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ORIGINAL ARTICLE

IL-8 as a potential biomarker in Guillain-Barre Syndrome

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ABSTRACT. This pilot study was designed to compare the levels of interleukin-8 (IL-8), a pro-inflammatory chemokine, in the cerebrospinal fluid (CSF) of patients with Guillain-Barre syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), non-inflammatory polyneuropathy (PNP), and other non-inflammatory neurological diseases (functional syndrome or migraine). The results show elevated CSF IL-8 levels in GBS compared to the other groups ($p < 0.05$). IL-8 could be considered a potential biomarker to differentiate GBS from CIDP. This distinction could be relevant in terms of therapeutic decisions and functional prognosis.

Key words: cytokine, chemokine, inflammation, cerebrospinal fluid, chronic inflammatory demyelinating polyneuropathy

INTRODUCTION

Guillain Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP) could present overlapping clinical features during the early stages. Compared to GBS, CIDP has a higher frequency of proprioceptive disorder and sensory ataxia [1], but the entities could be difficult to distinguish during the first 4 weeks. Clinical exam, cerebrospinal fluid (CSF) analysis, and electroneuromyography (ENMG) do not always solve the diagnosis dilemma at the disease onset.

GBS is an acute inflammatory demyelinating polyneuropathy that occurs classically in a postinfectious immune-mediated condition [2]. The triggered inflammatory burst targets the peripheral nervous system (PNS), leading to progressive-onset bilateral muscle weakness, areflexia, mild sensory changes, and dysautonomic features. The classical installation time lasts less than 4 weeks [2]. Prompt diagnosis is crucial, as treatment delay can lead to neurological deterioration of the patient. Diagnosis relies on several lines of evidence, such as clinical, electrophysiological, and CSF findings, namely albumin-cytologic dissociation [3]. The initial presentation of GBS may resemble other dysimmune conditions affecting the PNS, such as CIDP in its acute phase [4], whereas the treatment approach for both the conditions differs. The classic histopathological findings in GBS are inflammatory infiltrates consisting mainly of T cells, macrophages, complement activation and anti-ganglioside antibodies, and areas of segmental demyelination, often associated with signs of secondary axonal degeneration [2]; Hafer-Macko E.C., 1996). Even though the physiopathological mechanisms of GBS are not entirely understood, research indicates that cytokines and chemokines are involved in immune-mediated demyelination and axonal disease of the peripheral nerves [5].

CIDP is the most common chronic autoimmune neuropathy, but no pathogenic autoantibody or single triggering antigen has been identified. Diagnosis relies on electrophysiological testing and CSF analysis. Electrophysiological testing demonstrates various typical features of demyelination in motor and sensory fibers (e.g., slow conduction velocity, prolonged distal motor or sensory latencies, prolonged F wave latencies, and conduction block). CSF analysis demonstrated up to 6-fold elevation of protein levels and limited pleocytosis, usually in the presence of a coexisting infection. The current standard of care involves corticosteroids, intravenous immunoglobulin (IVIg), and/or plasmapheresis, which provide short-term benefits. Maintenance therapy with IVIg can induce

Abbreviations

CSF	cerebrospinal fluid
IL-8	interleukin-8
GBS	Guillain-Barre syndrome
CIDP	chronic inflammatory demyelinating polyneuropathy
PNP	polyneuropathy
ENMG	electroneuromyography
IVIg	intravenous immunoglobulin
ROS	reactive oxygen species
AP-1	activator protein 1
HNPP	hereditary neuropathy with pressure palsy

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sustained remission, increase the quality of life, and prevent further axonal loss [6].

Binding of chemokines and their cognate receptors plays a major role in mediating the activation and trafficking of immune cells during the innate and adaptative responses. Interleukin 8 (IL-8) is a pro-inflammatory chemokine whose expression is stimulated by various cytokines (IL-1, IL-6, IL-12, TNF), hypoxia, reactive oxygen species (ROS), bacterial particles, and environmental stress. All of these factors activate intracellular processes that lead to IL-8 synthesis, such as the NF-KB and activator protein 1 (AP-1) pathways [7]. The primary cells capable of secreting IL-8 are circulating monocytes and local macrophages. IL-8, or CXCL8, binds CXCR1 or CXCR2 receptors expressed at the inflammatory cell surface, especially polymorphonuclear neutrophil cells, monocytes, mast cells, and endothelial cells [8]. IL-8 plays a crucial role in the inflammatory cascade by inducing a chemotaxis gradient, cell proliferation, motility and migration, and neutrophil cell granule release and oxidative burst [9, 10].

CIDP can be described as the chronic counterpart of GBS due to various electrophysiological, histological, and immune similarities, but differs from GBS by its time course, disease course, prognosis, and responsiveness to steroids.

This pilot study aimed to assess whether CSF IL-8 levels may help differentiate GBS and CIDP at disease onset.

MATERIALS AND METHODS

Patients

This monocentric retrospective pilot study is based on a cohort of patients followed in the Neurology Clinics of the University Hospitals of Geneva between 2010 and 2018. This study aimed to understand the role of different CSF cytokines in patients diagnosed with peripheral neuropathy of inflammatory or non-inflammatory origin who underwent a lumbar puncture to search potential differential diagnoses. The tested group included GBS, CIDP, noninflammatory polyneuropathy, migraine, and functional syndromes. This study was performed with the approval of the local ethics committee.

CSF analysis

We performed CSF cellular repartition and determined the levels of proteins and glucose, IgG index, albumin quotient, and IgG intrathecal synthesis if present.

Determination of IL-8, IL-6, and TNFα levels in the CSF

IL-8, *IL-6, and TNFα* were measured by commercially available multiplex bead immunoassays (Fluorokine MAP Multiplex Human Cytokine Panel, R&D Systems, Minneapolis, USA) and read by the Bioplex 200 array reader (Bio-Rad Laboratories, Hercules, CA, USA) using Luminex xMAP^{TM} Technology (Luminex Corporation, Austin, TX, USA).

Determination of CRP levels in the serum

Serum CRP was quantified with the Cobas e701 automate using electrochemiluminescence from Roche diagnostics (Roche, Rotkreuz, Switzerland). The upper reference limit was set at 10 mg/l.

Statistical analysis

To detect significant differences between the compared groups, the mean and standard deviation were calculated, and ANOVA was used for quantitative features to compare each group with the others. The level of significance was set to $p < 0.05$.

RESULTS

Patients

Four patients with GBS were included in the study (mean age 51 years old, disease duration 1-2 months, mean 1.25 months); three were females. Three patients exhibited ascending sensorimotor deficit and one had purely sensory symptoms with limb ataxia. Two patients presented with vasomotor signs (orthostatic hypotension or hypertension) and two presented facial nerve palsy (unilateral or bilateral). ENMG showed signs of either myelinic or axono-myelinic neuropathy. The CSF analysis is detailed in *table 1*.

Five patients with CIDP were included in the study (mean age 59 years, disease duration 8-72 months, mean 30 months); two were female. Four patients presented with classical CIDP and one patient was diagnosed with multifocal acquired demyelinating sensory and motor neuropathy (MADSAM). ENMG showed axono-myelinic neuropathy. Anti-MAG antibody was negative in all the five cases.

Four patients with noninflammatory polyneuropathies were included in the study (mean age 63 years, disease duration 3-84 months, mean 38.2 months); two patients were female. The etiologies of polyneuropathy comprised two drug-related origins (statin and chemotherapy), one cryptogenic cause, and one hereditary neuropathy with pressure palsy (HNPP).

Six patients with functional neurological disorders were included in the study (mean age 39 years, disease duration 1-3 months, mean 1.7 months); four were female. They presented with several neurological complaints, including sensory claims in the four limbs (one) or the hemibody (three), retro-orbital pain (one), and cognitive complaints (one). The functional neurological disorder was determined retrospectively according to positive criteria [11] and complementary examinations were negative (brain and medullar MRI, ENMG, CSF analysis depending on the clinical complaints).

Three patients with migraine with aura according to the ICHD-3 classification were included in the study (mean age 38 years, disease duration 1-3 months, mean

Table 1
Demographic and CSF characteristics of the GBS and control groups.

				CSF Analysis			
	Gender	**Age (years)**	**DD (months)**	**WBC**	**IgG index**	**Alb Quot**	**Prot.**
GBS	F	69	1	3	0.6	19.7	1
	F	45	1	1	0.6	11.9	0.7
	M	49	2	29	0.7	17.6	1.1
	F	42	1	1	0.5	11.8	0.6
CIDP	F	58	8	2	0.5	8.8	0.6
	F	69	6	1	0.5	5.8	0.5
	M	70	16	1	0.5	5.4	0.4
	M	56	72	1	0.5	7.3	0.5
	M	43	48	1	0.5	5.7	0.4
Non-inflammatory PNP	M	60	84	2	0.4	11.9	0.7
	F	70	60	1	0.5	6	0.5
	M	76	6	2	0.5	15.4	0.9
	F	47	3	2	0.5	6.6	0.4
Migraine	M	36	1	1	0.6	4	0.3
	F	52	3	1	0.5	9	0.6
	F	27	3	1	0.5	3.1	0.2
Functional	F	41	1	1	0.5	4.7	0.2
	M	42	2	1	0.5	4.8	0.4
	F	19	1	1	0.5	4.1	0.4
	M	51	2	1	0.5	4.6	0.3
	F	54	1	1	0.5	3.3	0.2
	F	26	3	1	0.5	3.7	0.3

DD = disease duration; CSF = cerebrospinal fluid; WBC = white blood cell count; GBS = Guillain-Barre syndrome; CIDP = chronic inflammatory demyelinating polyneuropathy; PNP = polyneuropathy

2.30 months); two were female. Brain MRI and CSF analysis were normal.

CSF IL-8 analysis

The mean CSF IL-8 concentration was 106 pg/ml (SEM 21.9; 95% CI 60.1-151.4) in GBS, 43 pg/ml (SEM 3.6; 95% CI 35.5-50.6) in CIDP, 53 pg/ml (SEM 4.2; 95% CI 44.1-61.4) in noninflammatory polyneuropathy, 28 pg/ml (SEM 4.2; 95% CI 1.1-36.7) in the functional group, and 36 pg/ml (SEM 10.1; 95% CI 14.8-56.9) in migraine. *Figure 1* depicts the IL-8 concentration in each patient in each group.

ANOVA revealed significant differences between the GBS group and the others: CIDP ($p = 0.003$), noninflammatory polyneuropathy ($p = 0.02$), func-

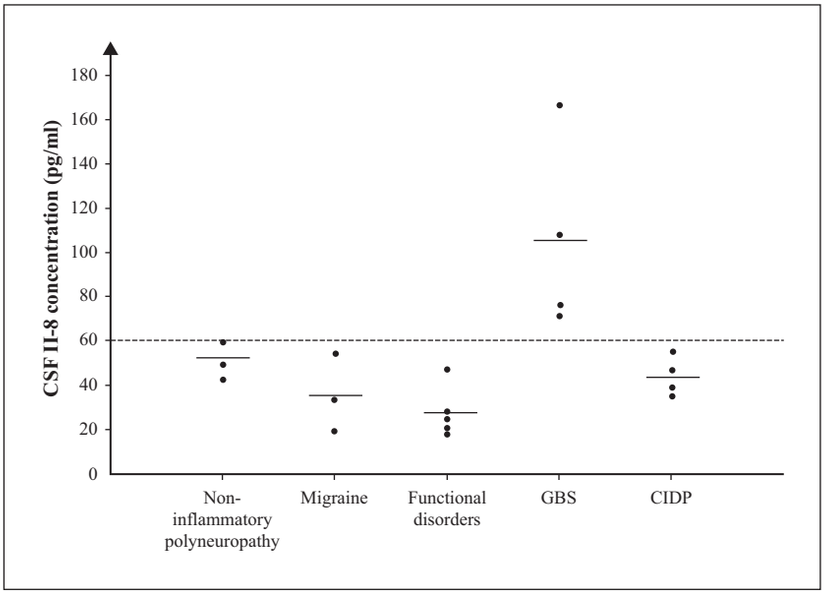

Figure 1
IL-8 concentration in the CSF. Individual CSF IL-8 levels are depicted per patient and distributed by group. The dotted black line indicates a virtual cutoff of 60 pg/ml. Horizontal black lines correspond to the mean of the dots per category. Patients diagnosed with GBS had higher concentrations of IL-8 compared to other inflammatory and noninflammatory causes.

tional (p = 0.0001), and migraine (p = 0.004). A cutoff was arbitrarily set at > 60 pg/ml.

CSF IL-6 analysis

The mean CSF IL-6 concentration was 2.0 pg/ml (SEM 0.9; 95% CI 1.1-2.9) in GBS, 3.0 pg/ml (SEM 1.54; 95% CI 1.6-4.3) in CIDP, 2.6 pg/ml (SEM 1.9; 95% CI 0.8-4.5) in noninflammatory polyneuropathy, 1.0 pg/ml (SEM 0.2; 95% CI 0.7-1.3) in the functional group, and 0.7 pg/ml (SEM 0.4; 95% CI 0.2-1.2) in migraine. *Figure 2*a depicts the CSF IL-6 concentration in each patient in each group.

CSF TNFα analysis

The mean CSF TNFα concentration was 0.8 pg/ml (SEM 0.6; 95% CI 0.2-1.4) in GBS, 0.5 pg/ml (SEM 0.0) in CIDP, 0.51 pg/ml (SEM 0.03; 95% CI 0.49-0.54) in noninflammatory polyneuropathy, 0.7 pg/ml (SEM 0.3; 95% CI 0.4-0.9) in the functional group, and 0.6 pg/ml (SEM 0.1; 95% CI 0.5-0.7) in migraine. *Figure 2*a depicts the CSF TNFα concentration in each patient in each group.

Serum CRP analysis

The mean serum CRP concentration was 1.9 mg/l (SEM 1.6; 95% CI 0.3-3.5) in GBS, 1.6 mg/l (SEM 0.9; 95% CI 0.8-2.3) in CIDP, 1.6 mg/l (SEM 0.7; 95% CI 1.0-2.3) in noninflammatory polyneuropathy, 2.3 mg/l (SEM 1.9; 95% CI 0.7-3.8) in the functional group, and 3.1 mg/l (SEM 1.8; 95% CI 1.0-5.1) in migraine. *Figure 2*b depicts the serum CRP concentration in each patient in each group.

CSF IL-6, CSF TNFα, and serum CRP analyses did not show any significant differences between GBS and CIDP patients.

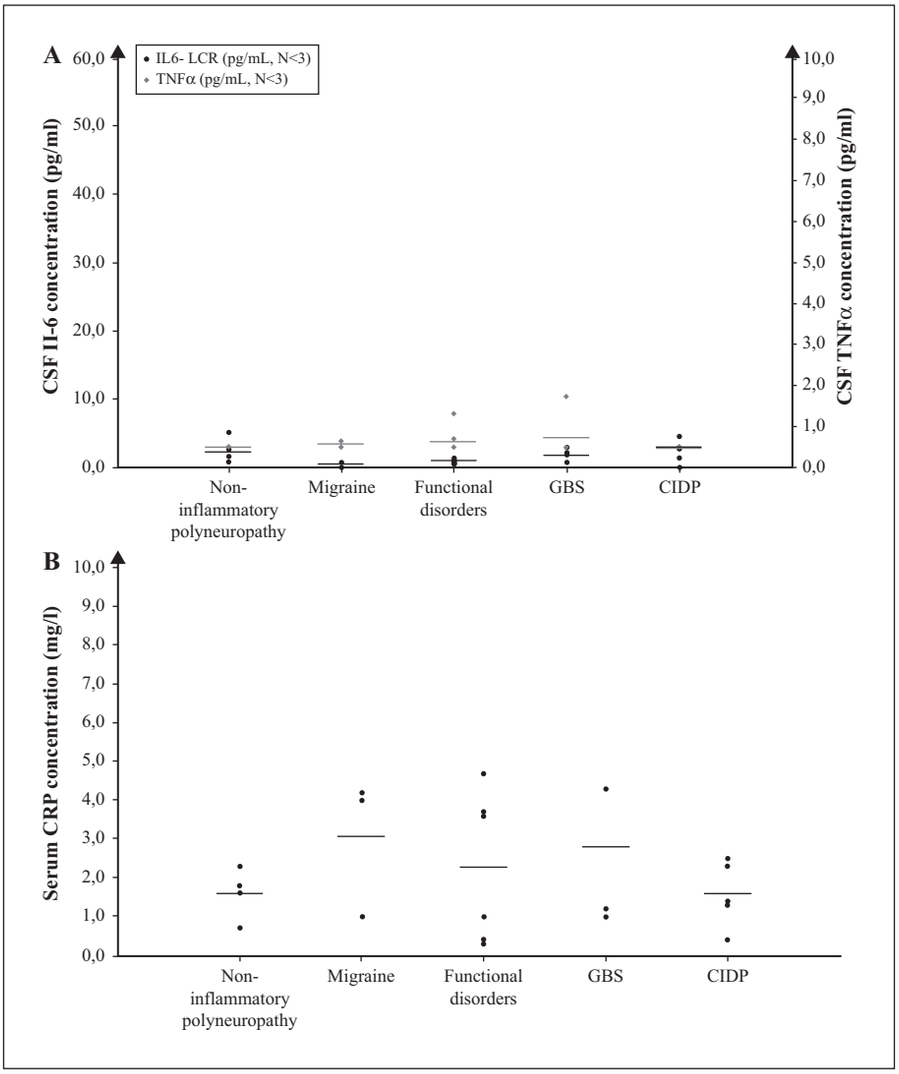

Figure 2

A) IL-6 and TNFα concentrations in the CSF. Both individual CSF IL-6 (black dots) and CSF TNFα (gray squares) levels are depicted per patient and distributed by group. Horizontal lines (black for CSF IL-6 and gray for CSF TNFα) correspond to the mean value per category. CSF IL-6 and CSF TNFα analyses did not show any significant differences between each group, especially when GBS patients were compared to CIDP patients. **B)** CRP concentrations in the serum. Serum CRP concentration levels (black dots) are depicted per patient and distributed by group. Black horizontal lines correspond to the mean value per category. Serum CRP concentration analyses did not show any significant differences between each group, especially when GBS patients were compared to CIDP patients.

DISCUSSION

Our pilot study shows that the CSF IL-8 concentration is significantly increased in GBS compared to the other neurological diseases investigated, including CIDP.
GBS is a monophasic disease in which the time to reach nadir is, by definition, within 4 weeks. In CIDP, the initial progressive phase lasts more than 2 months, after which the course may be relapsing-remitting, steadily progressive, or monophasic. However, not all the patients fulfill the diagnostic criteria for GBS or CIDP. Sixteen percent of patients with CIDP have been reported to have rapidly progressive weakness, with a nadir within 8 weeks from the onset of disease, followed by a chronic course. These patients are considered to have acute-onset CIDP [4].
CSF albumin-cytologic dissociation is the typical immunological alteration in GBS but is also described in CIDP. Several studies have examined the role of potential biomarkers and cytokines in GBS [12] and CIDP [10], including IL-8 [13]. Specific biomarkers are still lacking, either in the blood or CSF, to ensure the diagnosis of GBS [12] and CIDP [14]. CSF biomarkers, such as IL-8, could be helpful in differentiating GBS from CIDP.
As blood immune protein concentrations may evolve following the circadian rhythm to help the organism anticipate daily changes in activity [15], we hypothesize that IL-8 is much more reliable when measured in the CSF than the blood. In addition, human blood neutrophil concentrations fluctuate over the nychthemeron according to cell maturation and activation [16], possibly related to fluctuating IL-8 pathway stimulation.
Better defining GBS and CIDP may allow better medical treatment to prevent disease progression. IVIg and plasmapheresis are indicated in both diagnoses, but steroids are efficient only for CIDP [17] and may worsen GBS outcome [18].
Studies including a larger number of patients are required to confirm these results and to establish a standard cutoff for CSF IL-8 concentration (arbitrarily set at > 60 pg/ml in this study) to distinguish GBS and CIDP.

CONCLUSION

Though clinical history, examination, and ENMG are crucial in the assessment of GBS and CIDP, diagnostic uncertainty may persist. Our pilot study showed that CSF biomarkers, such as IL-8, could help differentiate GBS from CIDP.

Disclosure. None to report.

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