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Cerebrospinal fluid prognostic biomarkers in Multiple Sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune and disabling disease affecting the central nervous system (CNS) of unknown etiology. As a diagnostic marker for MS does not exist, prognostic biomarkers of disease severity become clinically relevant. Several CSF biomarkers related to the pathogenesis of brain lesions of MS are displayed. Neurofilament light protein, glial fibrillary acidic protein, and chitinase 3-like 1 protein are presented as future useful clinical markers in MS. Difficulties in performing and comparing studies of biomarkers in MS are discussed.

Key words: Multiple sclerosis, CHI3L1, GFAP, neurofilament, prognostic biomarkers, disability progression, YKL-40.

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INTRODUCTION

Multiple sclerosis (MS) is classically described as a demyelinating disease of the central nervous system (CNS). However, not only myelin and oligodendrocytes are implied in MS pathogenesis, but also axonal and neuronal damage, as well as astroglial activation and cytokines release that are important features in the pathogenesis of MS [1–4]. Even though the anatomy of brain lesions in MS is well known, the etiology of MS remains unknown. Even if the implication of the immune system has been fully described in MS and it is considered a chronic autoimmune condition, it is unclear whether the activation of immunological pathways is a primary or a secondary response to primary brain damage. Regardless of this, there is evidence that the pathology of MS is characterized by an inflammatory reaction in close relationship with diffuse neurodegenerative processes [5].

Several studies in MS patients have evaluated biomarkers in different body fluids such as blood, cerebrospinal fluid (CSF), urine, and tears. However, CSF is the closest body fluid to brain tissue and reflects better the brain damage in MS.

At present, a diagnostic marker for MS does not exist. Thus, prognostic biomarkers become relevant in MS to predict disability progression from the early stages of the disease. The availability of a well-standardized prognostic marker in MS could guide decisions on the election of MS treatment and on the therapy response monitoring.

Due to the heterogeneity of the disease both clinically and analytically, it is difficult to find a specific biomarker for MS. Therefore, a profile of biomarkers that represents the variety of cells involved in the pathogenesis of MS would be more consistent. Moreover, biomarkers of neurodegenerative processes would especially have advantages over intermittent inflammatory markers [6].

The present review focuses on CSF prognostic biomarkers in MS which could be clinically relevant in the near future. Several biomarkers related to the different components in brain lesions of MS are explained.

NEURONAL BIOMARKERS

Neurofilaments

Neurofilaments (NF), fibrillary proteins specific of the axoskeleton, are composed of light (NFL), medium (NFM), and heavy (NFH) chain subunits. NF functionally maintain the neuronal caliber and play a role in the intracellular transport through axons and dendrites. They are found in CSF as a product of axonal degradation due to focal demyelination as well as primary inflammation directly to the axon.

NF-light (NFL) are the most abundant, smallest, and most soluble. Due to their low molecular weight and no phosphorylation, they diffuse better in the brain parenchyma and in the CSF, but are susceptible to degradation by proteases. Therefore, if there is continuous axonal damage, there is continuous release of NFL and they could be analyzed in the CSF. If the axonal damage stops, the release of NFL decreases and after degradation, the CSF NFL levels diminish. However, NFL become stable at room temperature and after several cycles of thawing. By contrast, NF-Heavy (NFH) are bigger and less soluble. Due to their high molecular weight and phosphorylation, NFH diffuse badly in the brain parenchyma and CSF, but are resistant to proteases. NF-medium (NFM) are difficult to analyze because of their molecular instability.

NFL and NFH have been associated with the course of MS. However, after a head-to-head comparison, due to the higher abundancy of NFL in CSF and the higher sensibility

of NFL assay, NFL seem to be more clinically relevant than NFH [7].

Different studies evaluated NF in CIS patients. CSF NF have the ability to discriminate between CIS and MS vs. healthy controls, with NFL having a higher ability than NFH [7]. Higher CSF NFL levels were found in those clinically isolated syndrome (CIS) patients who converted to clinically definite MS (CDMS) [8]. Furthermore, CSF NFL levels were significantly higher in those CIS patients who converted early to CDMS [9]. Moreover, in a head-to-head study between NFL and another 11 biomarkers including CHI3L1, it was shown that high CSF levels of NFL in CIS patients were the only independent risk factor associated with earlier conversion to CDMS [9]. Different studies showed that CSF levels of NFL increased after a relapse, with a brief delay in time [9,10]. In addition, CSF NFL levels correlated with MR gadolinium enhancement [11]. By contrast, several studies showed that CSF NFH levels are higher in progressive forms of MS [11,12]. CSF NF correlate with age in healthy controls, but this correlation is not clear in MS patients, as the influence of neurodegeneration in MS is higher than the influence of the physiological age process. In relation to treatment response, one study evaluated the effect of natalizumab treatment on the release of NFL to CSF in MS patients and showed that natalizumab led to a reduction of NFL levels to a value similar to that found in healthy control subjects [13]. Moreover, a comparative study between NFL and NFH showed that NFL were better than NFH as a therapeutic biomarker because NFL decreased after a period of treatment, by the cycle mechanism explained above, while CSF NFH levels seemed to be more constant [14]. Thus, NFL could be a good marker of disease course and treatment response.

14-3-3 Protein

14-3-3 protein owes its name to its electrophoresis pattern. The 14-3-3 protein is a chaperone protein present in all eukaryotic cells, whose function is to bind molecules to form proteins. The 14-3-3 protein is also a heat shock protein, so it increases during cell stress situations to repair abnormal proteins as well as modulating the action of proteins that are involved in cell cycle and apoptosis [15]. In a normal brain, the 14-3-3 protein is expressed in the neuronal somata, while in MS brain demyelinated lesions it has also been observed throughout the whole plaque area, both in oligodendrocytes and in astrocytes. Therefore, under certain pathological situations, the glia and oligodendrocytes overexpress the 14-3-3 protein to repair cell damage and to prevent apoptosis [16]. However, this pattern is not specific to MS being that it has also been observed in progressive multifocal leukoencephalopathy (PML) brain lesions and in brains with multiple system atrophy [16,17]. Consequently, the CSF 14-3-3 protein could be considered both a marker of neuronal damage and glial activation. High CSF levels of 14-3-3 protein have been observed in different neurological disorders as amyotrophic lateral sclerosis, MS, PML, and specially, in Creutzfeldt Jakob disease. Different studies in MS have shown that CSF levels of 14-3-3 protein could be a prognostic marker of conversion from CIS to CDMS and a marker of disability progression in MS patients [18,19]. Nevertheless, other studies failed to confirm the value of this protein in MS [20].

Tau Protein

Tau protein is a protein that stabilizes microtubules of the neuron. During neuronal damage, the Tau protein is released in the extracellular space and high concentrations of Tau protein could be found in the CSF. Some studies showed high CSF levels of Tau protein in MS patients compared with healthy controls and other neurological diseases, but no significant differences were found among different types of MS [21,22]. Other studies showed the CSF Tau protein as a marker of conversion from CIS to CDMS and as a marker of poor outcome in patients with early relapsing-remitting forms of MS (RRMS) [23,24]. On the contrary, different studies did not show differences in CSF levels of Tau protein between MS patients and controls. Also, no correlation with clinical presentation or disability progression was found [9,25].

GLIAL MARKERS

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is a filament protein only present in the astrocyte cytoskeleton. GFAP is involved in the mitosis and the movement of astrocytes as well as participating in the blood–brain barrier (BBB) function. GFAP dysfunction provokes degeneration and astrocyte aggregates. Brain histopathological studies in MS patients showed that GFAP is the principal component in chronic lesions of white and gray matter [26]. GFAP in CSF represents products of degradation of mature astrocytes. Several studies in MS patients showed a correlation between CSF levels of GFAP and disability scores as the Expanded Disability Status Scale (EDSS) [10,27]. Similarly, a recent study showed that high CSF levels of GFAP were independently associated with early progression to EDSS 3 in RRMS patients [9]. Furthermore, the highest CSF levels of GFAP were found in patients with secondary progressive forms of MS [10,27,28]. Therefore, CSF levels of GFAP could be a good marker to predict disability progression in MS patients. Notwithstanding, GFAP failed as a treatment response marker [13].

Interestingly, the CSF levels of GFAP were found remarkably elevated in acute exacerbations of neuromyelitis optica (NMO) [29]. On the contrary, GFAP levels were found significantly lower during acute relapses of MS, as compared to patients in remission [9]. Comparative studies showed significantly higher CSF levels of GFAP in the early stages of NMO than in MS [30]. The reason is that aquoporin 4 channel (AQP4) is located in the cell surface of astrocytes. Thus, when the NMO-specific autoantibody to AQP4 binds to the AQP4 channel, this provokes the breaking down of the astrocytes and a

fast release of GFAP to the environment, while in MS, the CSF GFAP levels increase as the disease progresses and the astrocytes degenerate. Considering this, CSF GFAP could be a marker to differentiate between NMO and MS at first neurological event. However, cell-based assays and an optimized immunohistochemistry technique are the best tests for NMO diagnosis [31]. As in MS, the CSF levels of GFAP in NMO correlate with EDSS and could be a marker of disability outcome [30].

Cihitinase 3-Like 1 Protein

Cihitinase 3-like 1 protein (CHI3L1) also known as YKL-40 protein (YKL-40), from the three N-terminal amino acids and its molecular mass (40KDa), and Heparin binding cartilage-glucoprotein (HC gp-39), for its ability to bind to carbohydrates and proteins and for its interaction with heparin. The CHI3L1 is secreted by activated macrophages and neutrophils to regulate the inflammation and to prevent the apoptosis [32]. Many types of cancer cells also express the CHI3L1 protein [32]. Several studies showed a strong correlation between high levels of serum CHI3L1 and a poor outcome in different chronic inflammatory diseases and cancers, considering CHI3L1 as a disease severity marker [33–37]. Furthermore, some studies showed a correlation between serum CHI3L1 levels and synovial fluid CHI3L1 levels in arthritis, as well as between serum and bronchoalveolar lavage fluid in asthma [38–39].

Similarly, increased expression of CHI3L1 is related to a variety of neurological disorders [2]. However, intrathecal synthesis of the CHI3L1 protein has been described appropriately. Histopathological studies showed that in brain tissue, the CHI3L1 is released by activated astrocytes and microglia to the environment [2,32]. In MS, brain lesions with high inflammatory activity revealed CHI3L1 expression by reactive astrocytes and microglial cells, while non-neurological brain controls were negative for CHI3L1 staining [40]. Moreover, the evaluation of blood–CSF barrier dysfunction in samples from CIS patients showed no correlation between the albumin CSF/serum ratio and CHI3L1 protein levels in CSF [40]. Furthermore, a study of pooled CSF from CIS patients compared CSF CHI3L1 levels and serum CHI3L1 levels between two groups: CIS patients who converted to CDMS, had a brain magnetic resonance (MR) that fulfilled Barkof criteria and had positive oligoclonal bands (OCB) in CSF; and CIS patients who did not convert, had negative OCB and normal brain MR. High levels of CHI3L1 were observed in the CSF of CIS patients who converted to CDMS, while no differences were found in serum CHI3L1 among both groups [41]. In addition, CSF CHI3L1 levels were found significantly increased in patients with RRMS and NMO compared with healthy controls, but no significant differences were observed in serum levels among different groups [42]. Overall, it denotes that CSF CHI3L1 is mainly brain-derived. In CIS patients, high CSF levels of CHI3L1 were associated with a shorter time period in conversion to CDMS [9,40]. Notwithstanding, in a head-to-head comparison study of CIS patients who fulfill Barkof or McDonald criteria, CSF NFL levels were more powerful in predicting an early conversion to CDMS than CSF CHI3L1 [9]. Nevertheless, a study of 109 CIS and 201 RRMS patients showed the CSF CHI3L1 as the best independent marker for disability progression in MS and the marker less influenced by relapses [9]. In addition, the evaluation of the influence of drugs in CSF CHI3L1 levels of MS patients showed decreased levels of CSF CHI3L1 after natalizumab or mitoxantrone treatment, while no changes were found in serum levels [43]. Overall, the CSF CHI3L1 protein is postulated as a good prognostic marker of disease severity and treatment response in MS.

CONCLUSION

The research focusing on biomarkers for MS has been very extensive over recent decades. Even though many biomarkers have been suggested as relevant in MS, their validation becomes complex due to several limitations. The continuous improvement of the sensitivity of the assays makes it difficult to compare studies. The different procedures of collecting and storing samples as well as the different number of patients included in each study could entail contradictory results. In addition, the regular reviews of MS diagnostic criteria have led to different inclusion criteria for MS studies. For instance, patients who were previously classified as CIS, nowadays, can be diagnosed as RRMS by McDonald 2010 criteria. Furthermore, the CIS definition can differ from one study to another. While some studies consider CIS as the first neurological event suggestive of demyelinating CNS disease along with normal brain MR image and negative CSF OCB, others consider CIS when, after a first neurological event, the patient presents brain white matter lesions suggestive of MS regardless of being OCB positive or negative.

Fortunately, thanks to the multi-analyte immunoassays that allow the analysis of several biomarkers in many samples at the same time, along with the consensus protocol for the standardization of CSF sampling and the international McDonald MS diagnostic criteria [44,45], a tendency is expected for more comprehensive and collaborative studies which will allow the validation of biomarkers in MS in the near future. Recent studies on NFL, GFAP, and CHI3L1 are proof of that tendency.

Therefore, CSF levels of NFL from diagnostic lumbar puncture could be considered an independent marker for earlier conversion from CIS to CDMS. CSF NFL levels before and after a treatment could be a good marker for treatment response in clinical trials of RRMS patients. CSF levels of GFAP from diagnostic lumbar puncture could be considered an independent marker for disability progression in MS. CSF levels of CHI3L1 from diagnostic lumbar puncture in CIS patients could be associated with shorter time to CDMS. Finally, high CSF levels of CHI3L1 could be considered as an independent risk factor for earlier disability progression in MS patients, independent of the time of the lumbar puncture, since CSF levels of CHI3L1 have been shown as the less influenced by relapses. Moreover, CSF levels of CHI3L1 have been postulated as a good therapeutic biomarker. Hence, it is anticipated that the combination of these CSF biomarkers leads to the availability of a profile of

biomarkers that allows the identification of patients who are at risk of a severe form of MS from the early stages of the disease and that these CSF biomarkers become promising markers in MS treatment trials.

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Conflict of Interest

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