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Critical illness polyneuropathy: clinical findings and cell culture assay of neurotoxicity assessed by a prospective study

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Abstract *Objective*: First, to evaluate the role of typical intensive care-related conditions like sepsis, prolonged ventilation, drug effects and metabolic disorders in the pathogenesis of critical illness polyneuropathy (CIP); second, to investigate the possible significance of patient serum neurotoxicity assessed by an in vitro cytotoxicity assay with respect to CIP development.
Design: Prospective study.
Setting: Neurological intensive care unit.
Patients and participants: Twenty-eight patients who were on mechanical respiratory support for at least 4 days during a 21-month study period.
Results: Diagnosis of CIP was established by clinical and electrophysiological examination in 16 (57 %) of 28 patients. Patients were investigated on days 4, 8 and 14 of mechanical ventilation. Two of 16 CIP patients had clinical signs of polyneuropathy at initial examination. Factors that correlated significantly with the development of CIP were: the multiple organ failure score on day 8 of ventilation, the total duration of respiratory support, the presence of weaning problems and the manifestation of complicating sepsis and/or lung failure. The in vitro toxicity assay showed serum neurotoxicity in 12 of 16 CIP patients. Electrophysiological investigations yielded false positive results of the toxicity assay in six patients (not developing CIP) and false negative results in four patients (developing clinical and electrophysiological signs of CIP). Statistical analysis did not reveal a significant correlation between the diagnosis of CIP and the finding serum neurotoxicity.
Conclusion: The results support the hypothesis of a multi-factorial aetiopathogenesis of CIP. We observed serum neurotoxicity in the majority of CIP patients, indicating the possible involvement of a so far unknown, low-molecular-weight neurotoxic agent in CIP pathogenesis.

Key words Critical illness polyneuropathy · Prognostic factors · Neurotoxicity · Cell culture assay · Electromyography

Introduction

The term "critical illness polyneuropathy" (CIP), introduced by Bolton at al. [1] in 1984, refers to a primary distal axonal degeneration of motor and sensory fibres leading to severe limb weakness and difficulty in weaning from the ventilator in critically ill patients. The aetiology of CIP is still unknown. Sepsis and multiple organ failure (MOF), which are present in up to 90 % of CIP patients, are considered the chief underlying factors [2, 3, 4]. In addition, antibiotics, neuromuscular blocking agents, nutritional deficiencies, hypoxia, metabolic disorders and hypotension are discussed as being implicated in the pathogenesis of CIP [5, 6, 7, 8, 9, 10]. Currently, it is postulated that CIP is a toxic side effect of the inflammatory response which also causes other organ failures in MOF [4, 10, 11, 12]. CIP has recently been described in patients without MOF as well, however [13, 14].

Previous concepts on the aetiopathogenesis of CIP are predominantly based on retrospective studies. The aim of the present investigation was prospectively to evaluate the role of different conditions typically implicated in the course and treatment of critical illness, e. g. sepsis, prolonged respiratory support, administration of aminoglycosides, steroids and neuromuscular blocking agents, and metabolic abnormalities, with regard to the patient's risk of developing CIP.

Since several authors [11, 15, 16] have suggested an unknown neurotoxic agent to be responsible for CIP manifestation, we additionally investigated the neurotoxicity of patient sera during development of CIP with an in vitro assay.

Patients and methods

All patients who had received mechanical respiratory support for more than 4 days while being treated on our neurological Intensive Care Unit (ICU), University of Erlangen-Nürnberg, during a 21-month period from 4/1997 to 12/1998 were enrolled in the study. The spinal cord culture studies were performed at the Department of Biochemistry, University of Erlangen-Nürnberg. Admission diagnosis was ischaemic stroke in 12 and intracerebral haemorrhage in 16 of the 28 patients included. The presence of CIP was assessed by clinical, electrophysiological, laboratory and cell culture evaluation on days 4, 8 and 14 of mechanical ventilation. Patients with suspected pre-existing peripheral neurological diseases (other forms of polyneuropathy, myopathies) were excluded from the study, as were patients with quadriplegia due to spinal cord injury or brainstem infarction.

During the time of artificial ventilation all patients received i. v. analgesic and sedative medication with fentanyl (1–1.5 mg/h) and midazolam (5–15 mg/h). Patients were clinically evaluated when a steady neurological state had been achieved after interruption of sedative drugs.

The clinical signs of CIP were progressive muscle weakness with only minimal movement or complete paralysis in response to painful stimulation and reduced or absent deep tendon reflexes. In patients with central hemiparesis, only the unaffected side was assessed. The clinical state of patients was scored according to a functional disability score (FDS) as shown in Table 1. The Glasgow Coma Score (GCS) was used to grade the severity of coma [17].

Organ failure was classified using a modified Goris Score [18] with the exception of CNS functions, which were assessed according to the GCS. Multiple organ failure (MOF) was defined as severe dysfunction of two or more organ systems including the cardiovascular, respiratory, haematological, hepatic, renal and gastrointestinal systems [4, 6]. Sepsis was defined as the systemic inflammatory response to infection characterised by two or more of the following conditions: (1) temperature above 38 °C or below 36 °C; (2) heart rate more than 90 beats/min; (3) respiratory rate more than 20 breaths/min or $PaCO_2$ less than 32 mmHg; (4) white blood cell count above 12,000/mm^3 or below 4,000/mm^3; (5) more than 10 % immature (band) forms [19]. Complicated weaning was defined as a delay in tapering off use of the ventilator for more than 2 days after the first weaning attempt.

In patients receiving steroids, aminoglycosides and/or neuromuscular blocking agents, we determined doses, days of administration and type of the drugs used. When aminoglycosides had to be employed, regular measurements of the aminoglycoside serum level served to reduce the risk of toxic side effects. Laboratory examinations on days 4, 8 and 14 of ventilation included serum levels of vitamins B_1 and B_{12}, thyroid-stimulating hormone, electrolytes, glucose, albumin, phosphate, magnesium and creatine kinase (CK) [4, 20]. Arterial PO_2, PCO_2, pH, HCO_3 and lactate levels were regularly obtained.

Standard total parenteral nutrition (TPN) was provided for the first 4 days of mechanical ventilation followed by a uniform enteral nutrition regimen provided via nasogastric tube. This enteral nutrition regimen was kept unchanged until study end in all patients.

The electrophysiological examinations of the limbs were performed with a transportable electromyographic machine (MS 92, Medelec) on days 4, 8 and 14 of ventilation. The electromyogram (EMG) protocol included nerve conduction studies of the median, tibial, peroneal and sural nerves with assessment of nerve conduction velocity and amplitudes of the compound muscle and sensory nerve action potentials (CMAPs, SAPs) at distal and proximal supramaximal stimulation. Concentric needle examination was performed at multiple insertion points including the m. abductor pollicis brevis, m. biceps brachii and m. tibialis anterior. The presence of spontaneous activity (e. g. fibrillation potentials, positive sharp waves) was graded on a scale from 0 to 4, with 0 indicating absent, 1–2 mild to moderate and 3–4 substantial, amounts of spontaneous activity [21]. Repetitive stimulation at 3 Hz of the median or ulnar nerves with recording from thenar or hypothenar muscles was only performed in patients with clinically suspected neuromuscular

Table 1 Functional disability score (FDS, maximum 7 patients)

Deep tendon reflexes	0 = normal	1 = reduced	2 = absent	
Weakness	0 = normal	1 = mild	2 = moderate	3 = severe
Atrophy	0 = absent	1 = present		
Fasciculations	0 = absent	1 = present		

transmission defects due to administration of neuromuscular blocking agents.

Electrophysiological criteria for differentiation of primary demyelinating and axonal polyneuropathy were applied as described elsewhere [22, 23]. Patients with markedly reduced nerve conduction velocities were not included in the study. According to the criteria by Bolton et al. [1] and Zochodone et al. [10], we established the diagnosis of CIP in the case of acute or diffuse axonal dysfunction as suggested by the abnormal spontaneous muscle activity of at least two extremities, in combination with diminished amplitudes of compound muscle and sensory nerve action potentials. We separated patients into two groups, one without polyneuropathy and one with signs of acute axonal polyneuropathy, referred to as "without CIP" and "with CIP", respectively. These two groups were compared in terms of clinical**,** respiratory, metabolic, drug-related and electrophysiological characteristics. Additionally, we investigated the neurotoxicity of patient sera in both groups by an in vitro cell culture assay. The study was approved by the local ethics committee.

Sample preparation

Sera of CIP patients and controls were fractionated corresponding to their molecular weight by centrifugation on molecular sieve filters with 5 and 20 kilodalton (kDa) cut-off (Centricon; Sartorius, Germany) and the filtrates of less than 5 kDa, 5–20 kDa and more than 20 kDa were tested for neurotoxic effects on cultured rat spinal cord neurons.

Cell culture

Primary motoneuron cultures were prepared from the foetal spinal cord of E14 rat embryos. Following the procedures of Herkert et al. [24], cells were dissociated by mild trypsination (0.25%) in the presence of DNase I (0.1 mg/ml) after dissection of the spinal cord. After 30 min incubation, 10% horse serum was added to block the trypsin activity and the tissue was triturated gently with a fire-polished glass pipette. Thereafter, the cells were washed in Neurobasal Medium (Gibco) and plated on polylysine-coated 96 well plates (0.05 mg/ml) with a density of 3.5×10^5 cells per well. Motoneurons were allowed to differentiate for 7 days in Neurobasal Medium with B27 supplement (Gibco, Germany).

Bioassay of neurotoxicity

Neurotoxicity was assessed following the addition of serum filtrates to the cultured rat motoneurons (5 or 10% final serum concentration). A detailed description of this procedure will be available elsewhere (Herkert et al., manuscript in preparation). In brief, the viability of cells was determined microscopically and colorimetrically using a cytotoxicity assay (CellTiter AQueous; Promega, Germany) after a period of 20 h. This assay is based on the bioreduction of tetrazolium salt MTS (Owen's reagent) into a soluble formazan product by mitochondrial dehydrogenases of viable cells. Bioreduction of the reagent was quantified by an ELISA plate reader at 490 nm. The measured extinction is directly proportional to the number of living cells in culture. Serum toxicity was calculated as the percentage of survival in the range from 100% (untreated controls) to 0% (addition of 300 μM glutamate for 24 h). Each serum was analysed in at least two independent experiments, each with triplicate values.

Statistical analysis

Mean values for age are given with standard deviation. For rank-scaled data and data without assumption of normal distribution, median values are given with interquartile range (IQR). Relative frequencies of unpaired samples were compared using Fisher's exact test. Unpaired samples of rank-scaled data were compared using the Mann-Whitney U-test. Two-sided p values of equal to or smaller than 0.05 were considered significant. Correction for multiple comparisons was performed according to Hammel (1988). All calculations were carried out using SPSS for Windows, Version 9 (SPSS, Chicago, USA).

Results

Twenty-eight patients who had received at least 4 days of mechanical respiratory support while being treated in our ICU were included in the study. Patient characteristics, including mean ages, gender and clinical characteristics are summarised in Table 2. CIP was diagnosed in 16 (57%) of the 28 patients. Comparable age and sex distributions and similar mean GCS and MOF scores at initial examination were present in the two groups. Physical examination on day 8 revealed significantly higher mean MOF scores of the CIP patients compared to patients without CIP ($p = 0.017$). All but one of the 16 CIP patients had clinical signs of sepsis. Lung failure was present in 15 CIP patients (94%). One patient of the CIP group with the primary diagnosis of intracerebral haemorrhage died on day 14 due to acute cardiovascular failure. Two other CIP patients in whom a ventricular catheter had to be inserted for intracranial pressure monitoring developed ventriculitis in the later course.

Ventilation and weaning characteristics, metabolic parameters and details of drug administration are summarised in Table 2. Total duration of ventilation and weaning was significantly higher in CIP patients. Weaning problems occurred more often in the CIP group, with only three CIP patients not presenting such difficulties. The arterial blood gases and pH measured, as well as levels of serum bicarbonate and phosphate did not differ significantly between the two groups. No clear differences were found in medical treatment duration and dosages of midazolam, intravenous aminoglycosides and corticosteroids. Neuromuscular blocking agents were only used in the form of single injections for short-term procedures, like tracheal intubation, bronchoscopy or tube changing. Six of the ten patients who were receiving catecholamines developed CIP.

Mean levels of magnesium, phosphate, calcium, albumin, vitamins B1 and B12, and thyroid-stimulating hormone were similar in the two groups. There was no evidence for concomitant myopathy in either group. Repeated CK measurements were normal in most cases and did not differ significantly between the groups.

Table 2 Clinical data and patient characteristics of both study groups, referred to as "with" and "without CIP", respectively (*M/F* proportion of male to female, *FDS 1* functional disability score at first examination, *FDS 2* functional disability score at second examination, *GCS* Glasgow Coma Score, *MOF 1* multiple organ failure at first examination, *MOF 2* multiple organ failure at second examination) Data given as median values and interquartile range, for age as mean value with standard deviation

	Without CIP	With CIP	*p* value
Numbers of patients	12	16	
M/F	8/4	10/6	
Age (years)	63.6 ± 8.2	69.6 ± 7.5	0.02
FDS 1	0	0 (0.5)	0.35
FDS 2	0 (0.5)	2.0 (1.75)	0.009
GCS	5 (3.0)	5 (2.0)	0.51
MOF 1	2 (2.0)	2 (1.5)	0.49
MOF 2	0 (1.0)	1 (2.0)	0.009
Sepsis syndrome	6	15	0.05
Respiratory failure	8	15	0.005
Ventilation days	8 (5.0)	15 (5.5)	0.005
Weaning days	1 (2.0)	5 (4.0)	0.002
Weaning problems	2	13	0.002
PO_2 (mmHg)	104 (14.8)	94 (8.4)	0.09
PCO_2 (mmHg)	36.8 (2.9)	35.2 (2.2)	0.62
HCO_3 (mmol/l)	28.4 (2.2)	29.7 (2.7)	0.08
pH	7.47 (8.0)	7.48 (8.2)	0.45
Creatine kinase (normal value: 10–70 U/l)	42 (39.0)	41.7 (140.0)	0.44
Phosphate (normal value: 2.7–4.5 mmol/l)	3.5 (0.9)	3.9 (0.6)	0.59
Midazolam (days of administration)	8 (6.0)	11 (5.5)	0.06
Aminoglycosides (days of administration)	0 (0)	0 (8.5)	0.09
Aminoglycosides (mg/day)	0 (0)	0 (400.0)	0.085
Corticosteroids (days of administration)	1 (5.0)	0 (1.0)	0.16
Corticosteroids (mg/day)	32 (64.0)	0 (250)	0.47

The results of nerve conduction studies are shown in Table 3. Nerve conduction velocities were normal or only slightly reduced in all patients and did not differ significantly between patients with and without CIP. Amplitudes of compound muscle and sensory nerve action potentials were significantly reduced in CIP patients compared to patients without CIP. Spontaneous abnormal muscle activity was found in five patients of the CIP group as early as 4 days after the start of mechanical ventilation. Fibrillation potentials and positive sharp waves of grades 1–2 were present in the m. biceps brachii in two of these patients, in the m. abductor pollicis brevis in three and in the m. tibialis anterior in all patients. EMG revealed no axonopathic features in the patients without CIP.

Table 3 Electrophysiological findings in patient groups "with" and "without CIP" (*NCV* nerve conduction velocity, *CMAP* compound muscle action potential, *SNCV* sensory nerve conduction velocity, *SAP* sensory action potential, *n.s.* not significant) Data given as median values with interquartile range

	Without CIP	With CIP	*p* value
Number of patients	12	16	
N. medianus NCV (m/s)	54.5 (8.0)	52.5 (6.7)	n.s.
CMAP (mV)	6.2 (7.8)	3.2 (4.9)	0.036
N. tibialis NCV (m/s)	43 (3.0)	44.7 (7.8)	n.s.
CMAP (mV)	5 (3.6)	3.0 (1.9)	0.047
N. peroneus NCV (m/s)	46 (13.0)	45.7 (6.8)	n.s.
CMAP (mV)	5 (4.8)	2.8 (2.0)	0.039
N. suralis SNCV (m/s)	49 (14.5)	46.3 (4.3)	n.s.
SAP (μV)	4.7 (4.5)	2 (2.5)	0.029

Recently, a preliminary study has demonstrated increased serum neurotoxicity of CIP patients compared to healthy controls, as assessed by a bioassay containing cultured spinal cord neurons [25]. In the present study we prospectively investigated the significance of this bioassay with regard to the diagnosis of CIP in neurological ICU patients. Since the clinical appearance of CIP shows a preponderance for motor impairment, cultured neurons from rat spinal cord were selected as an in vitro test system for serum neurotoxicity. Furthermore, neuronal primary culture systems are distinguished from currently available systems (e. g. cell lines derived from neural crest progenitor cells) by a high degree of morphological differentiation.

The results of this in vitro serum toxicity assay and the clinical investigation are shown in Table 4. Since only low-molecular filtrate less than 5 kDa turned out to mediate toxic effects, further investigations were focused on these 5 kDa filtrates. At initial examination absent tendon reflexes were found in only two of 16 CIP patients and signs of limb weakness were not present in any patient. Twelve of 16 patients with the electrophysiological diagnosis of CIP had neurotoxic sera as shown by one or more assays of the 5 kDa serum filtrate. Serum was not toxic in six of 12 patients without CIP. In the remaining four and six patients of the respective "with CIP" and "without CIP" groups, electrophysiological investigations yielded "false" negative or positive results of neurotoxicity testing, i.e. electrophysiological signs of CIP were present in patients without

Table 4 Cytotoxicity of low-molecular-weight filtrates of patient sera on day 4, 8 and 14 of mechanical ventilation in patient groups "with" and "without" CIP as defined by clinical and electromyographic criteria

1st examination on day 4 (n = 28)		2nd examination on day 8 (n = 20)*		3rd examination on day 14 (n = 10)*		Cytotoxicity assay
With CIP	Without CIP	With CIP	Without CIP	With CIP	Without CIP	
12, 17, 23	3, 4, 8, 10, 13, 18, 20, 21, 22, 25, 27	3[c], 8[a,c], 14[c], 17[c], 23[c], 26[a,b]	11[c], 22	3[c], 5[a,c], 8[a,c], 11[c], 23[c], 28[a,c]	∅	Not neurotoxic
6, 26	1, 2, 5[c], 7, 9,11, 14[c], 15[b],16[c],19, 24, 28	4[a,c], 5[a,c], 6, 9[b], 10[b], 12, 25[a,c], 28[a,b]	2, 7, 13, 16[c]	2[a,c], 6[a,c], 9[c],12[a,c]	∅	Neurotoxic

[a] distal weakness, [b] hyporeflexia, [c] areflexia
* patient drop outs due to lethal complications, extubation or transfer to peripheral hospitals

neurotoxicity and absent in those with toxicity of serum. Four of the six patients with positive serum toxicity but absent signs of CIP dropped out after the initial examinations and were therefore lost to follow-up investigations, which might have revealed CIP manifestation at a later point in time. Statistical analysis did not reveal a significant correlation between the diagnosis of CIP and serum neurotoxicity ($p = 0.664$). In six patients who did not develop signs of CIP until the time of the second examination, serum neurotoxicity had been demonstrated already at the initial investigation when neither clinical nor electrophysiological signs of CIP had been present.

Discussion

The clinical diagnosis of CIP might be difficult in the early stages of neuropathy. The severity of the underlying illness, effects of neuromuscular blocking agents and mechanical ventilation impair the recognition of beginning symptoms [24]. The earliest sign of neuropathy is difficulty in weaning from the ventilator; then distal weakness and reduced deep tendon reflexes appear [26]. The reported frequency of decreased or absent reflexes varies considerably from 30 to 100 % [27].

In our study, absent tendon reflexes were found at initial examination in only two of 16 CIP patients. No signs of limb weakness were present at this time in any patient. As expected, the mean duration of mechanical ventilation and weaning was significantly higher in the CIP group compared to the group of patients without CIP. According to Zochodone et al. [10] failure to wean might be non-specific, however, due to the possibility of central respiratory depression, especially in neurological patients. Electrophysiological examination is considered the most important tool in the diagnosis of CIP [10]. However, despite some exceptional cases in which denervation signs have already been found on the 5th day [28], spontaneous EMG activity can not be expected before the 10th and 14th days of acute denervation [26].

The aim of this prospective study was, first, to investigate the prognostic value of previously suggested risk factors of CIP and, second, to assess the possible significance of serum neurotoxicity for development of CIP in ventilated, critically ill patients. The role of patient serum neurotoxicity was investigated for the first time in this context. The majority of reports have linked the critical illness neuropathy to sepsis and MOF [1, 2, 5, 10, 29, 30, 31]. There are, however, reports on CIP in patients without sepsis, as well. MOF was present in most of these patients, with respiratory failure being the most frequently described complication [4, 11, 14]. In our study, sepsis was present in 15 of 16 CIP patients. The MOF score on day 8 of ventilation was significantly higher in the CIP group compared to the group without CIP. Considering the results of the present, and those of previous, CIP studies, sepsis and MOF are very common and thus obviously important factors in CIP development even though neither condition seems to be obligatory in the prediction of this type of neuropathy [32, 33, 34, 35].

Infusion of non-depolarising neuromuscular blocking agents has been suggested as a potentially causative factor of neuropathy [4, 7]. The form of axonal neuropathy described by these authors has subsequently been documented in a lot of CIP patients who had not received neuromuscular blockers, however [1, 2, 36, 37]. We kept a complete record of all drugs administered during ICU stay and were particularly interested in the effects of the administration of corticosteroids, muscle relaxants and aminoglycosides. There was no evidence that any of these drugs was associated with CIP. Our results are in agreement with those of Latronico et al. [31], Witt et al. [4] and Coacley et al. [32], who did not see a connection between the drugs and the manifestation of CIP either.

Further factors which might be involved in the pathogenesis of CIP are electrolyte disturbances, elevated blood glucose, CK levels, acidosis and nutritional factors [4, 20, 38]. No severe disturbances of serum electrolytes, CK and blood glucose levels were present in any patient, and standardised nutritional support was provided in both groups.

So far, the exact aetiology of CIP is still unknown. There are theories on the pathogenetic role of a sup-

posed axonopathic 'autotoxin' in patients with MOF that may disturb enzymatic processes in the neuron, affecting the axonal transport of nutrients and degradation products, causing a functional axonopathy at distal sites which can be compared with the 'dying back' phenomenon [15, 16]. If the inflammatory response subsides, levels of the putative toxin will drop and axonal transport will restore itself [11]. The autotoxin hypothesis of MOF has brought the recognition of a variety of immune factors that may be candidates for CIP as well as other organ autotoxins [39, 40]. The question remains which substances may be essential for the initial steps of CIP.

Cytokines are primarily responsible for producing MOF and ARDS [33, 34, 41, 42, 43, 44, 45] and it seems possible that cytokines may have a direct toxic effect on peripheral nerves [35]. One of the cytokines, tumour necrosis factor (TNF), is a key mediator of MOF and ARDS [33, 34, 41, 42, 43, 44, 45]. Bolton et al. [43] found no elevated levels of TNF in five patients with CIP, but it is known that these levels rise only transiently within the range of a few hours with episodes of sepsis. TNF is known to decrease the resting transmembrane potential of skeletal muscle fibres in vitro [46]. Prolonged neuromuscular agents also decrease this potential and induce enlarged end-plates [9]. Op de Coul et al. [7] speculate that this might be the explanation for the reported relation of muscle relaxant drugs to CIP in some patients. Considering these reports, as well as the numerous descriptions of sepsis, MOF and hypoxia [5] in CIP patients, a combination of exogenous and endogenous factors seems to be the most likely cause for the development of CIP.

One of our study objectives was to elucidate further the suggested role of a possible neurotoxic serum factor in the development of CIP. Serum samples were subjected to an in vitro cytotoxicity assay using primary motoneuron cultures from the foetal spinal cord of rat embryos. In a previously investigated control group of 22 healthy subjects neurotoxicity had not been induced by any low-molecular-weight filtrate of these sera (Herkert et al., manuscript in preparation). In the present study, the low-molecular-weight filtrate of 18 patients showed toxic effects in vitro. Twelve of these patients developed CIP. Four of the remaining six patients dropped out after the initial examinations so that it was left unclear whether or not these patients might have developed CIP in the later course. A possible explanation for the frequent "false" positive result of neurotoxicity testing (6/12 patients without CIP) is the speculative release of a structurally undefined toxic agent in consequence of brain injury, since all patients in the present homogenous collective were suffering from cerebral infarction or haemorrhage.

Due to the fact that our collective exclusively included neurological intensive care patients, it must be assumed that the clinical features of patients clearly differed from the groups recruited from general medical and surgical ICUs referred to in previous publications [21, 25, 31, 34]. Proinflammatory cytokines IL-1 alpha, IL-1 beta, IL-6 and TNF-alpha are produced within the CNS and can play a role in CNS damage [43, 47, 48, 49]. Cytokines exacerbate brain injury by several mechanisms and activate the synthesis of acute-phase reactants [49]. In contrast to the previously suggested potentially nerve toxic agents belonging to the group of proinflammatory cytokines with molecular masses exceeding 10 kDa, the molecular weight of the speculative cytotoxic agent in the present case must be below 5 kDa as patient samples were subjected to size exclusion centrifugation with a cut-off of less than 5 kDa. Such a centrally released low-molecular-weight toxin could explain the frequent finding of positive serum toxicity in our neurological patients, in contrast to the exclusively negative serological results in a previously investigated group of healthy persons. One might speculate that such a toxic agent could be involved in the pathomechanism of CIP, since in the present study most of the patients with demonstrated serum neurotoxicity developed axonal polyneuropathy. Statistically there was no significant correlation of neurotoxicity and CIP, however. Therefore, further studies are required to define the role of serum toxicity with respect to the manifestation of CIP in greater detail.

It is noteworthy that six of our patients who developed CIP had serological evidence of neurotoxicity as early as on the 4th day of mechanical ventilation, when electrophysiological signs of polyneuropathy were still absent. Consistent with a potential "hit and run" effect, this observation implies the possibility of a speculative serological agent being present before manifestation of sepsis and/or MOF in these patients, and of "induction" of its potentially neurotoxic effects on the basis of multifactorial complications in critical illness.

With regard to previous publications on myopathy or disturbances of the neuromuscular junction in critically ill patients, most cases have been related to the administration of neuromuscular blocking agents, especially vecuronium, corticosteroids or aminoglycosides, and have been accompanied by mildly to moderately elevated serum CK levels [7, 31, 50, 51, 52, 53, 54, 55]. In our study, however, no patient had signs of concomitant myopathy. Neuromuscular blocking agents were exclusively used as single injections in preparation for short-term procedures and no clear differences were found in treatment duration and dosages of former medications among the patients in the present study. Repeated serum CK levels were normal or only slightly, statistically non-significantly, elevated. The reduced compound muscle action potentials and sensory action potentials observed in most of our CIP patients can be explained by neurogenic, but not by myogenic, abnormalities. Nevertheless,

definite differentiation between CIP and critical illness myopathy would have required muscle and nerve biopsies, which were not performed in the present study due to missing prognostic and therapeutic relevance [31, 51].

In summary, the present study supports the assumption of a multi-factorial pathogenesis of CIP, in which both sepsis and MOF play an important role. In consideration of the serum neurotoxicity demonstrated in the majority of CIP patients, we assume that a so far unknown low-molecular toxic agent might be involved in CIP development. Due to the low diagnostic significance of neurotoxicity testing as performed by a cell biological in vitro assay in the present study, we still consider electrophysiological evaluation as the mainstay in CIP diagnosis.

References

1. Bolton CF, Gilbert JJ, Hahn AT, Sibbald WJ (1984) Polyneuropathy in critically ill patients. J Neurol Neurosurg Psychiatr 47: 1223–1231
2. Bolton CF, Young GB (1986) Sepsis and septic shock: central and peripheral nervous system. In: Sibbald WJ, Spring CL (eds) New horizons: perspectives on sepsis and septic shock. Soc Crit Care Med, pp 157–171
3. Hotz M, Mewes J, Biniek R (1997) Komaassoziierte Neuropathie. Nervenarzt 68: 659–663
4. Witt NJ, Zochodone DW, Bolton CF, Grand-Maison F, Wells G, Young GB, Sibbald WJ (1991) Peripheral nerve function in sepsis and multiple organ failure. Chest 99: 176–184
5. Barat M, Brochet B, Vital C, Mazaux JM, Arné J (1987) Polyneuropathies au cours de séjours prolongés en réanimation. Rev Neurol (Paris) 143: 823–831
6. Gooch JL, Gray L (1989) Prolonged neuromuscular blockade after pancuronium. Muscle Nerve 12: 776–777
7. Op de Coul AAW, Lambregts PCLA, Koeman J, Van Puyenbroek MJE, Ter Laak HL, Gabreels Festen AAWM (1985) Neuromuscular complications in patients given pavulon (pancuronium bromide) during artificial respiration. Clin Neurol Neurosurg 87: 17–22
8. Rivner MH, Sok Kim, Greenberg M, Swift TR (1983) Reversible generalized paresis following hypotension: a new neurological entity. Neurology 33 (Suppl 2):240
9. Wokke JHJ, Jennekens FGI, Van den Oordt CJM, Veldman H, Van Gijn J (1988) Histological investigations of muscle atrophy and end plates in two critically ill patients with generalized weakness. J Neurol Sci 99: 95–106
10. Zochodone DW, Bolton CF, Wells GA, Gilbert JJ, Hahn AF, Brown JD, Sibbald WA (1987) Critical illness polyneuropathy. A complication of sepsis and multiple organ failure. Brain 110: 819–842
11. Leijten FSS, De Wird AW (1994) Critical illness polyneuropathy – a review of literature, definition and pathophysiology. Clin Neurol Neurosurg 96: 10–19
12. Hund EF, Fogel W, Krieger D, DeGeorgia M, Hacke W (1996) Critical illness polyneuropathy: clinical findings and outcomes of a frequent cause of neuromuscular weaning failure. Crit Care Med 24 (8):1328–1333
13. Coakley JH, Nagedran K, Ormerod IED, Ferguson CN, Hinds CJ (1992) Prolonged neurogenic weakness in patients requiring mechanical ventilation for acute airflow limitation. Chest 101: 1413–1416
14. Gorson KC, Ropper AH (1993) Acute respiratory failure neuropathy: a variant of critical illness polyneuropathy. Crit Care Med 21: 267–271
15. Ochs S, Brimijoin WS (1993) Axonal transport. In: Dyck PJ, Thomas PK (eds) Peripheral neuropathy. Vol. 1, 3rd edn. Saunders, Philadelphia, pp 331–360
16. Spencer PS, Sabri ML, Schaumberg HH, Moore CL (1997) Does a defect of energy metabolism in the nerve fibre underlie axonal degeneration in polyneuropathies? Ann Neurol 5: 501–507
17. Teasdale G, Jennett B (1974) Assessment of coma and impaired consciousness. Lancet i:81–84
18. Goris RJA, Boekhorst A, Nuytinck JKS, Gimbrere JFS (1985) Multiple organ failure. Arch Surg 120: 1109–1115
19. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RMH, Sibbald WJ (1992) Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 101: 1644–1655
20. Knochel JP (1982) Neuromuscular manifestations of electrolyte disorders. Am J Med 72: 521–535
21. Zifko UA, Zipko HAT, Bolton CF (1998) Clinical and elektrophysiological findings in critical illness polyneuropathy. J Neurol Sci 159: 186–193
22. Kimura J (1984) Nerve conduction studies and electromyography. In: Dyck PJ, Thomas PK, Lambert EH, Bunge R (eds) Peripheral neuropathy. Saunders, Philadelphia, pp 919–966
23. Stöhr M, Bluthardt (1993) Atlas der klinischen Elektromyographie und Neurographie, 3. Auflage. Kohlhammer, Stuttgart Berlin Köln
24. Herkert M, Röttger S, Becker CM (1998) The NMDA receptor subunit NR2B of neonatal rat brain: complex formation and enrichment in axonal growth. Eur J Neurosci 10: 1553–1562
25. Hund E, Herkert M, Becker CM, Hacke W (1997) A humoral neurotoxic factor in sera of patients with critical illness polyneuropathy. Ann Neurol 40: 539
26. Bolton CF, Young GB, Zochodone DW (1993) The neurological complications of sepsis. Ann Neurol 33: 94–100
27. Neundörfer B (1997) Critically ill-Polyneuropathie. Fortschr Neurol Psychiatr 65: 446–450
28. Op de Coul AAW, Verheul GAM, Zomer E (1990) "Critical illness" polyneuropath. Ned Tijdschr Geneeskd 134: 1789–1792
29. Bolton CF, Brown JD, Sibbald WJ (1983) The electrophysiologic investigation of respiratory paralysis in critically ill patients. Neurology 33: 186
30. Roelofs RJ, Cerra F, Bielka N, Rosenberg L, Delaney J (1983) Prolonged respiratory insufficiency due to acute motor neuropathy: a new syndrome? Neurology 33 (Suppl 2):164–165
31. Latronico N, Fenzi F, Recupero D, Guaneri B, Tomelleri G, Tonin P, De Maria G, Antonini L, Rizzuto N, Candini A (1996) Critical illness myopathy and neuropathy. Lancet 347: 1579–1582
32. Coakley JH, Nagendran K, Honavar M, Hinds CJ (1993) Preliminary observations on the neuromuscular abnormalities in patients with organ failure and sepsis. Intensive Care Med 19: 323–328

33. Leijten FSS, Harinck-De Weerd AW, Poortvliet DCJ, De Weerd AIW (1995) The role of polyneuropathy in motor convalescence after prolonged mechanical ventilation. JAMA 274: 1221
34. Op de Coul AAW, Verheul GAM, Leyten ACM, Schellens RLLA, Teepen JLJM (1991) Critical illness polyneuromyopathy after artificial respiration. Clin Neurol Neurosurg 93: 27–33
35. Souron V, Chollet S, Ordroneau JR, Chailleux E (1995) Dèficits neuromusculaires secondaires en reanimation. Secondary neuromuscular dèficits in ICU patients. Ann Fr Anesth Reanim 14: 213–217
36. Lycklama à Nijeholt J, Troost J (1987) Critical illness polyneuropathy. In: Vinken PJ, Bruyn GW, Matthews WB (eds) Handbook of clinical neurology. Vol.7, Neuropathies. Elsevier, Amsterdam, pp 575–585
37. Plaschke M, Ohly A, Trenkwalder C, König E, Lechner C (1999) Critical illness-Polyneuropathie bei Intensivpatienten: Klinische Befunde, elektrophysiologisch Diagnostik und klinischer Verlauf. Akt Neurologie 26: 72–80
38. Aggarval A, Gutmann L, Gutierrez A (1994) Electrophysiological features of vecuronium induced prolonged neuromuscular blockade. Muscle Nerve 17: 251–252
39. Baue AE (1992) The horror autotoxicus and multiple-organ failure. Arch Surg 127: 1451–1462
40. Trunkey DD (1988) Inflammation and trauma. Arch Surg 123: 1517
41. Beutler B (1989) Cachectin in tissue injury, shock and related states. Crit Care Clin 5: 353–367
42. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA (1988) Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 318: 1481–1486
43. Bolton CF (1995) Critical illness polyneuropathy. In: Asbury AK, Thomas PK (eds) Peripheral nerve disorders. Vol. 2. Butterworth-Heinemann, London, pp 262–280
44. Old L (1988) TNF. Sci Am, May:41–51
45. Tracey K (1989) Cachectin/TNF and other cytokines in infectious disease. Curr Opin Immunol 1: 454–461
46. Tracey HJ, Lowry SF, Beutler B, Cerami A, Albert JD, Shires GT (1986) Cachetin/tumor necrosis factor mediates changes of skeletal muscle plasma membrane potential. J Exp Med 164: 1368–1373
47. Carlson NG, Wieggel WA, Chen J, Bacchi A, Rogers SW, Gahring LC (1999) Inflammatory cytokines IL-1 alpha, IL-1 beta, IL-6 and TNF-alpha impart neuroprotection to an excitotoxin through distinct pathways. J Immunol 163 (7):3963–3968
48. Ferrarese C, Mascarucci P, Zoia C, Cavarretta R, Frigo M, Begni B, Sarinella F, Frattola L, De-Simoni MG (1999) Increased cytokine release from peripheral blood cells after acute stroke. J Cereb Blood Flow Metab 19 (9):1004–1009
49. Vila N, Filella X, Deulofeu R, Ascaso C, Abellana R, Chamorro A (1999) Cytokine-induced inflammation and long-term stroke functional outcome. J Neurol Sci 162 (2):185–188
50. Hirano M, Ott BR, Raps EC, et al. (1992) Acute quadriplegic myopathy: a complication of treatment with steroids, nondepolarizing blocking agents, or both. Neurology 42: 2082–2087
51. Lacomis D, Smith TW, Chad DA (1993) Acute myopathy and neuropathy in status asthmaticus: case report and literature review. Muscle Nerve 16: 84–90
52. Ramsay DA, Zochodone DW, Robertson DM, et al. (1993) A syndrome of acute severe muscle necrosis in intensive care unit patients. J Neuropathol Exp Neurol 52: 387–398
53. Sher JH, Shafiq SA, Schutta HS (1979) Acute myopathy with selective lysis of myosin filaments. Neurology 29: 100–106
54. Zochodone DW, Ramsay DA, Shelly S, et al. (1994) Acute necrotizing myopathy of intensive care: Electrophysiological studies. Muscle Nerve 17: 285–292
55. Hund E (1999) Myopathy in critically ill patients. Crit Care Med 27 (11):2544–2547