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Protein biomarkers in multiple sclerosis

Jun-Soon Kim[1,2]

[1]Department of Neurology, Seoul National University Bundang Hospital, Seongnam, Korea
[2]Department of Neurology, Seoul National University College of Medicine, Seoul, Korea

This review aimed to elucidate protein biomarkers in body fluids, such as blood and cerebrospinal fluid (CSF), to identify those that may be used for early diagnosis of multiple sclerosis (MS), prediction of disease activity, and monitoring of treatment response among MS patients. The potential biomarkers elucidated in this review include neurofilament proteins (NFs), glial fibrillary acidic protein (GFAP), leptin, brain-derived neurotrophic factor (BDNF), chitinase-3-like protein 1 (CHI3L1), C-X-C motif chemokine 13 (CXCL13), and osteopontin (OPN), with each biomarker playing a different role in MS. GFAP, leptin, and CHI3L1 levels were increased in MS patient groups compared to the control group. NFs are the most studied proteins in the MS field, and significant correlations with disease activity, future progression, and treatment outcomes are evident. GFAP CSF level shows a different pattern by MS subtype. Increased concentration of CHI3L1 in the blood/CSF of clinically isolated syndrome (CIS) is an independent predictive factor of conversion to definite MS. BDNF may be affected by chronic progression of MS. CHI3L1 has potential as a biomarker for early diagnosis of MS and prediction of disability progression, while CXCL13 has potential as a biomarker of prognosis of CIS and reflects MS disease activity. OPN was an indicator of disease severity. A periodic detailed patient evaluation should be performed for MS patients, and broadly and easily accessible biomarkers with higher sensitivity and specificity in clinical settings should be identified.

Keywords: Biomarkers, Cerebrospinal fluid, Intermediate filaments, Multiple sclerosis

Introduction

Multiple sclerosis (MS) is a common autoimmune demyelinating disease that can affect the entire central nervous system. Most patients develop a 'relapsing' form, while some develop a 'secondary relapsing progressive form,' wherein the overall neurological function steadily deteriorates with repeated relapses [1,2]. Because the burden of acute phase treatment due to relapse and functional impairment due to progressive neurodegeneration are social/medical economic burdens, including a long-term decline in quality of life, early diagnosis and treatment of MS have been consistently studied [3,4]. According to long-term pathophysiology studies, an autoimmune-mediated inflammatory response involving B cells and T cells is the main pathological phenomenon of MS [5,6]. Hence, various therapeutics have been introduced, from interferon-beta (IFN-β) injections over the past several decades to recent high-efficacy drugs (e.g., cladribine, alemtuzumab, and natalizumab) [7]. Moreover, many published study results elucidated the need to identify high-risk MS patient groups at an early stage of disease onset and to actively start treatment to avoid long-term progression. In fact, the McDonald's diagnostic criteria, which are widely used internationally, are being revised to increase the efficiency of early diagnosis [8]. Accordingly, although studies on biomarkers that can be used for 'early diagnosis/prediction of disease activity/monitoring of treatment response' are limited, there have been recent attempts to maximize the efficiency of the process from diagnosis to treatment. Therefore, this review focuses on protein biomarkers in body fluids, including blood and cerebrospinal

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Correspondence: Jun-Soon Kim
Department of Neurology, Seoul National University Bundang Hospital, 82 Gumi-ro 173beon-gil, Bundang-gu, Seongnam 13620, Korea
E-mail: bigai300@gmail.com
ORCID: https://orcid.org/0000-0001-7685-2793

fluid (CSF), among the recently published results of biomarker studies.

Main Subjects

Neurofilament proteins

Neurofilament proteins (NFs) are responsible for maintaining cytoskeletal integrity throughout the nervous system and are composed of neurofilament light chain (NfL), neurofilament medium chain, neurofilament heavy chain, and alpha-internexin [9]. NF levels can increase in pathophysiological situations, leading to axonal nerve injury, and they are the most extensively studied biomarker candidates in a wide variety of neurological diseases, including MS. Among the various NFs, NfL has shown the highest efficacy as a biomarker, as NfLs are released into the CSF either by damage to the cell membrane or by active secretion through multivesicular bodies [10]. Then, some NfLs enter the blood through the glymphatic system or periarterial drainage [11]. The NfL levels in the blood and CSF had a significant correlation regardless of measurement platform [12]. Physiologically, neurodegeneration occurs with aging, and as the blood-brain barrier integrity is disrupted, lower-than-normal concentrations of NfL can be detected in normal body fluids [13]. However, in various pathological conditions, including MS, its level is more likely to increase. Since NfL is the most studied substance as a biomarker of MS, we aim to describe it in terms of its role in diagnosis, disease activity, and therapeutic monitoring (Table 1 [14-37]).

Diagnosis

Since NfL levels have been shown to increase not only in MS but also in other inflammatory nervous diseases, it would not be appropriate to use only NfL for diagnosis of MS [38,39]. However, in MS research, efforts are being made to shorten the time from onset of symptoms to diagnosis of MS. Hence, studies have been conducted on the use of NfL for the purpose of early discovery of patients who transition from clinically isolated syndrome (CIS) or radiologically isolated syndrome (RIS) to clinically definite MS (CDMS).

Recently, when CSF NfL levels were measured in RIS patients, patients who later converted to CDMS showed higher levels than those who did not [14]. A prospective study in adult and pediatric CIS patients in a Dutch cohort also showed that the higher the CSF NfL level, the higher the risk of later conversion to relapsing-remitting MS (RRMS) [15]. Moreover, a 15-year longitudinal follow-up study predicted future transition to secondary progressive MS (SPMS) with a very high level of accuracy (93.3% sensitivity, 46.1% specificity) depending on the baseline serum NfL level (> 7.62 pg/mL) [19]. Various studies have reported that increase in CSF or serum NfL levels helps predict later conversion to MS in patients at a first demyelinating event.

Disease activity

Evaluating disease activity reflects the severity of an acute attack at a certain point in time; however, it is also useful to predict long-term prognosis and changes in advance during follow-up. When evaluating disease activity in terms of relapse activity, which is the most commonly used clinical indicator, a high baseline serum NfL level was associated not only with past relapse activity [20] but also with future relapse [21]. Studies showed that the direction of dynamic change is critical, in addition to the concentration measured unilaterally. There was a case report wherein, after measuring baseline CSF NfL levels in RRMS patients, follow-up measurements were performed after 6 and 28 weeks; the patient experienced clinical relapse at 15 weeks, and the CSF NfL level measured at 6 weeks was three times higher than baseline [40]. In addition, a study elucidated that some relapsed patients with highly active MS treated with alemtuzumab showed serum NfL level increase at 5 months before clinical onset [34]. Referring to the above findings, from the perspective of "predicting" long-term disease activity, regular follow-up evaluation of NfL levels is essential.

Many associations with findings related to brain magnetic resonance imaging (MRI) have been studied and are used as indicators to evaluate the activity of important diseases in clinical practice and clinical trials. Exploring T1-enhancing lesions, CSF NfL assessment showed that high baseline NfL level was associated with many baseline T1-enhancing lesions [22] and high possibility of future T1-enhancing lesions [17]. Similarly, a close correlation between baseline serum NfL levels and baseline T1-enhancing lesions/future T1-enhancing lesions has been reported [23,26], and an increase in serum NfL levels (not baseline serum levels) was associated with new T1-enhancing lesions [24,34]. Similar results have been reported for T2 lesions. Baseline NfL CSF and serum levels can predict the overall T2 lesion burden and the occurrence of new T2 lesions in the future [25,26]. However, some studies have shown that serum NfL levels are unrelated to T1-enhancing lesions or T2 lesion burden [24,41]. In interpreting this result, it is necessary to consider the limited possibility of conventional MRI because high NfL levels are significantly associated with decreased fractional anisotropy and

Table 1 Summary of representative studies with NfL chain as a biomarker of MS

Subtype (No.)	Body fluid	Assay method	Finding	Reference
Diagnostic marker				
RIS (75)	CSF	ELISA	High NfL levels (cut-off value, 619 ng/L) were associated with a significantly shorter time to MS ($p = 0.017$)	[14]
CIS (adults, 88; children, 65)	CSF	ELISA	Increased NfL levels were associated with a shorter time to CDMS diagnosis (pediatric: HR, 3.7; $p = 0.007$ / adult: HR, 2.1; $p = 0.032$)	[15]
CIS (222)	Serum	ECL	Converters to MS showed higher NfL baseline levels compared to non-converters (median, 30.2 pg/mL vs. 9.7 pg/mL; $p < 0.001$)	[16]
CIS (32)	CSF	ELISA	Converters to MS showed higher NfL baseline levels compared to non-converters (median, 812.5 pg/mL vs. 329.5 pg/mL; $p = 0.002$)	[17]
MS (MS, 60; control, 60)	Serum	Simoa	NfL levels of MS patients were higher compared with matched controls in samples drawn a median of 6 years before clinical onset (median, 16.7 pg/mL vs. 15.2 pg/mL; $p = 0.04$), and a within-person increase was associated with higher MS risk (rate ratio ≥ 5 pg/mL increase, 7.50; 95% CI, 1.72–32.80; $p = 0.007$)	[18]
MS (67)	Serum	Simoa	Those with baseline NfL levels less than 7.62 pg/mL were 4.3 times less likely to develop an EDSS score ≥ 4 ($p = 0.001$)	[19]
Disease activity				
Past relapse (RRMS, 47)	CSF	ELISA	Baseline NfL levels correlated with the number of relapses occurring in the previous six ($R = 0.565$, $p < 0.001$) and 12 months ($R = 0.758$, $p < 0.001$)	[20]
Future relapse (RRMS, 607)	Serum	Simoa	High baseline NfL levels (above the 80th percentile) could predict relapse in the short-term (60 days) (OR, 1.98; 95% CI, 1.12–3.37; $p = 0.015$) and long-term (1 year) (OR, 1.67; 95% CI, 1.27–2.18; $p < 0.001$)	[21]
T1-enhancing lesion on brain MRI (RRMS, 34)	CSF	ELISA	NfL levels were higher in patients with T1-enhancing lesions in brain MRI compared to those without lesions (median, 3,970.5 pg/mL vs. 1,530.0 pg/mL; $p < 0.001$)	[22]
T1-enhancing lesion on brain MRI (RRMS, 85)	Serum	Simoa	Patients with T1-enhancing lesions had significantly higher serum NfL levels than patients without MRI disease activity (mean difference, 12.6 pg/mL; $p < 0.01$)	[23]
T1-enhancing lesion on brain MRI (RRMS, 42)	Serum	ELISA	10-fold higher NfL baseline levels were associated with 2.9-fold more frequent enhancing lesions over time (95% CI, 2.2–3.8; $p < 0.001$). A 10-fold increase in NfL over time was associated with a 4.7-fold increase in number of new enhancing lesions (95% CI, 3.3–6.9; $p < 0.001$)	[24]
T2-weighted lesions on brain MRI (RRMS, 52)	CSF	Simoa	Patients with CSF NfL above the cut-off (807.5 pg/mL) 1 year after treatment had a relative risk of 5.0 for relapse and/or new T2-weighted lesions on MRI ($p < 0.001$) during the first year of treatment	[25]
T2-weighted lesions on brain MRI (RRMS, 142)	Serum	ELISA	Serum NfL levels were associated with number of contrast-enhancing and T2 lesions on brain MRI (beta coefficient = 3.00 and 0.75, respectively; both $p < 0.001$)	[26]
Therapeutics monitoring				
Glatiramer acetate (RRMS, 20) & INF-β (RRMS, 12)	Serum	Simoa	NfL levels remained high in nonresponders with clinical relapse, whereas NfL decreased significantly during follow-up (24 months) in patients with a relapse-free course	[27]
DMF (RRMS, 52; HC, 23; placebo, 52)	CSF	Simoa	RRMS patients had higher NfL levels at baseline compared to HC (mean, 2,368 pg/mL vs. 417 pg/mL; $p < 0.001$), and 72% of samples showed a reduction to levels comparable to HCs after 1 year of treatment	[25]
DMF (DMF, 27; placebo, 27)	CSF	ELISA	Mean change in CSF NfL level did not differ between groups (mean difference, 99 ng/L; 95% CI, −292 to 491; $p = 0.61$)	[28]
Fingolimod (RRMS, 36)	CSF	ELISA	Fingolimod proved effective in decreasing NfL levels in RRMS (−326 pg/mL, 83.3% with reduction, $p = 0.002$), and the NfL levels one year after treatment were higher in patients with relapse during the study vs. those without (mean, 1,448 pg/mL vs. 384 pg/mL; $p = 0.014$)	[29]
Natalizumab (RRMS, 96)	Serum	Simoa	In the second year after natalizumab treatment, patients who later developed PML had significantly higher NfL levels than non-developers (mean, 10.1 vs. 7.1 pg/mL; $p = 0.03$)	[30]
Natalizumab (RRMS, 92)	CSF	ELISA	Significant decrease in NfL levels after 12 months of Tx (3-fold reduction: from a mean value of 1,300–400 ng/L; $p < 0.001$)	[31]
Natalizumab (SPMS, 748)	Serum	Simoa	NfL concentrations at weeks 48 and 96 were significantly lower in natalizumab versus placebo participants (ratio, 0.84; 95% CI, 0.79–0.89; $p < 0.001$ and ratio, 0.80; 95% CI, 0.7–0.85; $p < 0.001$, respectively)	[32]
Alemtuzumab (RRMS, 354)	Serum	Simoa	Alemtuzumab reduced serum NfL levels significantly (baseline, 31.7 pg/mL; year 2, 13.2 pg/mL), which was sustained at long-term follow-up (year 7, 12.7 pg/mL)	[33]
Alemtuzumab (RRMS, 15)	Serum	Simoa	Low NfL levels (< 8 pg/mL) correlated with stable disease status, whereas increased NfL levels (> 20 fold) showed an association with T2 lesion progression and development of new T1-enhancing lesions	[34]
Cladribine (progressive MS, 2)	CSF	ELISA	NfL levels were significantly reduced 1 year after treatment (73% and 80%)	[35]
Siponimod (SPMS, 525)	Serum	Simoa	SPMS patients revealed decreased (−5.7%) NfL levels 21 months after treatment, while the placebo group showed increased NfL levels (+9.2%)	[36]
Ofatumumab (RRMS, 936)	Serum	Simoa	In ASCLEPIOS I, NfL levels were lower in the ofatumumab group than in the teriflunomide group by 27% at month 12 and by 23% at month 24. In ASCLEPIOS II, the corresponding differences were 26% and 24%	[37]

NfL, neurofilament light chain; MS, multiple sclerosis; RIS, radiologically isolated syndrome; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; CIS, clinically isolated syndrome; CDMS, clinically definite multiple sclerosis; HR, hazard ratio; ECL, electrochemiluminescence immunoassay; EDSS, Expanded Disability Status Scale; RRMS, relapsing-remitting multiple sclerosis; OR, odds ratio; MRI, magnetic resonance imaging; INF, interferon; HC, healthy control; DMF, dimethyl fumarate; Tx, treatment; SPMS, secondary progressive multiple sclerosis.

increased diffusivity (for the entire normal-appearing white matter [NAWM]; $\rho = -0.49$, $p = 0.005$) when measuring the diffusion tensor index in NAWM in 79 MS patients [42]. Although there is no routine T1 or T2 lesion, a study [42] showed the possibility of determining the progress of overall diffuse white matter damage through NfL level measurement.

Therapeutic response monitoring

As various MS therapeutics are developed and utilized clinically, one of the most critical issues is appropriately verifying the effectiveness of therapeutics. A practically used method is to assess whether a patient has a clinical relapse or to follow up with MRI annually to monitor the presence of newly developed lesions. However, the medical cost may not be the only dilemma, as disease activity may not necessarily be revealed as a change in the image. Accordingly, there has been an expectation that NfL measurement can be used as an auxiliary indicator to reflect subclinical disease activity, and studies on this have been conducted recently.

Exploring drugs that are usually selected as first-line agents in Korea, in 32 RRMS patients treated with glatiramer acetate or IFN-β, NfL levels were decreased in those who responded to treatment, whereas those with increased levels showed lesions on MRI and frequent clinical relapses [27]. With dimethyl fumarate, baseline NfL levels in both the CSF and serum were high in treatment-naïve RRMS patients; however, after 1 year of treatment, these levels in treatment-naïve RRMS patients were the same as those of the healthy control group, and CSF NfL levels were more sensitive in reflecting clinical relapse or MRI activity than were blood NfL levels [25]. However, when the same drug was used to analyze CSF NfL levels in primary progressive MS (PPMS) patients, no significant difference was found in the levels at baseline or after follow-up compared with those of the placebo group [28].

After administration of fingolimod, CSF NfL level in RRMS patients decreased and was correlated with the relapse rate [29]. Interestingly, CSF NfL level was significantly decreased when using fingolimod as first-line treatment [43] but was unchanged when treatment was switched to fingolimod after using natalizumab [44]. This finding demonstrates the use of NfL to provide information on the efficacy of therapeutic agents and to simply monitor the treatment response.

Natalizumab is one of the most frequently prescribed high-efficiency drugs, and CSF NfL level decreased significantly after 12 months of administration in RRMS patients [45]. The CSF NfL level was stable when the disease activity was stable, but it increased rapidly upon relapse [46]. When prescribing natalizumab in clinical practice, one of the critical considerations is the risk of progressive multifocal leukoencephalopathy (PML). When following up with patients prescribed natalizumab, serum NfL levels decreased with stabilization of the disease after initial administration, and results obtained in the second year showed higher serum NfL levels in the group of patients who developed PML than in the group of patients who did not develop PML [30]. This is a valuable finding because serum NfL levels can be used as an adjuvant to determine the risk of PML in John Cunningham virus (+) patients and when deciding to stop natalizumab treatment.

An alemtuzumab-related study identified significantly lower serum NfL levels after administration in RRMS patients after 2 years, and this effect was maintained until the 7th year [33]. In addition, when using alemtuzumab in highly active MS patients ($n = 15$), there was no sign of relapse or new lesion on brain imaging in a small cohort of patients with low serum NfL levels after administration, whereas increase in serum NfL levels was associated with increase in T2 lesion burden and occurrence of new T1-enhancing lesions on brain MRI [34]. In a study comparing alemtuzumab with dimethyl fumarate, fingolimod, natalizumab, teriflunomide, and rituximab, treatment with alemtuzumab showed the lowest plasma NfL levels and the most significant decrease in NfL levels compared to baseline [47], and NfL levels are believed to reflect clinical drug efficacy.

In addition, cladribine [35], which was recently introduced in Korea, and siponimod [36] and ofatumumab [37], which have not yet been introduced, decreased CSF or serum NfL levels according to RRMS or progressive MS types, indicating NfL levels as a possible indicator reflecting treatment response in progressive MS.

Others

As MS progresses, overall brain atrophy progresses, which indicates overall deterioration of the patient's long-term neurological function. Hence, studies to predict future brain atrophy are being conducted. Several studies have shown a correlation between higher CSF NfL levels and severe brain atrophy [48], some have shown an association with gray matter (GM) atrophy [49], and another showed correlation with thalamus and nucleus accumbens volumes rather than overall brain volume [50]. In addition, a study showed that the baseline level of serum NfL and the degree of increase during follow-up could predict future brain volume changes [24].

In diagnosing and treating patients with MS, interest in systemic symptoms that can affect the quality of life of patients, as well as clinical relapse in the form of actual focal neurological deficit, is increasing. In the case of fatigue, the most representative MS symptom, a study of CIS and RRMS patients (n = 38) showed no significant association between serum NfL levels and fatigue [51]. However, since another study showed a correlation between baseline serum NfL levels and baseline quality of life measured using the Multiple Sclerosis Quality of Life-54 questionnaire [52], further studies are needed.

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is a type III intermediate filament protein expressed in the *GFAP* gene located on chromosome 17 and is found in large amount in the cytoplasm of mature astrocytes in the central nervous system. Although it plays various roles, the most important is to maintain the cytoskeleton of astrocytes and provide mechanical tension [53]. CSF GFAP level was increased in conjunction with astrocytosis that occurs in brain trauma, toxic damage, and various genetic diseases. Similarly, CSF GFAP level was increased in MS patients compared with healthy controls. According to the most recently published meta-analysis [54], a mean difference of 0.62 (95% confidence interval [CI], 0.56–0.88; $p < 0.001$) in CSF GFAP level was reported between the RRMS patient group and healthy control group, and a very large mean difference of 103.83 (95% CI, 68.09–139.57; $p < 0.001$) was reported between the remission and relapse periods within the RRMS group. Although CSF GFAP level was significantly lower in the progressive MS patient group than in the RRMS patient group, no difference was noted between the SPMS and PPMS groups. Additionally, CSF GFAP level has been positively correlated with duration of disease ($\rho = 0.3$, $p = 0.014$), reflecting the phenomenon of astrogliosis alongside disease progression [55].

Few studies have measured GFAP level in the blood compared with CSF. Patients with PPMS showed higher blood but not CSF GFAP level than patients with RRMS ($p < 0.05$), and blood GFAP level was correlated with disease severity ($\rho = 0.5$, $p < 0.001$) [56]. However, since the literature on this topic is limited, follow-up studies with a larger cohort are needed to clarify the role of blood GFAP level.

In summary, the pattern of GFAP CSF level differs by MS subtype, which is expected to aid in early classification of PPMS and RRMS. In particular, it may be a helpful biomarker for determining disease severity and progression.

Leptin

Leptin is a protein consisting of 167 amino acids expressed by the *ob* gene and is mainly produced in adipocytes, enterocytes, T-lymphocytes, and bone marrow cells. It has been shown to have a wide range of effects on angiogenesis, wound healing, energy balance, and fat storage by acting through type I cytokine receptors [57]. In addition to its role in immune system regulation, leptin is gaining attention in the field of autoimmune diseases, including MS. Mechanisms acting on the immune system have been reported to promote the proliferation of autoreactive T cells, inhibit the proliferation of T-reg cells, and promote the secretion of proinflammatory cytokines [58,59]. Some studies have shown conflicting results for circulating leptin level in MS patients. However, the largest recently published meta-analysis (including 645 MS patients and 586 controls from nine studies) showed that MS patients had significantly higher blood leptin level than individuals in the control group (standardized mean difference [SMD], 0.70; 95% CI, 0.24–1.15) [60]. A follow-up study showing that overweight young adults (20 years old) had a greater than two-fold higher risk of developing MS supports this finding [61]; however, some studies have reported contradictory results depending on sex/age. For example, in a Swedish biobank-based study, the higher the blood leptin level in men, the higher the MS risk (odds ratio [OR], 1.4; 95% CI, 1.0–2.0; $p = 0.04$), but the higher was the leptin level in women in their 30s, the lower was the risk of MS (OR, 0.74; 95% CI, 0.54–1.0; $p = 0.05$) [62]. A study in Kuwait, where the prevalence of obesity is high, reported that leptin level was significantly lower in MS patients than in individuals in the control group [63].

The diverse study results may be attributed to limitations such as small sample sizes, a heterogeneous mixture of factors known to affect leptin level (age, sex, smoking status, body mass index, and treatment status including steroids), and inconsistent sampling timing (fasting vs. non-fasting). The use of leptin as a valuable biomarker in MS depends on the results of subsequent studies controlling the various confounding factors.

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family, with splicing pattern depending on the type of stimulation, and approximately 30 types of messenger RNA transcripts are produced. BDNF is widely expressed in the central nervous system and plays a vital role in neuronal development and long-term potentiation of synapses by regulating survival, growth, differentiation, and death of neurons

and various types of cells through the receptors TrkB and p75 [64,65]. BDNF in MS has been associated with the single-nucleotide polymorphism (SNP) rs6265, with alteration in some domain structures of BDNF by substituting methionine for valine at codon 66 (Val66Met). This change attenuated BDNF release and receptor binding [66]. Controversial results have been reported regarding the association between this genetic variation and MS. While studies have reported that the Val66Met polymorphism results in more severe GM atrophy in the brain than that of Val/Val carriers (mean GM volume, 812.92 mL vs. 846.42 mL; $p = 0.005$) [67], some studies have shown low BDNF expression in Val66Met carriers, with a protective effect on cognitive decline ($p = 0.027$) [68]. These differing results led to the hypothesis that the polymorphism itself is not essential compared to the direction of "epigenetic regulation" (i.e., the methylation status of the *BDNF* gene). This hypothesis was supported by a recently reported study in an Italian cohort [69]. According to that study, disease severity and presence of the rs6265 SNP were unrelated, and the lower the methylation ratio of the *BDNF* gene, the higher the severity of the disease and the faster the progression. This is probably because the more active is the disease and the stronger the inflammation, the greater the demethylation of BDNF as a defense mechanism and the greater BDNF translation, maximally suppressing inflammation. The expression of BDNF and its receptors in or near MS plaques is increased in the brain pathology tissues of patients with MS, but it decreases in older chronic plaques [70].

Regarding studies of BDNF level at various stages of MS, some studies showed slightly elevated BDNF level in the serum of patients with relapse [71]. However, compared with that of the control group, BDNF level was decreased in MS patients (mean, 60.7 ng/mL vs. 23.9 ng/mL; $p = 0.013$) [72]. This suggests a possible effect of chronic progression of MS by reducing the overall capacity of the nerve repair mechanism due to decreased levels of neurotrophic factors, such as BDNF, in the long-lasting chronic inflammatory phase.

Chitinase-3-like protein 1

Chitinase-3-like protein 1 (CHI3L1) is a glycoprotein secreted from various types of cells, including macrophages, astrocytes, smooth muscle cells, and chondrocytes, and plays an essential role in various inflammatory responses, tissue damage, fibrosis, and extracellular tissue remodeling [73]. In the central nervous system, most CHI3L1 is secreted by astrocytes, activated microglia, and macrophages at sites of inflammatory lesions and reactive gliosis [74]. Many studies have measured the concentration of CHI3L1 in the CSF and blood in patients with MS, with similar results. A recent meta-analysis of 486 patients with MS and 228 healthy controls identified significantly higher CHI3L1 level in the CSF of MS patients compared to a healthy control group (SMD, 0.964; 95% CI, 0.795–1.133; $p < 0.001$) [75]. Furthermore, CIS patients had higher CHI3L1 level than the healthy control group, and increased concentration of CHI3L1 in the blood/CSF of CIS patients was an independent predictive factor of conversion to definite MS (hazard ratio, 1.6; $p = 3.7 \times 10^{-6}$) and rapid disability development ($p = 1.8 \times 10^{-10}$) [76]. In another study, the higher the CHI3L1 level, the higher the number of T2 and Gd+ contrast-enhancing lesions on brain MRI [77]. Furthermore, CSF CHI3L1 level was reduced when natalizumab or fingolimod was administered in patients with RRMS [44,78] and in those who responded to treatment with IFN-β ($p = 0.013$) [79].

Although no significant difference was observed among MS subtypes, CHI3L1 showed potential as a biomarker for early diagnosis of MS and prediction of disability progression. This conclusion requires validation in a larger sample size including patients with homogeneous disease phenotypes.

C-X-C motif chemokine 13

C-X-C motif chemokine 13 (CXCL13) is a chemokine and the most potent B-cell chemoattractant, which is a ligand protein of the B-cell receptor CXCR5 [80]. It is responsible for organization of B cells in the lymphoid follicle and is involved in formation of ectopic meningeal B-cell follicles in the central nervous system, which is very important for forming intrathecal autoimmunity in MS [81]. Since B-lymphocytes are one of the most critical factors in development and progression of MS, CXCL13 has received attention as a candidate early biomarker for MS.

The CSF CXCL13 level has been reported to be associated with CSF pleocytosis and immunoglobulin G (IgG) oligoclonal band (OCB)-positive findings in CIS patients, and high CXCL13 level increased the risk of conversion to CDMS [82]. In RRMS patients, IgG index, CSF white blood cell count, and degree of cerebral cortical atrophy were significantly correlated with CSF CXCL13 level [83] and with disease activity and levels of other biomarkers (NfL and CHI3L1) in progressive MS [84,85]. In addition, when the CXCL13 index ($[CSF_{CXCL13} / serum_{CXCL13}]$ / $[CSF_{alb} / serum_{alb}]$) was introduced, it showed better accuracy than OCB in predicting future disease activity (CXCL13 index: sensitivity/specificity, 91%/64%; OCB: sensitivity/specificity, 81%/30%) [86].

Among patients on high-efficacy disease-modifying therapies, CSF CXCL13 level was increased in some of those who were stable without clinical/imaging relapse (RRMS, 39%; progressive MS, 50%) [87]. This finding suggests that CXCL13 can be used to assess disease activity more sensitively than can clinical indicators or MRI.

Studies have also reported CXCL13 as a marker of response to MS treatment, with levels of both CXCL13 and CCL19 chemokines being significantly reduced in the CSF after rituximab administration and after natalizumab or methylprednisolone treatment [88,89]. It was also reported that baseline serum CXCL13 level before administration of fingolimod was significantly lower in the group that responded to fingolimod than in the group that did not (mean level of responders vs. nonresponders, 58.25 pg/mL vs. 127.2 pg/mL; $p = 0.009$). This suggests that serum CXCL13 level indicates treatment response to fingolimod [90].

In summary, CXCL13 has potential as a biomarker of prognosis of CIS and reflects disease activity in MS. Furthermore, after validation in larger cohorts, CXCL13 is expected to be used as a biomarker related to treatment response (particularly for B-cell-depleting agents).

Osteopontin

Osteopontin (OPN) is an extracellular matrix glycoprotein, a substance secreted by many cell types in different tissues. It is involved in various physiological functions, such as bone remodeling, wound healing, and immune cell activation. In the immune response, it promotes interleukin (IL)-1b, IL-12, and IL-17 production and inhibits IL-10 expression, contributing to transformation of the overall cytokine balance into a proinflammatory state [91]. Because OPN is widely expressed in both the neurons and glia of the brain, it has attracted attention in neuroinflammatory diseases, including MS.

A recent meta-analysis (including 27 previous studies) showed that, regardless of MS subtype, OPN level in MS patients was significantly increased in both the CSF (SMD, 0.65; 95% CI, 0.28–1.01; $p < 0.01$) and blood (SMD, 0.61; 95% CI, 0.34–0.87; $p < 0.01$) compared with that in the control group. In addition, RRMS had the highest level among MS subtypes, followed by PPMS, CIS, and SPMS in that order [92]. Another study has shown that CSF OPN level increased during the acute phase of the disease and decreased after the acute phase, indicating it may as an indicator of disease activity [93]. However, in the meta-analysis, no significant difference was noted in CSF OPN level between MS patients and other inflammatory nervous system disease groups ($p = 0.079$), hindering clinical use as a diagnostic marker of MS. Nevertheless, decrease of an indicator reflecting disease severity or CSF OPN level after natalizumab administration in progressive MS (–65 ng/mL; 95% CI, –34 to –96; $p < 0.001$) [89] indicates the possibility of its use as a marker to evaluate the effect of treatment.

Conclusion

With the development of various therapeutic agents for MS within the past 20 years, the relapse rate has significantly decreased compared with that of the past, and it has become possible to reduce damage caused by MS to the nervous system. However, since MS has a heterogeneous phenotype and complex pathophysiology, it requires 'treatment and control' for the remaining lifetime. A periodic detailed patient evaluation should be performed, and it is essential to have a system to detect subclinical disease activity and respond in advance. In addition to the biomarker proteins mentioned in this review article, there is need for broadly and easily accessible biomarkers with higher sensitivity and specificity. Furthermore, valuable study results are expected in the future, not only in the field of proteins but also for genomic markers, including microRNAs.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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