during Modafinil treatment ($p < 0.02$).There were no significant differences between the mean MSLT and the SF-36 subscale mean scores during the two treatment phases.

Modafinil was well tolerated by all patients. The most frequent side effect was headache (4 patients).

Conclusion: Modafinil is a well-tolerated, wake-promoting agent in DM. At a daily dosage of 200 mg it appears to reduce the subjective perception of somnolence (ESS) in patients with DM without modifying MSLT scores. The subjective ESS and the objective MSLT are believed to evaluate different, complementary aspects of sleepiness. The Modafinil-induced change in EDS does not appear, however, to improve the quality of life in DM patients.

P782

**Causes, clinical presentation, diagnosis and treatment of rhabdomyolysis in a neurological intensive care unit. Report on 11 cases.** C. Gaul, S. Neudecker, J. Sommer, M. Winterholler, S. Zierz, Department of Neurology (Halle/Saale, Erlangen-Nuremberg, Rummelsberg, D)

Background: Intensive care medicine is necessary in a part of patients with acute rhabdomyolysis. About 30% of them need haemodialysis. Painful muscles and swelling oedema of the muscle in addition to beer-brown urine with myoglobinuria are clinical signs of rhabdomyolysis. In laboratory diagnostic more than five times elevated creatin-kinase (CK) and often additionally electrolyte disturbances will be found.

Methods: In a five year period patients with rhabdomyolysis treated in two Neurological Intensive Care Units were retrospectively evaluated for causes, clinical presentation, diagnostic procedures and treatment of rhabdomyolysis.

Results: In that period 11 out of about 2500 patients were treated for rhabdomyolysis. Rhabdomyolysis was caused in most cases by intoxication (4/11). Other causes were infections and sepsis (2/11), striking exercises, heat stroke (1/11), and acute arterial occlusion of a lower limb (1/11). One patient had water intoxication with hyponatremia due to psychosis. Status epilepticus-caused rhabdomyolysis occurred only in one patient (1/11). Troponin I elevation can give evidence for cardiac rhabdomyolysis (1/11).

Complications were compression of peripheral nerves caused by a compartment syndrome, which can occur very early in the course of the disease (1/11). Focal rhabdomyolysis can be shown in computed tomography (CT) (1/11).

Discussion: Morphological diagnosis due to a muscle biopsy is normally not needed for diagnosis of rhabdomyolysis itself, because it shows only non-specific necrosis of the muscle. In nearly half of our cases an intoxication was the explanation for rhabdomyolysis, in none of these patients an underlying myopathy was diagnosed. If muscle enzymes stay on a high level in the post acute phase of disease and pain persists, a muscle biopsy should be performed. Especially diagnosis of Carnitin-Palmityl-Transferase II deficit and other metabolic myopathies requires biochemical in addition to morphological analysis. In some cases the cause of rhabdomyolysis still remains unclear. Menace condition in rhabdomyolysis is acute renal failure caused by tubular necrosis. Intravenous fluid administration and alkalisation of urine shall avoid haemodialysis. If there is a nerve compression through a compartment syndrome, an early fasciotomy can be necessary. With early and aggressive therapy including haemodialysis rhabdomyolysis may have a prognosis ranging from good to favourable.

P783

**Mycophenolate mofetil for myasthenia gravis: a safe and promising immunosuppressant.** L. Gogovska, R. Ljapcev, Clinic of Neurology (Skopje, MK)

Objective: To investigate the short-term efficacy and safety of low-dose Mycophenolate mofetil (MM) in patients with Myasthenia Gravis (MG).

Background: Mychophenolate mofetil (MM) which blocks purine synthesis in activated T and B lymphocytes and selectively inhibits their proliferation has been successfully used for treatment of allogeneic transplants and other immune mediated diseases.

MG is currently treated with several immunosuppressive agents including corticosteroids, azathioprine, cyclosporin A, cyclophosphamide. But, all these agents carry serious side effects and some patients do not respond adequately to them.

Method/Design: In an open-label study, 12 patients with refractory MG or who were taking corticosteroids and required additional immunosuppression, received MM (CellCept) in an oral dose of 250 mg twice daily for 6 months. Most patients were with late-onset MG, with Class IIIb and IVb according to MGFA Clinical Classification and Quantitative MG score for disease severity (QMG) of at least 15, and two patients with Class V. An improvement was defined as a reduction of at least 3 points in the QMG or a reduction of at least 50 % in steroid dose for at least 3 months. Safety was assessed by physical and laboratory examination and regular monitoring of adverse effects.

Results: Nine of 12 patients (75 %) improved presented by a reduction of at least 3 points in the QMG Score. Improvement started early, between 3 weeks and 2 months and persisted throughout the 6 months. Eight of 12 patients taking steroids at the time MM treatment was begun, were able to decrease the steroid dose without worsening, but only 5/12 decreased their steroid use by 50 % for at least 3 months without worsening of the QMG Score. No major side effects were observed and all patients were able to tolerate the drug.

Conclusions: Low-dose MM appeared to be effective as adjunctive therapy in the treatment of refractory and steroid-dependent MG. It can also be used as a steroid-sparing drug.

The safe side effect profile and the rapid onset of therapeutic effect would make MM a very attractive alternative to other currently available immunosuppressants for MG.

Larger, prospective controlled trials are warranted to assess long-term efficacy and safety and the potential role of MM as a sole therapy in MG.

P784

**Multiple MtDNA deletions in a patient with facioscapulohumeral muscular weakness.** M. Filosto, M. Mancuso, G. Fontanini, A. Rocchi, G. Siciliano, L. Murri, Neurological Institute, Oncology (Verona, Pisa, I)

Objectives: To describe a 52-year-old woman with a myopathy mimicking facioscapulohumeral muscular dystrophy (FSHD) and multiple mitochondrial DNA (mtDNA) deletions in muscle.

Methods: We screened DNA for the diagnosis of FSHD. We performed histochemical and biochemical studies of the mitochondrial respiratory chain in muscle, sequenced the 22 mitochondrial tRNA genes, and looked for multiple mtDNA deletions by long PCR.

Results: The patient did not harbor rearrangements within the subtelomeric region of 4q. Muscle histochemistry showed a myopathic pattern with several cytochrome c oxidase (COX)-negative fibers. Biochemical studies showed decreased activities of complexes I, III and IV, and molecular analysis revealed multiple mtDNA deletions.

Conclusions: Our data further expand the clinical spectrum associated with multiple mtDNA deletions, which should now be considered in patients with the FSHD phenotype but without rearrangement within the subtelomeric region of 4q.

P785

**Muscle mtDNA alterations during ribavirin therapy.** F. J. Authier, G. Bassez, V. Zarrouk, C. Jardel, A. Mallat, A. Lombes, R. K. Gherardi, CHU Henri-Mondor, INSERM U523 (Creteil, Paris, F)

Mitochondrial toxicity accounts for most adverse effects of nucleoside analogue reverse transcriptase inhibitors (NRTIs). Ribavirin is a nucleoside analogue currently used in treatment of hepatitis-C virus (HCV) infection. Increased mitochondrial toxicity with ribavirin was reported in HIV/HCV coinfection.

We report here a patient infected by HCV alone and treated by ribavirin and IFN-alpha who presented with myalgias and increased CK level during drug administration. Muscle biopsy showed striking muscular mitochondrial dysfunction assessed by the presence of numerous Cox-negative fibers (31 %) and ragged-red fibers (RRF) (7 %). Immunofluorescence study showed, in COX deficient fibers, a decrease of mitochondrial DNA-encoded COX2 subunit expression, contrasting with the persistence of nuclear-encoded COX4 subunit expression. Enzymatic analyses showed severe defect of complex I, III and IV, while activity of succinate dehydrogenase and citrate synthase were normal. Mitochondrial DNA analysis disclosed a severe mtDNA depletion with multiple large size deletions.

Myopathological changes observed in our patient were very similar to those induced by AZT in HIV-infected patients. Furthermore, the association of severe, combined, respiratory chain defect with mtDNA depletion and multiple deletions has been encountered in HIV patients treated with nucleosides analogs. IFN-alpha/ribavirin bitherapy is at present the cornerstone of anti-HCV treatment. Physicians need to be aware of a possible ribavirin-induced mitochondrial toxicity. We recommend to carefully monitor CK levels and lactacidaemia during ribavirin treatment and perform muscle biopsy in case of muscle involvement.

P786

**Late onset myasthenia gravis.** D. Lavrnic, P. Djukic, V. Stojanovic, Z. Stevic, S. Pavlovic, I. Basta, A. Vujic, S. Apostolski, University of Belgrade (Belgrade, YU)

We have analyzed the clinical features and the response to therapeutic procedures in the population of patients with myasthenia gravis (MG), starting with the age of 50 years, which was designated as the late onset MG.

During the 5 year period (1992 to 1996), 398 new cases of MG were diagnosed within the entire territory of Serbia. In 150 (37.7 %) of them the first symptoms developed after the age of 50 years. This subgroup of late onset MG was characterized by the predominance of male patients (1.46:1) and similar mean age for both sexes (60.1 for males and 62.2 for females). In the majority of these patients (70.7 %) there was no provoking factor for the onset of the disease. Most of them were non-smokers (72 %) and did not consume alcohol (88.7 %). The most frequent initial symptoms were extraocular (62 %) and bulbar (15.3 %) muscle weakness as well as the combination of both of them (8 %). According to Osserman's classification, 35 (23.3 %) patients had MG1, 41 (27.3 %) MG2A, 69 (46 %) MG2B, and 5 (3.3 %) patients had acute fulminating myasthenia, MG3. Anticholinesterase and immunosuppressive drugs (prednisolone and/or azathioprine) were administered to all patients. Only 47 (31.3 %) patients with generalized MG (14 MG2A; 30 MG2B, 3 MG3) underwent thymectomy. Histological analysis revealed thymoma in 15 (31.9 %), thymic hyperplasia in 11 (23.5 %) and involution of thymic tissue in 21 (44.7 %).

After the follow-up period of 6 to 10 years, 22 (14.7 %) patients were in stable remission, 83 (55.3 %) significantly improved, 39 (26 %) were unchanged and 6 (4 %) patients worsened. The effect of thymectomy was compared with the outcome in 30 sex and age matched non-thymectomized patients with generalized MG. No significant difference in the final outcome was registered between thymectomized and nonthymectomized patients.

P787
**Merosin-positive congenital muscular dystrophy: a relatively benign form (stick form) in a Spanish family.** A. Pou-Serradell, J. M. Corominas Torres, Hospital del Mar (Barcelona, E)

Background/Objective. Classical forms of congenital muscular dystrophies (CMD) in western countries are classified in merosin-positive (MP-CMD) and merosin-negative forms. The objective is to present the study of a family with a rare form of MP-CMD

Patients: We studied a mother (48-year-old-woman) and her daughter (a 13-year-old-girl) with CMD: Both had "generalised muscular weakness and hypotonia at birth" without arthrogryposis. Both presented delayed motor milestones but normal intelligence.The mother could still walk, but with difficulty, at the age of 30. Since then, she got worse and became confined to wheelchair. The daughter presents weakness of lumbopelvic girdle muscles, diffuse mild amyotrophy, prominent scapular winging. No calf-muscle hypertrophy has observed. An ojival palate in the daughter and a mild contracture of elbows in the mother were noted. Brains' MRI and serum CK were normal. Muscle biopsy showed advanced dystrophic changes (interstitial fibrous tissue, adiposis and variation in fiber size) particulary in the mother and a mild type-2 fiber predominance without fibre-type disproportion. Immunohistochemistry revealed normal expression of the dystrophin and laminin-alpha-2 chain (merosin).

Conclusion: Since the molecular defect causing merosin-positive CMD is not yet identified, we concluded that the family here presented is clinically and genetically distinct from the already mapped forms of CMD and confirms that merosine-positive CMD are generally milder and more slowly progressive than merosin-negative form of CMD.

P788
**Homoplasmic T3394C mtDNA mutation and genetically documented CPT deficiency in a patient with myoglobinuria and evidence of ragged red fibres at muscle biopsy.** M. Sciacco, A. Prelle, G. P. Comi, G. Fagiolari, P. Ciscato, E. D'Adda, A. Bordoni, A. Di Fonzo, M. Crimi, S. Jann, N. Bresolin, M. Moggio, Ospedale Maggiore IRCCS, University of Milan, Ospedale Niguarda (Milan, I)

We report the case of a 45 y. o. male patient who came to our observation after an episode of acute renal failure due to myoglobinuria, accompanied by intense myalgias and generalized weakness. Serum CK levels were over 30,000 U/L at onset of symptoms (normal values < 195 U/L) and still around 8,000 U/L one week later, after he had undergone dialysis. He completely recovered, with normalization of serum enzymes, in a few weeks. In the past, the patient had presented similar episodes of myalgias and exercise intolerance with dark urine. Neurological examination was normal and family history negative.

We performed left biceps muscle biopsy, which was normal except for the presence of some both COX-positive and COX-negative ragged red fibers. The clinical picture was highly suggestive for a Carnitine Palmitoyl-Transferase (CPT) deficiency, but, given the morphological pattern, we also considered the possibility of a respiratory chain disorder with particular attention to cytochrome b gene mutations (Andreu et al. 1999).

Biochemical CPT assay showed a marked CPT deficiency (isotope 64.5 pmol/min/mg, nv 452 ± 160; forward 147 pmol/min/mg, nv 367 ± 110) and genetic screening (1p32 gene) revealed that the patient is heterozygous for the common S113L substitution. Search for the other mutation is underway. Complete mtDNA sequence did not reveal any cytochrome b gene mutations, but disclosed a homoplasmic T3394C point mutation, which is normally associated with a Leber's hereditary optic neuropathy (LHON) phenotype and which has been described in association with the A3243G mutation in patients with diabetes mellitus (Ohkubo et al. 2001).

Two further patients with identical clinical and morphological features are presently being studied.

P789
**Myasthenia gravis in the elderly.** A. Barbaud, R. Verdier, B. Carlander, M. Pagès, Hopital Gui de Chauliac (Montpellier, F)

Objective: To compare late-onset myasthenia gravis (MG) with the common form of the disease.

Background: Myasthenia gravis is a disease which is classically observed in young people. Its occurrence in old patients is well known but reports on late-onset MG are rare.

Material and methods: In a retrospective study, we selected cases of MG with a follow-up which lasted more than 1 year which were observed in 2 departments of neurology from 1980 to 2002.

Patients were divided into 2 groups according to the age of onset of the disease (more or less than 65 years).

Statistical analysis was performed to compare epidemiological, clinical, electrophysiological and biological data, clinical outcome and responsiveness to treatment of these two groups, in order to identify the main characteristics of MG in the elderly.

Results: 81 patients with MG were observed, with a follow-up varying from 1 to 29 years. 28 (34%) were older than 65. 17 were male and 11 female, aged from 65 to 85 when MG occurred. 4 of them had a thymoma and 4 died from MG during the follow-up.

When compared with early onset MG, there was a trend to a faster course towards generalised symptoms for elder patients. Initial symptoms were not statistically different in the two groups although bulbar signs were more common in the older group.

Older patients were treated earlier than younger people with corticosteroids and/or azathioprine, with a similar response to treatment.

The number of myasthenic crises, remissions and death-rate due to myasthenia gravis were not statistically different in younger and older populations.

Conclusion: Late onset MG is common and the occurrence of MG in old people is not a factor of poor prognosis.

P790
**Is the expression of MHC I in muscle fibres specific for polymyositis?** D. Heuss, C. Berghoff, S. Probst-Cousin, T. Leuschner, M. Haslbeck, B. Neundörfer, Institute of Neurology (Erlangen, D)

Background: Concerning criteria for making the diagnosis of idiopathic polymyositis, it is always stated that MHC I expression of muscle fibers is a characteristic hallmark.

It is suggested, that MHC I plays a pathogenetic role in antigen presentation.

Objective: To determine which muscle fibers express MHC I in idiopathic polymyositis.

Methods: Immunofluorescence colocalisation studies using monoclonal antibodies for the detection of CD8 + cells, the neural cell adhesion molecule N-CAM and the major histocombatibility complex I. 8 biopsies of patients with polymyosits, 3 biopsies of patients with dermatomyositis and 2 biopsies of patients with Duchenne muscular dystrophy are investigated.

Results: In polymyositis MHC I expression N-CAM was observed in N-CAM + regenerating fibers, but not in fibers surrounded or invaded by CD8 + cells. In dermatomyositis and Duchenne muscular dystrophy MHC I expression was also observed in N-CAM + fibers.

Conclusion: MCH I is expressed in regenerating muscle fibers in polymyositis, dermatomyositis and Duchenne muscular dystrophy. Muscle fibers surrounded by cytotoxic CD8 + cells are negative for MCH I. Therefore we suggest that in polymyositis MHC I expression is unspecific and is part of the program of muscle regeneration also in this disease.

Thus, MHC I expression should consequently no longer be regarded as a hallmark for making the diagnosis of polymyositis.

P791
**The role of parents' sex in intergenerational changes of CTG repeats in patients with myotonic dystrophy type 1.** V. Rakocevic-Stojanovic, D. Savic, S. Pavlovic, D. Lavrnic, S. Romac, S. Apostolski, Institute of Neurology, Faculty of Biology (Belgrade, YU)

Introduction: Myotonic dystrophy type 1 (DM1) is a multisystem disease caused by an expansion of a CTG repeat, located in the 3'untranslated region of the DMPK gene. The aim of this study was to analyze the role of parents' sex in intergenerational changes of CTG repeats in 50 patients with DM1.

Methods: DNA was isolated from white blood cells or chorionic villi using a standard protocol. All subjects were studied by polymerase chain reaction (PCR)and Southern blotting.

Results: Out of 50 investigated DM1 patients, 28 inherited the DM1 mutation from their mothers and 22 from their affected fathers. Analysis of variance showed that the mean of the smallest CTG expansion was smaller in patients with paternal inheritance (192 CTG repeats) than in those with maternal inheritance (284 CTG repeats). The same was found when comparing the means of the average expansions. Conversely, there was no significant influence of parents' sex on the value of the largest CTG expansion. All 10 mother-child pairs exhibited increased CTG repeat expansion in the children (from 63 to 146 repeats in the mothers and 129 to 596 CTG repeats in the children). We detected a prenatal mutation (more than 2000 CTG repeats) in a woman with 429 repeats. This pregnancy was terminated. We also found an increased CTG repeat expansion in two out of three father-child pairs. In the third pair there was a reduction (contraction) in size of the CTG repeats (from 113 in the father to 96 in the child).

Conclusion: This study shows that maternal transmission of the DM1 mutation results in a greater number of CTG repeats than does paternal transmission. This suggests a greater instability of mutant alleles in female meiosis. On the other hand, a reduction of CTG repeats (contraction) was present only in paternal transmission of the DM1 mutation.

P792
**Association of myasthenia gravis with epilepsy.** Ü. Börü Türk, A. Koçer, C. Bilge, H. Tutkan, Dr.Lutfi Kirdar Education Hospital (Istanbul, TR)

Current prevalence of seizures ranges from 1% to 10% in myasthenia gravis (MG) patients. In this study we report two cases of MG with epileptic seizures.

Case I: A 22-year-old woman developed complex partial seizures at the age of 2 years and was treated with carbamazepine, oxacarbazepine, and lamotrigine. She developed myasthenia gravis at the age of twent. EEG showed diffused slowing and continued epileptiform activity in right centro-temporal area. Case II: A 58-year-old woman came to outpatients clinics with developed complex partial seizures and secondary generalized tonic-clonic seizures. She had a historiy of epilepsy started at the age of 39 years and MG for 2 years. She was treated with carbamazepine. EEG showed generalized slowing and paroxysmal spike wave activities.

Descriptions of seizures in MG patients appeared as knowledge accumulated about the role of brain cholinergic systems. Both excitatory and inhibitory synapses are directly modulated by nicotinic acetylcholine receptor (nAChR) activity. Depending on the neural area and stage of development, nAChRs of multiple subtypes will have varying degrees of importance in regulating neuronal excitability. Associations between MG and epilepsy appear to be either coincidental or the result of uncontrolled MG, although an increased incidence of epilepsy and electroencephalographic (EEG) abnormalities has been noted in patients with MG. An increased association of MG with epilepsy has not been found in most large studies of patients with MG, so we reported these cases with seizures preceding the appearance of myasthenic symptoms.

P793
**Early diagnosis and treatment reverse clinical features in Hoffmann syndrome due to hypothyroid myopathy: a case report.** M. Ozdag, H. Ipekdal, E. Eroglu, U. Ulas, Z. Odabasi, O. Vural, Gulhane Military Medicine Faculty (Ankara, TR)

A man, age of 22, had been described progressive weakness in his arms and legs during exercise for two months and complained about generalized muscle cramps and pains during daily activities for the last two weeks of period.

His medical history explains that he stopped the thyroid hormone replacement therapy five months ago which was given for the treatment of primary hypothyroidism diagnosed at the age of five.

He had presented a well developed musculature; strength was normal in manual muscle testing. There was no deficit in his sensation and deep tendon reflexes were normal. Labaratory studies pointed out that his TSH, muscle enzymes CPK and LDH were significantly elevated and Free T3, Free T4 levels were severely decreased. Bilateral mild CTS was detected in EMG examination. Needle EMG was normal. Pathological examination of muscle biopsy specimen of the right vastus lateralis was normal.

He was diagnosed as Hoffmann Syndrome due to stopping the hormone replacement therapy prescribed for primary hypothyroidism. He was restarted the thyroid hormone replacement therapy and after a two months period, his complaints were remarkably decreased except some stiffness in his legs. His hormone and muscle enzyme levels turned to normal. CTS findings on EMG were completely improved. His muscle bulk turned to normal appearance.

Hoffmann Syndrome is a rare complication of hypothyroidism.

This report suggests to study the thyroid hormones of the patients who were suspected of myopathy. Early diagnosis and treatment of hypothyroid myopathy helps patients recover completely.

## Neurobiology

P794
**The relationship between microglial activation, early axonal injury and permanent brain damage.** S. Golde, A. Compston, Otto-von -Guericke-Universität, University of Cambridge (Magdeburg, D; Cambridge, UK)

In multiple sclerosis, an autoimmune disease of the central nervous system, inflammation is thought to cause demyelination and transsection of axons. Microglia, the CNS resident cells of monocyte lineage, are among the first cells to become activated by an inflammatory stimulus. In vitro, such activated microglia have been shown to secrete potent neurotoxins. We demonstrated previously that rat microglia stimulated with IFN-g + lipopolysaccharide (LPS) kill primary rat cortical neurones by secreting high amounts of nitric oxide (NO). NO can kill neurones either directly or by causing glutamate release and excitotoxicity. Here, we investigate in vivo whether microglial activation after a single injection of IFN-g + LPS into rat cortex leads to damage of axons, neuronal cell bodies and oligodendrocytes. We show that intracortical injection of 5u IFN-g + 2,5microg LPS causes widespread activation of microglia as demonstrated by morphological change, upregulation of CD11b and expression of inducible nitric oxide synthase (iNOS) after 24hrs. At this early timepoint accumulation of amyloid precursor protein (APP) in axon endbulbs is more marked in IFN-g + LPS- injected animals compared to controls, indicating axonal injury which correlates well with the degree of inflammation. This injury however, is unchanged by inhibition of NO-production with aminoguanidine or blockage of NMDA-receptor activation with MK801. Surprisingly, 7 days after injection of IFN-g + LPS no permanent damage to axons, neuronal cell bodies or oligodendrocytes can be identified with immunostaining against phosphorylated neurofilaments, the neuronal marker NeuN and the oligodendrocyte-specific RiP, respectively. These results indicate that microglial activation and iNOS expression do not necessarily cause brain damage. Most likely, the duration of inflammation and a high concentration of microglial toxins, such as NO, are critical.

P795
**Generation of neural progenitors from murine bone marrow.** F. Locatelli, S. Corti, C. Donadoni, L. Montesissa, M. Guglieri, F. Capra, S. Strazzer, S. Salani, R. Del Bo, F. Fortunato, N. Bresolin, G. P. Comi, University of Milan, I. R. C. C. S. Eugenio Medea (Milan, Lecco, I)

Recent evidences suggest that cells from bone marrow (BM) can acquire neuroectodermal phenotype in cell culture or after transplantation in animal models and also in human brains. These observations may be relevant for the development of cell replacement treatment for a variety of neurodegenerative disorders. However several basic science issues need to be addressed before considering such applications.

One of there issues is the isolation of the BM cell subpopulation with neuronal differentiation potential. To isolate and expand neural progenitor from whole murine BM, BM was obtained from hind limb bone of C57Bl6 mice and plated in culture with neuronal medium without serum (NSA). After 5–7 days in culture, cellular spheres similar to brain neurospheres appeared either floating or attached in the culture dishes. These spheres were collected, dissociated and expanded. The BM-derived spheres were positive at the immunocytochemistry for nestin and, after exposure to neuronal differentiative medium, cells positive for the neuronal markers tubulin III (TuJ1) and Neurofilament (NF) were detected.

Neurosphere-like cell aggregate derived from BM of green fluorescent protein (GFP) transgenic mice (that express GFP under alpha-actin promoter in all tissues) were transplanted by direct injection into lateral ventricle of newborn C57Bl6 mice. One month after transplantation, the animals were sacrificed and the brain analyzed by confocal fluorescence microscopy. GFP + cells coexpressing neuronal markers (TuJ1, NeuN, NF, Microtubule Associated Protein) were found in cortical areas of all treated mice.

Isolation and expansion of BM derived neurospheres could be a possible cell source for transplantation as a therapy for central nervous system diseases.

P796
**Intra-cerebellar administration of copper increases extra-cellular levels of gamma-aminobutyric acid (GABA) by sodium independent mechanisms: a microdialysis study in the rat.** M. Manto, M. Pandolfo, M.-A. Laute, Hôpital Erasme (Brussels, B)

We encountered recently 4 patients presenting a disabling cerebellar syndrome associated with increased levels of copper and normal levels of

ceruloplasmin in blood. Genetic studies were negative. Brain MRI demonstrated a cerebellar atrophy. The molecular basis of the disorder has not been established. A direct toxicity of copper on cerebellar structures was suspected. To better characterize the effects of copper on the cerebellum, we tested the hypothesis that copper impaired the concentrations of GABA in the extra-cellular space by a sodium dependent mechanism, as shown previously for other cerebellotoxic substances. We analysed the effects of local administration of copper upon GABAergic transmission in the cerebellum of the rat, using the technique of microdialysis. Microdialysis was performed in 12 freely moving Wistar rats (weight: 250–350 gr). The probe (CMA/12; CMA, Sweden) was put in the lateral cerebellum using stereotaxic coordinates, was secured by dental cement and was perfused at a rate of 2 µL/min. Dialysates were analysed for GABA content by HPLC-electrochemical detection (Bioanalytical systems, USA). Histological location of the probe was identified following the experiments. Two studies were performed: [1] administration of copper sulfate (71.2 µM) alone in 5 rats, [2] administration of a combination of copper sulfate (71.2 µM) and tetrodotoxin (10 µM), a sodium channel antagonist. Analysis of baseline samples showed stable levels of GABA for both studies. In the first experiment, mean values of GABA contents increased markedly from 100% to 199.3 ± 98.6% (+ 10 min), 364.1 ± 107.7% (+ 20 min), 375.9 ± 128.9% (+ 30 min), 293.4 ± 91.4% (+ 40 min) (ANOVA: $p < 0.001$). In the second experiment, mean values of GABA concentrations increased from 100% to 253.1 ± 88.1% (+ 10 min), 327.2 ± 117.6% (+ 20 min), 352.5 ± 122.5% (+ 30 min), 620.4 ± 220.7% (+ 40 min) ($p = 0.006$). The increase of GABA contents tended to be higher in the second group, but without reaching a significant level ($p = 0.31$). Our study demonstrates that intra-cerebellar administration of copper exerts a potent effect upon the extra-cellular concentration of GABA. An increased neuronal activity is unlikely the cause of the increase of GABA concentrations. A direct effect of copper on GABA receptors should be considered. Our study confirms the vulnerability of cerebellar gabaergic pathways to copper.

P797

**Chemokines differentially modulate the proliferation of oligodendrocyte precursor cells.** M. Stangel, F. Zobel, M. Höpfner, D. Nguyen, Medical School Hannover, Free University (Hannover, Berlin, D)

Chemokines represent a family of chemotactic proteins that are best characterized in their action on inflammatory cells. Among other cell types, chemokine receptors have recently been shown to be expressed on oligodendrocyte precursor cells. We have determined the expression pattern for chemokine receptors on primary cell cultures of rat oligodendrocyte precursor cells. Fura-imaging was utilized for intracellular Ca2 + -monitoring to demonstrate functionality of the expressed chemokine receptors. To explore the effect on cell function, we tested the proliferation of oligodendrocyte precursor cells in the cell line CG4 in differentiating and in proliferating medium. While SDF-1a (ligand for the receptor CXCR4) promoted proliferation in both media, Fractalkine (ligand for CX3CR) increased proliferation only in differentiating medium, and Eotaxin (ligand for CCR3) had no effect on proliferation. These data support that chemokines are differentially involved in the regulation of oligodendrocyte precursor cells. This has implications on developmental processes of the central nervous system and may have implications for repair mechanisms in demyelinating diseases like multiple sclerosis.

P798

**Intra-peritoneal administration of tiagabine, a selective inhibitor of GABA transporter 1, counteracts the reduction of extra-cellular levels of gammaaminobutyric acid (GABA) induced by local infusion of ethanol in the cerebellum.** M. Manto, M. Pandolfo, M.-A. Laute, Hôpital Erasme (Brussels, B)

Cerebellum is highly vulnerable to ethanol consumption. It is established that ethanol is toxic for Purkinje cells and granule cells. Ethanol interferes with AMPA/kainate receptors and GABAA receptors. We showed previously that local administration of low doses of ethanol in the cerebellum strongly impairs gabaergic transmission in the cerebellum, inducing a marked reduction on the extra-cellular levels of GABA. We analysed the effects of tiagabine, a selective inhibitor of GABA uptake acting on GAT-1, on GABAergic transmission in the cerebellum of the rat. We tested the hypothesis that general administration of tiagabine prevents the reduction induced by local administration of ethanol on the extra-cellular contents of GABA. Microdialysis was performed in 14 freely moving Wistar rats (weight: 250–350 gr). Rats were anesthetized with chloral hydrate (400 mg/kg; ip). The probe (CMA/12; CMA, Sweden) was put in the lateral cerebellum using stereotaxic coordinates, was secured by dental cement and was perfused at a rate of 2 µL/min. Samples were collected every 20 minutes. Dialysates were analysed for GABA content by HPLC-electrochemical detection (Bioanalytical systems, USA). Histological location of the probe was identified following the experiments. Two studies were performed: [1] administration of tiagabine ip (1 mg/kg) alone in 7 rats, [2] administration of a combination of tiagabine ip (1 mg/kg) under the condition of continuous intra-cerebellar administration of ethanol 20 mM ($n = 7$ rats). Analysis of baseline samples showed stable levels of GABA for both studies. In the first study, mean values of GABA contents increased markedly from 100% up to a peak of 319.6 ± 117.2% (ANOVA: $p = 0.026$). In the second experiment, mean values of GABA concentrations increased from 100% up to a peak of 233.8 ± 56.7% ($p = 0.037$). The increase of GABA contents tended to be higher in the first group, but without reaching a significant level ($p = 0.24$). Our study indicates that general administration of tiagabine is associated with a marked increase in the concentrations of GABA in the extra-cellular space of the cerebellum. In addition, tiagabine prevents the decreasing effect induced by ethanol administered locally. The activity of GAT-1 seems preserved during ethanol administration. These findings extend previous findings on the gabaergic modulation in cerebellar areas during ethanol intoxication.

P799

**FK 506, but not cyclosporine A, may reverse the inhibitory effects exerted by 1-methyl-4-phenylpyridinium and 3-nitropropionic acid on the kynurenic acid synthesis – studies in vitro.** P. Luchowski, E. Luchowska, M. Wielosz, W. A. Turski, E. M. Urbanska, Medical University (Lublin, PL)

Kynurenic acid (KYNA), the only known endogenous glutamate antagonist able to prevent neurotoxicity and seizures in various experimental models, is produced in the brain by kynurenine aminotransferase (KAT) I and II. MPP + (1-methyl-4-phenylpyridinium) and 3-NPA (3-nitropropionic acid), are widely used toxins inducing parkinsonism-like symptoms and modelling Huntington's disease, respectively. Recently, we have shown that MPP + and 3-NPA diminish cortical synthesis of KYNA via interference with KAT I and II (Neurosci Lett. 2002, 330[1]:49–52). FK-506 and cyclosporin A (CsA) are neuroimmunophilin ligands interacting with calcineurin and effective in animal models of ischemia, trauma or neurodegenerative disorders. Both compounds may also ameliorate the experimental toxicity evoked by MPP + and 3-NPA.

Here we evaluated whether the alterations in KYNA production contribute to the action of FK-506 and CsA. KYNA synthesis in rat cortical slices in vitro and KATs activity in semi-purified brain homogenate were assessed. Produced KYNA was measured using High Performance Liquid Chromatography (HPLC) with fluorescence detector. FK-506 (10–60 µM), in contrast to CsA (up to 50 µM), enhanced the formation of KYNA in vitro. 30 µM FK-506 (but not 3 µM), and not CsA (50 µM), abolished the inhibition of KYNA synthesis evoked by MPP + and 3-NPA. Neither FK-506 nor CsA influenced the activity of KAT I and KAT II.

Obtained data suggest that protective action of FK506, as opposed to CsA, might include the stimulation of KYNA synthesis, the effect possibly not related to the interference with KATs or calcineurin activity.

Supported by grant from the Polish Committee of Research KBN K018/P05/2001.

P800

**Extracellular matrix effects on astrocyte phenotype: relevance to brain scarring.** N. J. Gutowski, J. L. Whatmore, J. E. Holley, Royal Devon and Exeter Hospital (Exeter, UK)

Astrocytes are the main supporting cells of the brain. They are normally relatively quiescent cells. Following various brain injuries astrocyte activation occurs, followed by the formation of a glial scar which inhibits brain repair. The scar consists of post-reactive astrocytes (SAs). In vivo we have found that adult human cerebral white matter SAs express the proteins embryonic neural cell adhesion molecule, epidermal growth factor receptor, and basic fibroblast growth factor. We wish to define factors that produce a human SA phenotype thereby finding mechanisms that inhibit scarring and assist repair. It would be advantageous to have an in vitro model to define these factors, but this requires a baseline quiescent astrocyte phenotype. In vitro, adult human cerebral white matter astrocytes in serum have a non-quiescent phenotype. In serum-free chemically defined medium astrocytes on the extracellular matrix poly-L-lysine only have a partially quiescent phenotype. By changing the extracellular matrix on which astrocytes are cultured we found striking differences in their antigenic phenotype. The extracellular matrices fibronectin, tenascin C, laminin, vitronectin, and collagen 1V, which are found in the human brain, were used. This has allowed us to closely mimic the phenotype of normal quiescent

astrocytes in vivo, therefore establishing a viable model for normal human quiescent astrocytes in vitro.

## Neuro-oncology

P801
**Stereotactic radiosurgery using dynamic micro-multileaf collimator vs IMRT vs circular collimators for small medium-size intracranial meningiomas.** I. Milanesi, M. Fumagalli, L. Brait, L. Fariselli, Istituto Neurologico C. Besta (Milan, I)

Introduction and purpose: A micro-multileaf collimator (DMLC, 3DLine) dedicated to dynamic conformal radiotherapy was installed on a Philips SL75–5 linear accelerator. DMLC has been designed for the treatment of small and irregular size tumors. In particular it can be used for fractionated stereotactic radiotherapy and for radiosurgery of brain tumors. The implementation of dynamic leaf motion on a micro-multileaf collimator system provides the capability for intensity-modulated stereotactic-radiosurgery (IMSRS). The aim of this study is to compare dose conformity to planning target volume (PTV) and dose reduction to surrounding healthy tissues and organs at risk (OR) relative to different radiosurgery techniques applied to the treatment of intracranical lesions: stereotactic radiosurgery with dynamic multileaf collimator (DC-SRS), intensity-modulated stereotactic-radiosurgery (IM-SRS), stereotactic radiosurgery with circular collimators (SRS).

Six patients with small medium-size intracranical meningioma (range 3–14 $cm^3$) and irregular shapes have been analyzed.

Patient plans relative to different treatment techniques have been quantitatively evaluated on basis of standardized index.

Methods and materials: PTV, brain tissue and OR volumes of interest (VOI) are delineated on CT-RM fused images. Treatment plans generated for these comparisons include noncoplanar dynamic fields with uniform-intensity beams (1 isocenter, 4–5 arcs of approximately 100° per arc) for techniques DC-SRS, and IM-SRS (1 isocenter, 4–5 arcs, approximately 120° per arc divided into smaller subarcsarc of 30°) and standard arcs (3–4 isocenters, each isocenter was assigned 4–5 arcs of approximately 100° per arc) with multiple circular collimators (Radionics). For each plan the dose-volume histogram (DVH) has been calculated for the PTV and OR. Conformity Index (CI) has been evaluated on basis of the definition contained in ICRU 62; maximum dose in PTV divided by prescription dose (MDPD) has been evaluated. The ERGO (3D-line) radiation treatment-planning system has been used for dose calculations and for input of contours for target volumes and normal critical structures on CT-RM fused images. The IMRT system for optimisation of intensity distributions is the Arc Modulation Optimization Algorithm (AMOA, 3D-line) which automatically optimize the weight of the arcs based on dose constraints to critical organs.

Results and conclusion: DC-SRS allows a better dose conformity than the other techniques in every case analyzed. It may not be necessary to use AMOA for every treatment plan. Non optimized dynamic arc treatment produces a good dose distribution, especially when no big concavities are present. SRS with circular collimators is competitive with DC-SRS and IM-SRS only when the lesions have a small volume (around 3 $cm^3$) and a spherical shape.

P802
**Secondary OCD and chronic headache in anti-Ma2 positive paraneoplastic limbic encephalitis.** R. Scheid, T. Guthke, R. Voltz, D. Y. von Cramon, University of Leipzig, University of Munich (Leipzig, Munich, D)

Introduction: Paraneoplastic limbic encephalitis (PLE) is a rare neurological disorder, most often occurring in association with small cell lung carcinomas and germ cell tumours. Antineuronal antibodies can be found in about 60 % of patients. We report a patient with anti-Ma2 positive PLE due to a metastasised testicular cancer with emphasis on the clinical course and the neuropsychiatric profile.

Case report: In December 1999 obsessive-compulsive disorder (OCD) was diagnosed in a 39-year-old previously healthy man. In January 2000 the patient noticed a lump in his right testicle. Orchiectomy and retroperitoneal lymph dissection followed. The histological examination showed a differentiated non-metastasised teratoma and a seminoma-in-situ. In August 2000 there was a first epileptic seizure. Since June 2001 memory deficits, gustatory sensory auras, and moderate chronic daily headache were reported. In November 2001 MRI showed a non-enhancing T2-hyperintense signal change in the left hippocampus. Anti-Ma2 antibodies were detected in serum and CSF. In March 2002 a thoracic CT showed a single (~8 mm) lesion in the right lung, which turned out to be a metastasis of the teratoma. Neither surgery of the lung metastasis nor additional chemotherapy were of any benefit with respect to the neuropsychiatric symptoms. The headache did not respond to analgesics or antidepressants.

Discussion: To the best of our knowledge, OC-symptoms and chronic headache have not been reported in PLE. We believe that the OC-symptoms were the real first sign of paraneoplasia in this patient. The assumption of secondary OCD is supported by recent neuroimaging studies. PET indicates that OC-symptoms are mediated by hyperactivity in frontostriatal circuits and fMRI shows that there is an involvement of paralimbic and limbic structures in OCD.

Neurological symptoms of PLE usually precede the diagnosis of cancer. In our patient "classical" symptoms of PLE (epilepsy and amnesia) appeared with a long latency after treatment of the primary tumour and possibly were due to occult lung-metastasis. With respect to the autoimmune hypothesis of PLE there might be prolonged immunological process and a longer interval might be used for non-tumour therapy than is assumed today. According to the literature, treatment of the tumour appears to be most effective, but there are also reports of a good clinical response to immunotherapies. In our patient antiepileptics and treatment with intravenous immunoglobulins (IVIg) were accompanied by a stabilization of memory-functions. No clinical improvement was documented after surgical removal of the metastasis and chemotherapy.

Conclusion: Our report underscores the clinical importance to consider paraneoplasia and tumour recurrence in patients with a history of malignancy and newly evolving neuropsychiatric symptoms. OC-symptoms and headache should be added to the spectrum of possible clinical abnormalities of PLE.

P803
**Brain metastases in children with musculoskeletal tumours.** I. Shchurovska, A. Abramyuk, A. Shchurovsky, Lviv Regional Specialised Children's Clinic (Lviv, UKR)

Objectives: According to literature, brain metastases (BM) are rarely reported in children with musculoskeletal tumours (MST).

The aim of study is to show the frequency of BM in MT patients, as well as its dependency from the diagnosis and location of a primary lesion.

Methods used: From January 1993 till December 2002 were treated 68 patients (42 boys and 26 girls) with MST. The ages ranged from 1 to 18 years (mean 13.8). Twenty one patients had 24 metastases in different locations, brain locations with being 29 % them. Among of them, 10 patients with Osteosarcoma had 3 brain metastatic lesions (30 %) and 8 patients with Ewing's Sarcoma had 4 brain metastatic lesions (50 %). In 6 patients with Ewing's Sarcoma the primary locations were pelvic bones.

Results: All BM were identified by imaging modality, 6 were histologically proven. At the time BM were diagnosed all patients had some neurological symptoms: headache (n = 6), headache and hemiparesis (n = 5).

Conclusions: BM more often occur in patients with Ewing's Sarcoma, especially in cases of central (pelvic) location of primary lesions.

P804
**Primary leptomeningeal sarcomatosis; a pathology proven case with challenging MRI and clinical findings.** K. Uluc, E. M. Arsava, A. Cila, F. Zorlu, E. Tan, Hacettepe University Hospital (Ankara, TR)

Leptomeningeal sarcomas are rare malignant neoplasms of the central nervous system accounting for less than 1 % of the primary intracranial tumors, presenting either with diffuse leptomeningeal involvement or large discrete lesions. Diffuse involvements of meninges are classified under the heading of primary leptomeningeal sarcomatosis. There is a wide range of clinical presentation and the diagnosis is generally based on histopathology, either by biopsy or autopsy. The clinical course is rapid and data about the efficacy of the recommended therapy, radiotherapy, are insufficient.

A 20-year-old male patient admitting with intractable seizures and progressive dementia is presented. Magnetic resonance imaging (MRI) examinations revealed diffuse leptomeningeal thickening, enhancement especially in the basal cisterns and multiple cystic formations in the brain stem, temporal lobes and basal ganglia. The pathologic examination from the right temporal lobe was consistent with leptomeningeal sarcoma. Marked regression of the symptoms and MRI lesions were detected following radiotherapy.

This is the first report with well-documented MRI findings of a biopsy proven case of primary leptomeningeal sarcoma involving the brain and the spinal cord. In addition to a diffuse leptomeningeal thickening, cystic lesions in the brain parenchyma provide the radiologic findings in this patient. The patient responded both clinically and radiologically to radiotherapy.

P805
**Basal-diencephalic tumours (clinical and neurophysiological correlations).** I. Voronina, The Burdenko Neurosurgery Institute, Moscow, (Moscow, RUS)

Objective: The purpose of the report was to reveal clinical and neurophysiological peculiarities in patients with basal-diencephalic tumors of different histology depending on a IIId ventricle tumor localization (anterior, posterior compartments and the floor).

Material and methods: 356 patients, aged 16–66 (male-217, female-139) entered this study.

Results: Tumors of anterior compartments of the IIId ventricle were predominantly gliomas and neurocytomas (114 cases). Hypertensive-hydrocephalic syndrome was marked in 99%, mental disorders and memory problems – 92%, generalized epileptic attacks – 40%, extrapyramidal syndrome – 40%, endocrine disturbances (hypogonadism, hypernatremia) – 35%. Thus, hypertensive-hydrocephalic syndrome, mental disorders, paroxismal, extrapyramidal and mild endocrine disturbances were thought to be indicative of the tumors invading anterior compartments of the IIId ventricle. Tumors occupying the IIId ventricle floor were mainly gliomas and craniopharyngiomas (107 cases). The expressed diencephalic syndrome was revealed: secondary hypothyrosis (82%), hypogonadism (65%), hypocorticoidism (50%), diabetes insipidus (36%), panhypopituitarism (30%), cachexia (17%). Chiasmal syndrome, mental disorders, visceral-vegetative paroxysms and "sleep-awake" pattern disturbances made up 87%, 68%, 45% and 33% correspondingly; hypertensive-hydrocephalic syndrome was marked in 41%, extrapyramidal syndrome – in 16%. Thus, endocrine, visceral-vegetative and vision disturbances, as well as hypertensive syndrome were thought to be indicative of the tumors occupying the IIId ventricle floor and chiasma. Posterior compartments of the IIId ventricle were occupied by germinal-cellular tumors and gliomas (135 cases). Hypertensive-hydrocephalic syndrome was marked in 95%; all patients developed brain stem symptoms; coordination disturbances were marked in 76%. Endocrine disturbances – secondary hypogonadism (33%), diabetes insipidus (21%), hypothyrosis (16%) – were less frequent. Mental disorders were marked in 60%; visceral-vegetative paroxysms – 50%, generalized epileptic attacks – 40% of patients. Thus, hypertensive-hydrocephalus syndrome, brain stem symptoms, mild mental disorders and endocrine disturbances were characteristic for the tumors occupying posterior compartments of the IIId ventricle. EEG analysis in half of observations revealed an alpha-rhythm predominance, 30% showed voltage decrease; 65% – teta-and delta-rhythms voltage increase, mainly in patients with IIId ventricle gliomas. Coherence appeared to be related to clinical syndrome peculiarities despite an alpha-rhythm and its voltage on EEG.

P806
**Vasogenic oedema from different brain metastatic tumours – MRI study.** R. Semnic, M. Semnic, K. Koprivsek, D. Kozic, B. Petrovic, D. Djilas-Ivanovic, S. Popovic, I. Miucin-Vukadinovic, Institute of Oncology, Institute of Neurology (Sremska Kamenica, Novi Sad, YU)

Introduction: MR phenomenon has supportive role in determination of different metastatic brain tumors (MBT) using their MRI (Magnetic Resonance Imaging) signal pattern, behaviour after Gadolinium dose injection and most of all, using MR spectroscopy.

Purpose of this study was to assess whether MBT from different primary malignant tumors (PMT) produce vasogenic edema in various extent which may be helpful in semiquantitative determination of MBT from unknown origin.

Patients and methods: brain MRI of 89 patients with histopathological proved PMT (35 with breast cancer (BCa), 31 lung cancer (LCa), 15 malignant melanoma (MM) and 8 colorectal cancer (CRCa)) was evaluated. Pial metastatic lesions were excluded. Volumes of MBT and peritumoral edema were manually traced and calculated from the system console and ratio between odema volume and MBT volume was expressed as edema producing index (EdPI). Four-grading system was established: a) regarding malignant capability of PMT to metastase into grade 1-LCa (the highest capability), 2-MM, 3-BCa, 4-CRCa and b) according to the value of EdPI, from the highest (grade 1) to the lowest (grade 4). Kruskal-Wallis ANOVA test was employed as statistical analysis in order to determine possible difference in edema production between different BMT and corresponding EdPI values.

Results: although EdPI values for each group of BMT differed: BCa – 5.11 (BMT mean volume 8.3 $mm^3$; oedema mean volume 42.49 $mm^3$)); LCa – 2.99 (18.66/55.87 $mm^3$); MM – 2.26 (20.4/46.23 $mm^3$); CRCa – 3.45 (10.13/34.97 $mm^3$), Kruskal-Wallis test did not confirm significant statistical difference between different BMT and EdPI ($H = 2.43$; $p > 0.05$).

Conclusion: different BMT do not produce edema in significantly different amount and this feature is of no importance regarding their distinction and predictability of BMT from unknown origin.

P807
**Paraneoplastic limbic encephalitis.** L. Guilloton, P. A. Renoult, Y. Billaud, J. Honnorat, A. Drouet, D. Felten, HIA Desgenettes (Lyon, F)

Paraneoplastic limbic encephalitis (PLE) is a manifestation of clinicopathological entity encephalo-myelo-neuropathy associated with antineuronal antibodies (anti-Hu). Isolated PLE is rare in patients with carcinoma, and is supposed after the exclusion of other causes of encephalopathy. An 81-year-old man developed difficulty with orientation, memory and name recognition over 3 months. He has not facial recognition troubles. He was treated for hypertension and had presented one stroke episode 3 years ago; heavy smoker, he was also treated with chemotherapy for a lobar inferior right lung small cell carcinoma (carboplatine; etoposide) 18 months before the start of cognitive impairments. Brain CT and cerebro-spinal fluid were normal, as were a full haematological and biochemical screen. Psychometry disclosed a mini mental test score of 15/30, with a pronounced deficit in short term memory. Neurological examination was otherwise normal. EEG disclosed an unilateral excess of slow activity in left fronto-temporal area. Magnetic Resonance Imaging (MRI) showed on T2-weighted and FLAIR axial sequences abnormal high signal within the limbic system; T1-weighted sequences revealed a low signal in this area without enhancement with gadolinium; these lesions are thought to be compatible with a PLE. Chest radiography was normal, whereas chest CT demonstrated a centimetric suspect nodule lesion in Fowler's segment, in the same area as anterior lung carcinoma. PLE was confirmed with the positivity of anti-Hu antibodies. After 6 months evolution, the patient died with infectious and metabolic complications. Post-mortem examination was declined. Limbic encephalitis is the term applied to encephalopathies arising in the setting of carcinoma which cannot be explained by metabolic, toxic infectious or other inflammatory processes. Although dementia is the most common clinical presentation for PLE, other manifestations include inappropriate affect, agitation, disorientation, hallucinations, seizures, ataxia, dysarthria and atrophy optic. PLE should be considered whenever mental status or psychiatric symptoms arise in the setting of a malignancy. Abnormalities on brain MRI have previously been reported: increased signal intensity in the limbic cortex early in the course of the disease with T2-weighted and FLAIR sequences and low signal with T1-weighted are described. As CT scan is usually negative, MRI must be the preferred examination.

P808
**Gliomatosis cerebri as a fatal subacute leukoencephalopathy: a diagnostic challenge.** J. M. Polo, J. Figols, A. González-Mandly, M. Ortega, A. Vadillo, P. Sánchez-Juan, J. Berciano, University Hospital Marqués de Valdecilla (Santander, E)

Background: Gliomatosis cerebri (GC) is an infrequent central nervous system tumor with a variable and mainly unspecific clinical picture. Objective: To describe a striking case presented as a fatal subacute leukoencephalopathy.

Case report: A 57 year-old woman began one month before admission with vague gait disturbances. Two weeks later a neurological evaluation was normal, but over the following days she developed unsteadiness and depression. On admission she appeared forgetful and showed slow thought processes although aware; her face was unexpressive with poor blinking; gait was unsure and clumsy, needing support; her extremities were rigid, with hyperreflexia and extensor plantar responses. The next few days she deteriorated rapidly, becoming progressively apathetic, withdrawn, unable to stand upright, with incontinence setting in. In a magnetic resonance imaging (MRI) performed eight days after admission, T2-weighted and fluid-attenuated inversion-recovery sequences revealed widespread and symmetrical high intensity of the complete white matter of both cerebral hemispheres; the corpus callosum was also affected throughout, appearing enlarged, this lesion spread through the fornix bilaterally. Electroencephalogram showed bursts of slow wave with occasional triphasic morphology, over a diffused background activity slowing. Cerebrospinal fluid was normal, including cytology, 14–3–3 protein determination and polyomavirus studies. An extensive etiological search was negative. In spite of various treatments, including levodopa and high-dose methylprednisolone, her clinical situation worsened rapidly. Three weeks after admission she reached akinetic mutism; myoclonus or other involuntary movements were absent. In two stereotactically obtained cerebral biopsies, two weeks apart, there were no histopathological changes enough to establish

a diagnosis: nonspecific astrogliotic proliferation and spongiotic changes; immunohistochemistry for prion protein was negative. Repeat brain MRI disclosed the same diffuse white matter changes with increased thickness of the corpus callosum. She remained unconscious the following weeks before dying of pneumonia four months after onset. At postmortem examination the diagnosis of GC involving both hemispheres and corpus callosum was made; on the right oval nucleus, a localized glyoblastoma multiforme was discovered.

Conclusion: the case of this patient illustrates just how dramatic GC may be, at its onset, becoming symptomatic when the tumor is already fully invasive, and in its evolution, leading to death in a subacute way.

**P809**

**Paraneoplastic limbic encephalitis: a diagnosis of suspect.** F. J. Ruiz-Ruiz, C. Iniguez, T. Castiella, J. L. Morales-Rull, J. A. Mauri, J. I. Perez-Calvo, Hospital Clinico Universitario (Zaragoza, E)

Background: Limbic encephalitis (LE) is a rare paraneoplastic disorder. Is most commonly associated with small-cell carcinoma of the lung. Definitive diagnosis is confirmed in autopsy. We report a case with uncommon clinical and imaging features.

Case report: A 49-year-old man presented with 4 weeks of increasing confusion and forgetfulness. Thereafter mild choreatetosis and dysautonomic features developed. Neurological examination showed a left lateral rectus paresis and mild left hemiparesis. Routine laboratory findings and serologies were normal. Antinuclear antibodies were positive (1/80). Computered tomography (CT) scan of his chest and abdomen were normal. Cultures were sterile. An electroencephalogram did not show evidence of seizure activity. The magnetic resonance imaging (MRI) brain scans showed marked abnormalities in hippocampal, subthalamic and right lenticular nuclei and high signal lesions in periventricular white matter on T2-weighted images.

The patient received methylprednisolone and neuroleptics. The choreatetosis improved and his mental status remained unchanged.

The next few months confusion, agitation and paranoid ideation increased. At about the same time, partial complex seizures, status epilepticus and intestinal pseudo-obstruction developed. Month after second admission he died of bronchopneumonia. Postmortem examination found out a small-cell lung cancer, brain lesions compatible with LE and neuronal degeneration of enteric plexures. Postmortem laboratory studies showed serum antineuronal nuclear (anti-Hu) positive.

Discussion: LE incidence is 3 cases each 1000 inhabitants per years. A high suspicion index is required for diagnosis, since behaviour disorders or psychiatric symptoms are the most frequent presentation form. Neurological manifestations can precede the cancer for months or years. The presentation of the disease was with choreatetosis suggestive of brainstem involvement. A further unusual feature was the predominance of several high density lesions in periventricular white matter and larger high-signal in the junction of the white and grey matter. We speculate that these lesions represent an early inflammatory component of LE. To the best of our knowledge, we believe this is the first report of periventricular white lesions caused by LE.

Paraneoplastic limbic encephalitis should be included in differential diagnosis in cases with neurologic and psychiatric symptoms and disseminate white matter lesions.

**P810**

**Intracerebral haemorrhage of a rare aetiology.** H. P. Grebe, D. Steube, D. Hartmann, Neurologische Klinik Bad Neustadt (Bad Neustadt, D)

We present the case of a 40 year-old female who was admitted in October 2001 with neck pain of sudden onset, accompanied by nausea and marked right arm paresis. A CT scan revealed left fronto-parietal cortico-subcortical haemorrhage. Cerebral angiography performed on the same day ruled out arterio-venous malformation as well as intracranial aneurysm. The patient was treated conservatively and thus could be well stabilised. She was started on a rehabilitation program and continued to improve.

Exactly two months after the initial event the residual paresis worsened and the patient developed neuropsychological deficits. A CT scan showed oedema around the original bleeding site, on MRI a solid lesion of 5 cm diameter with some cystic alterations and contact to the meninges could be seen. This lesion was considered to be a tumour, most likely oligodendroglioma.

A week later the tumour was surgically removed, removal at the time was considered complete. Histological analysis proved it to be embryonal rhabdomyosarcoma.

After surgery the patient started recovering from her neurological deficits once more. Almost three months after the operation she complained of intense left-sided headache. On CT a hyperdense left fronto-parietal lesion with positive enhancement could be seen, suggestive of recurrence, MRI confirmed this and showed bleeding in the rostral portion of the tumour as well as oedema.

The patient started radiation therapy with a total dose of 60Gy. Whole body image studies at the time failed to reveal any other neoplastic lesions.

Two months later (5 months after surgery) a CT scan showed continued tumour growth.

We present this case for representing a rare aetiology of intracerebral haemorrhage, more frequently associated with arterial hypertension or vascular pathology, as well as being an unusual manifestation of embryonal rhabdomyosarcoma. The latter occurs seldom in the brain and when it does, it usually has a primary site out of the CNS. Also the vast majority of patients (72%) are children, unlike the patient presented here.

**P811**

**Rare case of meningeal metastasis from breast adenocarcinoma in a male.** A. Rossidou, A. Kiamili, G. Tsambra, E. Antoniou, M. Gomousa, E. Tzamourani, General Hospital of Elefsina (Athens, GR)

A male patient of 52 years of age was admitted in the neurological clinic of our hospital for investigation of confusion episodes and walking instability. In the patient's history there is carcinoma of the left breast that was removed two years ago and followed up by two combinations of chemotherapy and twenty combinations of radiotherapy. Since then, the patient was in good health until three months ago, when he showed changes in mood with hyperirritability, sleep disturbances, paranoid fixations and mild locomotive instability with increased bladder reflexes. In his admission to our clinic, the neurological examination showed no pathological findings in the examination of cranial nerves. He was found with walking instability consisting of wide base walking, while the tendon reflexes were present and symmetric except those of Achilles tendon that were absent.

Finally incomplete caudaequina syndrome was found with "saddle" distribution hyposensitivity and urination and defecation due to overfilling. The patient was oriented in space and time out with scanning speech, hyporeactivity and generally mood depression.

From the laboratory examinations the MRI shows mild microischemic changes. The general hematological and biochemical blood examinations were physiological while the tumor markers were negative [-]. The lumbar puncture examination of cerebrospinal fluid shows 30 cells where 90% of them consist of lymphocytic type, physiological protein level and very low glucose level.

The Gram staining was negative [-], the cultures of cerebrospinal fluid for bacteria were also negative [-] as the orological examination of Syphilis, Listeria, Cryptococcus, Herpes virus and Mycobacteria tuberculosis.

The cytological examination of CSF shows a picture compatible with metastatic adenocarcinoma. The immunocytochemical staining with monoclonal antibodies Human milk fat globular protein and EMA was positive (+), while with the antibody Vinentin was negative [-].

The diagnosis of carcinomatous meningitis was made and the patient was admitted in oncological center where he is being treated with intrathecal chemotherapy.

## Early distorted emotions in neurodegenerative disorders

*C. Ballard*
Newcastle-upon-Tyne, UK

Mood disorders, including depression, anxiety and apathy are frequent in all neurodegenerative dementias. Hypomania occurrs but is much less frequent. When focussing upon mood symptoms that are prominent early in the course of a neurodegenerative disease, apathy is characteristic of basal ganglia dementias and some patients with Fronto-temporal dementia, probably related to disruption of key fronto-subcortical circuits. In Alzheimer's disease (AD) apathy is usually characteristic of moderate or severe dementia but depression is probably more frequent in patients with mild AD. The potential aetiology of depression in AD is complex. Whilst there is a moderate evidence base to support a link between depression and neuronal loss in monoaminergic nuclei, this is not specific to cases occurring early in the disease process. Life events and possibly retention of insight may also be important associations, both of which are highly relevant to the early stages of AD. More recent community studies also indicate an elevated frequency of mood disorders in community samples of

patients with mild cognitive impairment. Developing an evidence base for the treatment of these symptoms in people with neurodegenerative diseases is important, whilst there are several studies indicating the potential value of antidepressants in Parkinson's disease, the literature for the treatment of depression in AD is very contradictory, with the majority of studies indicating no significant benefit compared to placebo; although this may be a result of including patients with mild or self limiting depressive symptoms. The one placebo controlled study comparing a cognitive behaviour therapy intervention to an enrichment of daily activities and placebo, an appproach particularly suitable for people with mild dementia, showed a significant advantage for both psychological interventions. Further mechanistic and treatment studies are a priority.

## Abstracts received after deadline

501a

**Long-term effects of bilateral subthalamic nucleus stimulation in advanced Parkinson disease: a 4-year follow-up study.** Y. Temel, C. van der Linden, H. Celik, G. Spincemaille, V. Visser-Vandewalle (Maastricht, NL; Ghent, B)

High frequency stimulation (HFS) of the subthalamic nucleus (STN) is nowadays widely performed in advanced Parkinson disease (PD). More and more reports appear on the effects at long-term, most of them with a follow-up of 1 to 2 years. In the present study, we prospectively analyzed the effects of bilateral STN HFS in 20 patients, with a minimum follow-up of 4 years. Patients with idiopathic PDand, despite optimal pharmacological treatment, severe response fluctuations and/or dyskinesias were selected. Exclusion criteria were severe abnormalities on MRI, parkinsonism due to known causative factors, Hoehn and Yahr stage 5 at the best moment of the day, severe cognitive and affective disorders, and general contraindications for surgery. Surgery was performed under local anesthesia. The target was defined on computerized tomography (CT) with the following coordinates: 11–12 mm lateral to the AC-PC line, at the mid-commissural point, and 4 mm inferior to the inter-commissural line. The clinical response was evaluated by one blinded neurologist (CvdL) at each level of test stimulation. After determination of the optimum stimulation site the test electrode was replaced by the final quadripolar electrode (Medtronic, model 3389). The pulse generator was implanted at a second stage. UPDRS II(ADL), III(motor performance), IVa (dyskinesias), IVb (motor fluctuations), and Schwab and England scores were evaluated at preoperative med. on/off, 3 months postoperative (stim. on and med. on/off), and at four years postoperative (stim. on/med. on, stim. on/med. off, stim. off/med. off, stim. off/med. on). Thirty-two patients were included. Five patients died and 7 patients were lost to follow-up. Twenty patients (15 males, 5 females) were included in this study with a mean age of 60.9 ± 8.1 years. At 3 months, significant improvements were found on the total UPDRS III score, in the med. off (from 42.3 ± 9.3 to 19.5 ± 6.4), as well as the medication on (from 18.6 ± 12.2 to 10.1 ± 5.9) phase. All motor subscores improved significantly in the medication off phase. The UPDRS IV a and IV scores decreased significantly. At long-term follow-up, there were still significant improvements for the total UPDRS III motor score (from 42.3 ± 9.3 to 24.2 ± 13.2), as well as for all motor subscores, in the off phase, during stimulation. In the on phase, the only significant improvement was seen for rigidity. At 3 months and at long-term, there was a significant decrease on the UPDRS II score in the off phase as well as in the on phase. The Schwab and England scores increased significantly at 3 months and at long-term (from 23.0 ± 11.7 to 62.3 ± 14.1 at long-term, in the off phase, and from 65.8 ± 9.3 to 74.0 ± 9.6 at long-term, in the on phase).The LEU (levodopa equivalent dose) decreased significantly with 47.2% at long-term Complications included weight gain and behavioural changes.

Our results indicate that HFS STN results in long-lasting improvement of the motor symptoms, ADL activities and functional performance in patients suffering from advanced PD. The stimulation induced behavioral changes, however, need special consideration.

501b

**New selective therapies for multiple sclerosis.** L. Kappos, Department of Neurology, University Hospital, Kantonsspital Basel

Disease modifying therapies have up to now targeted the inflammatory component of the disease which is not necessarily directly linked to the other components of MS pathogenesis (demyelination, axonal loss, repair and/or scar formation). In placebo controlled studies efficacy of all three available interferons and glatirameracetate has been limited to the "magic" 30%-relapse rate reduction. While early treatment initiation is only an option for those who recently acquired the disease, other currently recomended means of improving the therapeutic yield like increasing dose and frequency of administration of interferons, as well as the use of more aggressive immunosuppressive treatment with cytostatic agents and/or autologous stem cell transplantation, seem to offer limited improvement. New, more selective immunomodulating drugs are now tested in phase II and III studies, and will be discussed with their specific merits and potential pitfalls. The most advanced and nearest to clinical practice is Natalizumab which is currently tested as monotherapy, and perhaps more promising as add-on therapy to ongoing interferon beta treatment. An increasing body of evidence supports the view that, in addition to immunomodulating and anti-inflammatory treatment, we need to address the more neurodegenerative part of disease pathogenesis. Due the disease's chronicity the therapeutic window in MS should be wider and allow for better chances of drugs that have been rather disapointing in catastrophic diseases like stroke or spinal cord injury or other mostly late diagnosed neurodegenerative diseases.

501c

**The treatment of acute neuro-inflammation.** Bernd C. Kieseier, MD, Heinrich-Heine-University, Duesseldorf, Germany

The prototypic example of an acute inflammatory disorder of the nervous system is the Guillain-Barré syndrome (GBS). It represents the most common cause of acute flaccid paralysis in the Western hemisphere, probably even world-wide, with an average incidence rate estimated to be 1.5/100.000.

Present treatment strategies in this acute disorder (Kieseier and Hartung, 2003) will be discussed in the lecture:

Plasmapheresis and intravenous immunoglobulins (IVIg) are the mainstay of immunomodulatory treatment of GBS at present. Both treatments have proven to exhibit beneficial effects in various controlled trials in favorably altering the natural course of the disease (Bril et al. 1996; French et al. 1987; Hughes et al. 2001; Raphael et al. 2001b; Ropper, 1992; The Guillain-Barré syndrome Study Group, 1985; van der Meche and Schmitz, 1992). In a large controlled trial combined treatment with plasmapheresis followed by IVIg did not provide additional significant benefit. The number of plasma exchanges required for effective treatment was determined in a large prospective study: two exchanges were sufficient to shorten the disease in mildly affected patients, whereas four exchanges were appropriate for patients with moderate to severe GBS, and additional exchanges did not provide any additional benefit (The French Cooperative Group on Plasma Exchange in Guillain-Barré Syndrome, 1997).

The role of glucocorticoids in the treatment regime of GBS might change. In contrast to other autoimmune diseases, glucocorticoids have no rational place in the treatment of GBS at present (Hughes and van Der Meche, 2000). However, in a Dutch not yet published, study comparing the combination of IVIg and glucocorticoids with the current IVIg therapeutic regime patients receiving the combination recovered faster. After six months the clinical outcome measures were similar in both treatment groups (*personal communication*).

Filtration of cerebrospinal fluid was recently investigated in a small prospective study of 37 GBS patients (Wollinsky et al. 2001). The repetitive removal of small volumes of CSF through a lumbar catheter followed by filtration through a millipore filter and re-infusion through the same catheter was well tolerated and found to be equally safe and equally effective compared to conventional plasmapheresis. The rational of this therapeutic approach rests in the notion that the nerve roots are prominently affected in some GBS cases and, therefore, filtration of CSF rather than whole plasma might be more efficient. This study would support the concept that soluble pathogenetic factors particularly present in the CSF, possibly including a small pentapeptide with sodium channel blocking properties (Brinkmeier et al. 2000), are of paramount importance in the pathogenesis of GBS. However, the pathology of GBS is not restricted to the CSF compartment, raising questions about the rationale of this treatment. Furthermore, serious methodological concerns have been voiced (Feasby and Hartung, 2001).

The empirical dose of IVIg generally used for the treatment of GBS is 0.4 g/kg/day for five days, based on practice in other autoimmune diseases. In a recent small randomised trial studying of 39 GBS patients, treatment for three and six days was compared. There was a non-significant trend towards a better outcome in the group receiving six treatments, and this trend reached significance when considering ventilated patients only (Raphael et al. 2001a). In another study, a second cycle of IVIg treatment was found to provide a beneficial effect after failure of a first cycle.

Various reports suggest that IVIg is superior to plasma exchange, especially in GBS patients with a preceding *C. jejuni* infection, an axonal and predominantly motor syndrome, GM1 or GM1b antibodies (Jacobs et al. 1996; Visser et al. 1995; Yuki et al. 2000). However, none of these correlations are absolute, and testing for ganglioside antibodies and preceding infections to guide therapeutical decisions may not be warranted in routine clinical situations (Hadden et al. 2001). However, IVIg is now considered as treatment of first choice by many authorities, given its efficacy, ease of administration, and the low incidence of side effects of this drug. The mode by which IVIg exhibit their immunomodulatory action still remains an immunological puzzle (*reviewed in* Hartung et al. 2000). Various mechanisms have been suggested, such as downregulation of the antibody production including autoantibodies by B lymphocytes, the acceleration of antibody metabolism, the neutralization of complement-mediated effects, an interference with antibody-dependent cellular cytotoxicity mediated by macrophages, modulation of nitric oxide production and microglial function, direct effects on T cell activation, inhibition of cell adhesion, and the induction of apoptosis. In a recent study Buchwald and colleagues could demonstrate another potentially relevant mechanism of action for IVIg. Using a perfused macropatch clamp electrode in a mouse nerve-muscle preparation they were able to show that serum form AMAN and MFS patients contains IgG antibodies that block quantal release and that these antibodies were neutralized in the serum obtained after therapy with intravenous immunoglobulin. Moreover, the Fab but not the Fc portion of IgG extracted from the same immunoglobulin lots used for treatment, neutralized the blocking antibodies in a dose-dependent manner (Buchwald et al. 2002). Besides blocking autoantibodies, immunoglobulins are known to exert a combined effect on complement, cytokines, and Fc receptors, mechanisms which might be clinically more relevant in AIDP (Dalakas, 2002). Since only around 60 % of GBS patients respond to plasma exchange or IVIg, other treatments are clearly warranted. Anecdotal observations suggest interferon-ß1a to be a useful adjunctive therapy (Créange et al. 1998; Schaller et al. 2001). However, further studies are clearly warranted before recommending the therapeutic application of this drug in GBS.

In a small, placebo-controlled pilot trial of 10 GBS patients examined a potential role for subcutaneous brain-derived neurotrophic factor, however no difference in the outcome between the groups were observable (Bensa et al. 2000).

## References


Bensa S, Hadden RD, Hahn A, Hughes RA, Willison HJ (2000) Randomized controlled trial of brain-derived neurotrophic factor in Guillain-Barré syndrome: a pilot study. Eur J Neurol 7:423–426

Bril V, Ilse WK, Pearce R, Dhanani A, Sutton D, Kong K (1996) Pilot trial of immunoglobulin versus plasma exchange in patients with Guillain-Barré syndrome. Neurology 46:100–103

Brinkmeier H, Aulkemeyer P, Wollinsky KH, Rudel R (2000) An endogenous pentapeptide acting as a sodium channel blocker in inflammatory autoimmune disorders of the central nervous system. Nat Med 6:808–811

Buchwald B, Ahangari R, Weishaupt A, Toyka KV (2002) Intravenous immunoglobulins neutralize blocking antibodies in Guillain-Barré syndrome. Ann Neurol 51:673–680

Créange A, Lerat H, Meyrignac C, Degos J-D, Gherardi RK, Cesaro P (1998) Treatment of Guillain-Barré syndrome with interferon-β. Lancet 352:368–369

Dalakas MC (2002) Blockade of blocking antibodies in Guillain-Barré syndrome: "unblocking" the mystery of action of intravenous immunoglobulin. Ann Neurol 51:667–668

Feasby TE, Hartung HP (2001) Drain the roots: a new treatment for Guillain-Barré syndrome? Neurology 57:753–754

French, Cooperative, Group, on, Plasma, Exchange et al. (1987) Efficacy of plasma exchange in Guillain-Barré Syndrome. Ann Neurol 22:753–761

Hadden RD, Karch H, Hartung HP, Zielasek J, Weissbrich B, Schubert J, et al. (2001) Preceding infections, immune factors, and outcome in Guillain-Barré syndrome. Neurology 56:758–765

Hartung HP Kieseier, BC, Gold R, Archelos JJ (2000) Intravenous immunoglobulins: impact on effector mechanisms in autoimmune neurological disorders. In: G. Said (ed) Treatment of neurological disorders with intravenous immunoglobulins. Martin Dunitz, London, pp. 1–17

Hughes RA, Raphael JC, Swan AV, van Doorn PA (2001) Intravenous immunoglobulin for Guillain-Barré syndrome (Cochrane Review). Cochrane Database Syst Rev 2:CD002063

Hughes RA, van Der Meche FG (2000) Corticosteroids for treating Guillain-Barré syndrome. Cochrane Database Syst Rev, CD001446

Jacobs BC, van Doorn PA, Schmitz PI, Tio-Gillen AP, Herbrink P, Visser LH, et al. (1996) Campylobacter jejuni infections and anti-GM1 antibodies in Guillain-Barré syndrome. Ann Neurol 40:181–187

Kieseier BC, Hartung HP (2003) Therapeutic strategies in the Guillain-Barré syndrome. Sem Neurol (in press)

Raphael JC, Chevret S, Harboun M, Jars-Guincestre MC (2001a) Intravenous immune globulins in patients with Guillain-Barré syndrome and contraindications to plasma exchange: 3 days versus 6 days. J Neurol Neurosurg Psychiatry 71:235–238

Raphael JC, Chevret S, Hughes RA, Annane D (2001b) Plasma exchange for Guillain-Barré syndrome (Cochrane Review). Cochrane Database Syst Rev 2:CD001798

Ropper AH (1992) The Guillain-Barré syndrome. N Engl J Med 326:1130–1136

Schaller B, Radziwill AJ, Steck A (2001) Successful tretament of Guillain-Barré syndrome with combined administration of interferon-β-1a and intravenous immunoglobulin. Eur Neurol 46:167–168

The French Cooperative Group on Plasma Exchange in Guillain-Barré Syndrome (1997) Appropriate number of plasma exchanges in Guillain-Barré syndrome. Ann Neurol 41:298–306

The Guillain-Barré syndrome Study Group (1985) Plasmapheresis and acute Guillain-Barré syndrome. Neurology 35:1096–1104

van der Meche FG, Schmitz PI (1992) A randomized trial comparing intravenous immune globulin and plasma exchange in Guillain-Barré syndrome. Dutch Guillain-Barré Study Group. N Engl J Med 326:1123–1129

Visser LH, Van der Meche FG, Van Doorn PA, Meulstee J, Jacobs BC, Oomes PG, et al. (1995) Guillain-Barré syndrome without sensory loss (acute motor neuropathy). A subgroup with specific clinical, electrodiagnostic and laboratory features. Dutch Guillain-Barré Study Group. Brain 118 (Pt 4):841–847

Wollinsky KH, Hulser PJ, Brinkmeier H, Aulkemeyer P, Bossenecker W, Huber-Hartmann KH, et al. (2001) CSF filtration is an effective treatment of Guillain-Barré syndrome: a randomized clinical trial. Neurology 57:774–780

Yuki N, Ang CW, Koga M, Jacobs BC, van Doorn PA, Hirata K, et al. (2000) Clinical features and response to treatment in Guillain-Barré syndrome associated with antibodies to GM1b ganglioside. Ann Neurol 47:314–321