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Serum and cerebrospinal fluid light neurofilaments and antibodies against them in clinically isolated syndrome and multiple sclerosis

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ABSTRACT

A release of light neurofilament subunits (NFL) into cerebrospinal fluid (CSF) and serum in multiple sclerosis (MS) may induce an immune response. We examined CSF and serum NFL levels and IgG antibodies against NFL in 19 patients with a clinically isolated syndrome (CIS) early converted into MS, 20 CIS-non-converters, 23 MS patients and 32 controls. CSF NFL levels were significantly higher in all patient groups. The highest CSF or intrathecally (IT) synthesized anti-NFL antibodies and CSF/serum ratios of anti-NFL antibodies were observed in CIS-converters. CSF NFL and CSF or IT anti-NFL antibodies could be surrogate biomarkers of axonal injury in early MS.

1. Introduction

Neurofilament protein triplets represent major components of the axonal cytoskeleton. The triplet is composed of three subunits — a 68 kDa low molecular weight neurofilament subunit (NFL), a 150 kDa medium-molecular weight neurofilament subunit and a 200 kDa high molecular weight neurofilament subunit (NFH) (Petzold, 2005).

Axonal pathology may be found in several neurological disorders (Trapp et al., 1998; Bartos et al., 2012; Beltran et al., 2012). An abnormal accumulation or aggregation of neurofilaments had been reported in a number of neurological diseases like Alzheimer's disease, dementia with Lewy bodies or amyotrophic lateral sclerosis (Julien et al., 1998; Petzold, 2005; Perrot and Eyer, 2009). Axonal injury is accompanied by release of neurofilament proteins into the extracellular space. These neurofilament subunits can leak into cerebrospinal fluid (CSF) and into blood (Malmestrom et al., 2003; de Jong et al., 2007; Jonsson et al., 2010; Kuhle et al., 2010; Singh et al., 2011). Extracellularly localized neurofilament structures become accessible to immune cells, which can trigger a humoral immune response against neurofilaments including the production of auto-antibodies (Couratier et al., 1998; Eikelenboom et al., 2003; Bartos et al., 2007b; Vyshkina and Kalman, 2008; Fialova et al., 2010; Beyer et al., 2012).

Neurofilaments in the CSF are considered to be a marker of neuroaxonal damage (Giovannoni and Nath, 2011). Several clinical studies demonstrated increased CSF levels of NFL or NFH in patients with multiple sclerosis (MS) (Lycke et al., 1998; Semra et al., 2002; Malmestrom et al., 2003; Norgren et al., 2003; Petzold et al., 2005; Teunissen et al., 2009a; Tumani et al., 2009; Kuhle et al., 2011). Lycke et al. (1998) was the first to notice elevated NFL proteins in the CSF of patients with relapsing–remitting (RR) MS. Subsequent studies showed higher NFL levels in the CSF of other subtypes of MS as well, and a significant increase during acute relapses in patients with RRMS (Semra et al., 2002; Malmestrom et al., 2003; Norgren et al., 2004; Teunissen et al., 2009a). Some studies, but not all, described a relationship between CSF NFL levels and patient disability (Semra et al., 2002; Malmestrom et al., 2003; Teunissen et al., 2009a; Axelsson et al., 2011). A recent follow-up study reported an association between higher levels of NFL in the CSF at the time of the diagnostic lumbar puncture (LP) relative to a later unfavorable MS prognosis (Salzer et al., 2010). Thus, CSF neurofilament levels may be utilized as a marker of severity and even as a prognostic marker for MS. Only one study regarding serum NFL exists, however, the authors failed to detect NFLs in the serum of patients with various neurological diseases using ELISA methods (Zhang et al., 2007).

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Not only antigens, but also antibodies against neurofilaments or other structures may be considered as biomarkers of axonal injury (Silber et al., 2002; Eikelenboom et al., 2003; Teunissen et al., 2005; Bartos et al., 2007a; Tewarie et al., 2012; Hvaring et al., 2013). Experimental animal models support the hypothesis that anti-cytoskeletal antibodies may contribute to axonal pathology (Huizinga et al., 2007). Several studies from our group and others have reported serum and CSF anti-NF antibodies in patients with definite MS (Silber et al., 2002; Eikelenboom et al., 2003; Bartos et al., 2007a, 2007b). Silber et al. (2002) described increased intrathecal production of IgG anti-NFL antibodies in patients with progressive forms of MS. Similar elevations were not observed in RRMS (Ehling et al., 2004; Bartos et al., 2007b). Intrathecal anti-NFL antibodies have been linked to magnetic resonance imaging (MRI) markers of inflammation and tissue destruction in MS (Eikelenboom et al., 2003). The results of Silber et al. (2002) and our study (Bartos et al., 2007b) regarding serum anti-NFL antibodies did not show differences between MS patients and control subjects. On the contrary, an elevation of serum IgG anti-NFL antibody levels during primary chronic progressive MS, compared with other neurological diseases or healthy controls, was demonstrated by Ehling et al. (2004). The contradictory results may be explained by the heterogeneity of MS patients in individual cohorts varying in the proportion of MS subtypes, therapy or duration of disease. Certain differences were also present in ELISA assays.

In our previous study, we measured antibodies against light chains of neurofilaments in patients with clinically definite multiple sclerosis (CDMS) (Bartos et al., 2007b). Now we focused on the relationship between anti-NFL antibodies and their target structure in an immune reaction — light chains of neurofilaments. We were interested to see if a relationship between antigens and corresponding antibodies against NFL, both in CSF and serum existed. If it did exist, did the relationship extend clinically useful information or if the counterparts contributed additional information about mechanisms of axonal damage in MS. In addition, we wanted to gather more information about the possibility of serum NFL determination by the commercial available ELISA kit and to consider its potential as another biomarker of axonal loss without LP.

Most of the previous studies regarding NFL and anti-NFL antibodies were focused on patients with different subtypes of MS. Only one study evaluated CSF NFL levels in patients with clinically isolated syndrome (CIS) (Teunissen et al., 2009a). In the majority of MS patients, the disease first presents as clinically isolated syndrome. Not all patients with CIS syndrome develop MS but those who convert into MS require early and effective therapy to delay irreversible changes in the central nervous system (CNS). Therefore it is important to identify CIS patients with a poor prognosis. Various prognostic biomarkers for CIS patients have been evaluated, such as MRI characteristics and various analyses of the CSF and/or serum (Brettschneider et al., 2006; Teunissen et al., 2009a; Brettschneider et al., 2010; Ferraro et al., 2013). New reliable predictive markers have been intensively studied. Recently, we found that CSF and intrathecal levels and CSF/serum ratios of antibodies against heavy subunits of neurofilaments were increased in the CIS patients early developing MS while NFH protein concentrations were not different among patients' groups and controls (Fialová et al., 2013). We aimed to assess whether anti-NFL antibodies together with NFL proteins might also serve as a predictive marker for CIS patients. Thus patients with incipient MS were best represented among the participants in our current study.

2. Participants and methods

2.1. Participants

We enrolled 39 patients with CIS, 23 patients with MS and 32 patients with other neurological diseases in a prospective study carried out by the MS Center and Department of Neurology, Charles University in Prague (Czech Republic). The basic demographic and CSF data from the participants are presented in Table 1.

Patients with CIS (n = 39) experienced the first clinical episode of a neurological disturbance suggestive of MS but did not meet the criteria for MS before lumbar puncture (LP) (Polman et al., 2005). Then we repeatedly evaluated their clinical status over a follow-up period of 3 years (median; interquartile range: 2.6–3.7 years) to determine if and when they fulfilled the MS criteria (either by a second relapse or by MRI criteria, which demonstrated dissemination of lesions over time) (Polman et al., 2005). The conversion to MS occurred in half of the CIS patients (19/39) during the follow-up (CIS–MS group). Median time to conversion was 10.6 months (interquartile range: 5.2–13 months). The other half of CIS patients remained stable (n = 20, CIS–CIS group). We also included 23 patients with relapsing–remitting MS using established criteria at the time of LP (6 patients during an attack and 17 patients during remission) (MS group) (Lublin and Reingold, 1996; Polman et al., 2005). Disease duration in MS patients until LP was 1.75 years (median; interquartile range: 0.46–5.0 years).

Most of patients with CIS and MS at the time of LP were not receiving corticosteroids or other immune modifying treatments (e.g. interferon or glatiramer acetate) (except for 9 CIS patients and 7 MS patients). Disability for all MS patients was assessed using the EDSS score (Kurtzke, 1983).

Controls were recruited from patients with heterogeneous neurological symptoms and non-inflammatory diseases (n = 32) who had undergone a LP as a part of a routine diagnostic process. Patients with dysesthesias (n = 12) and cephalea (n = 9) were most common. Other control patients included those with cervicocranial syndrome, vertigo, facial palsy and tinnitus.

All subjects gave written informed consents regarding study participation. The Ethics Committee of the Third Faculty of Medicine, Charles University, Prague approved the study.

Lumbar CSF and paired blood samples were collected, centrifuged, aliquoted in 1 mL polypropylene tubes and stored (at −80 °C) until analysis (on average within 1.5 h of sampling) in accordance with established guidelines (Teunissen et al., 2009b). Blood samples were allowed to clot to collect serum before centrifugation. The specimens were thawed just prior to NFL or anti-NFL antibody measurements.

3. Methods

3.1. Determination of NFL protein and IgG antibodies against NFL in serum and cerebrospinal fluid

Light subunits of neurofilaments in the CSF and serum were measured using commercial and validated kits NF-Light® Neurofilament ELISA RUO (UmanDiagnostics, Umeå, Sweden) (Petzold et al., 2010). The kit uses two highly specific monoclonal antibodies against NFL. The detection limit of the ELISA kit was 31 ng/L. The intra-assay and inter-assay coefficients of variation declared by the manufacturer were <6% and <9%, respectively. The ELISA procedure was performed according to the manufacturer's instructions.

The ELISA technique described by Silber et al. (2002) and modified according to our prior experience (Bartos et al., 2007a; Bartos et al., 2007b) was used for the determination of anti-NFL IgG antibodies in the CSF and serum. Microplate wells were coated with bovine neurofilament protein (68 kDa) (Progen, Heidelberg, Germany). CSF samples were analyzed undiluted while serum samples were diluted 1:400. The same pool of human sera diluted by geometric series was assayed as the standard curve in all of the analytical series for comparative purposes. Absorbances were transformed into arbitrary concentration units (AU) using the standard curve. Serum concentrations were multiplied by a dilution factor. The intra-assay and inter-assay coefficients of variation did not exceed 10%.