

# Biomarkers of Therapeutic Response in Multiple Sclerosis: Current Status

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**Abstract** Multiple sclerosis (MS) is an autoimmune disease of unknown cause, in which chronic inflammation drives multifocal demyelination of axons in both white and gray matter in the CNS. The pathological course of the disease is heterogeneous and involves an early, predominantly inflammatory demyelinating disease phase of relapsing–remitting MS (RRMS), which, over a variable period of time, evolves into a progressively degenerative stage associated with axonal loss and scar formation, causing physical and cognitive disability. For patients with RRMS, there is a growing arsenal of disease-modifying agents (DMAs), with varying degrees of efficacy, as defined by reduced relapse rates, improved magnetic resonance imaging outcomes, and preservation of neurological function. Establishment of personalized treatment plans remains one of the biggest challenges in therapeutic decision-making in MS because the disease prognosis and individual therapeutic outcomes are extremely difficult to predict. Current research is aimed at discovery and validation of biomarkers that reliably measure disease progression and effective therapeutic intervention. Individual biomarker candidates with evident clinical utility are highlighted in this review and include neutralizing autoantibodies against DMAs, fetuin-A, osteopontin, isoprostanes, chemokine (C-X-C motif) ligand 13 (CXCL13), neurofilament light and heavy, and chitinase 3-like protein. In addition, application of more advanced screening technologies has opened up new categories of biomarkers that move beyond detection of individual soluble proteins, including gene expression and autoantibody arrays, microRNAs, and circulating microvesicles/exosomes. Development of clinically useful biomarkers in MS will not

only shape the practice of personalized medicine but will also serve as surrogate markers to enable investigation of innovative treatments within clinical trials that are less costly, are of shorter duration, and have more certainty of outcomes.

## Key Points

In the past two decades several therapeutic options have become available for patients with multiple sclerosis. However, in individual patients it is difficult to determine the effectiveness of a given treatment because of the lack of objective measures that define efficacy.

Recently, a number of candidate biomarkers have emerged that can be used to measure ongoing treatment response. These include determination of protein levels that reflect disease activity and other aspects of pathophysiological processes such as oxidative stress and immune dysfunction as well as neural degeneration. Application of these biomarkers in clinical practice will help optimize therapeutic decision making.

## 1 Biomarkers in Multiple Sclerosis

### 1.1 Overview of Multiple Sclerosis

Multiple sclerosis (MS) is a chronic illness of the central nervous system (CNS) and is the leading non-traumatic cause of disability in young adults. Worldwide, over

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2.3 million people suffer from MS. The disease is characterized pathologically by an autoimmune attack directed primarily at myelin, the protective insulation surrounding nerve fibers in the brain and spinal cord. Demyelination, axonal degeneration, and scar formation (sclerosis) are characteristic of the inactive MS lesion. The clinical disease course consists of a several-year period of relapses and remissions of neurological deficits (relapsing–remitting MS [RRMS]) and evolves into a condition typified by progressive disability (secondary progressive MS [SPMS]). In a subset of patients (about 15 % of all patients with MS), the disease course from clinical onset involves steady worsening (primary progressive MS [PPMS]) [1].

The cause of MS is unknown, but multiple factors are involved in its pathogenesis, where a combination of genetics and environmental triggers are implicated. The strongest genetic predisposition correlates with the major histocompatibility complex, class II, DR beta 1 (*HLA-DRB1*)\*1501 allele, with some contribution from other alleles, such as interleukin 2 receptor (*IL2R*) and interleukin 7 receptor (*IL7R*) alleles [2]. Environmental agents associated with MS include exposure to infectious organisms (several candidate organisms have been investigated, with Epstein–Barr virus being the most widely implicated agent), vitamin D and its link to sunlight exposure and geographical latitude, and, possibly, antigenic determinants in the gut microbiome. Although an intrathecal cerebrospinal fluid (CSF) oligoclonal antibody response is seen in approximately 90 % of patients with MS, the antigenic trigger of this response remains unknown [1].

One of the complexities in understanding the pathogenesis of MS is related to disease progression from RRMS to SPMS. Unlike RRMS, which is associated with an increasingly well-characterized immune response and standardized magnetic resonance imaging (MRI) parameters, SPMS exhibits the hallmarks of a neurodegenerative phase, which is poorly understood and not easily quantified. Gray matter involvement, axonal degeneration, microglial activation, mitochondrial injury, and oxidative stress are likely associated with MS progression [3]. Currently, there are no therapies that are effective in reversing or slowing down the neurodegenerative process. Better understanding of the underlying mechanisms that drive disease progression will lead not only to discovery of new therapeutic targets but also to identification of biomarkers to measure disease progression, enabling more effective management of progressive disease.

## 1.2 The Need for Biomarkers in Personalized Medicine

One of the biggest challenges in therapeutic decision-making for MS is effective stratification (or personalization) of treatment in the face of an uncertain prognosis. A

major objective at the time of the initial diagnosis is to arrest the disease at the early inflammatory stage, with the hope that this will also delay disease progression and minimize future disability—a concept that has yet to be proven clinically [4]. The growing list of disease-modifying agents (DMAs) available to target inflammation in MS includes  $\beta$ -interferons (IFN $\beta$ ), glatiramer acetate, natalizumab, and rituximab, as well as newer oral medications, including fingolimod, teriflunomide, and dimethyl fumarate. Treatment decisions based on the risk to benefit ratios of each DMA are further complicated by the inherent disease heterogeneity exemplified by different MS subtypes and the rates of progression, the variety of clinical presentations (spinal cord, cerebellar, optic neuritis, cognition, fatigue, etc.), and the differences in pathological subtypes, implying different disease mechanisms [5]. The heterogeneity of MS is further reflected by the unpredictable efficacy of DMAs, which varies from patient to patient. Identification and validation of predictive biomarkers of therapeutic response are urgently needed to help guide optimal treatment management strategies in patients with MS.

At present, the clinical parameters that are used to assess disease activity and therapeutic efficacy depend on relapses rates, MRI outcomes, and changes in disability scores [1]. These assessments have limited sensitivity with respect to subclinical disease activity, especially when related to gray matter changes and spinal cord disease [6]. Effective stratification of treatment for individual patients with MS will ultimately depend on a new generation of assessment tools with better accuracy and predictability. Thus, there is a need for sensitive, specific, and relatively inexpensive biomarkers that can detect disease activity and serve as surrogate markers for assessing therapeutic efficacy [7]. For the purposes of this review, the definition of a biomarker is limited to measurable proteins, lipids, or nucleic acids in body fluids (such as blood or CSF) that reflect a disease-related or drug-related process. Some of the most promising candidate biomarkers that meet these criteria are listed in Table 1 and are discussed below. Despite the use of MRI in MS diagnosis, disease activity assessment, and therapeutic efficacy, a discussion of imaging techniques falls outside the scope of this review. Ultimately, accurate and sensitive biomarkers of subclinical disease activity will provide neurologists with more objective tools, in addition to MRI, to better assess and predict therapeutic outcomes in individual patients with MS.

## 1.3 Biomarkers in Blood and Cerebrospinal Fluid

Screening and clinical use of blood-based biomarkers has distinct advantages in many diseases, including MS. Blood collection is a minimally invasive procedure performed

**Table 1** Candidate biomarkers of therapeutic response in multiple sclerosis (MS)

Biomarker	Description	Utility in MS
NAbs	NAbs to IFN $\beta$ and natalizumab	Serum NAb testing is used to support lack of response to IFN $\beta$ or natalizumab
Fetuin-A	Secreted glycoprotein elevated in CSF of patients with MS; fetuin-A expression is associated with MS-specific brain pathology	CSF biomarker of subclinical disease activity and therapeutic response to natalizumab
Osteopontin	Matrix protein with pleiotropic functions, including pro-inflammatory cytokine; secreted by activated immune cells and abundantly expressed in MS lesions	CSF biomarker of disease activity, intrathecal inflammation, and therapeutic response to natalizumab
8-iso-PGF <sub>2<math>\alpha</math></sub>	Isoprostane byproduct of lipid peroxidation and a readout of oxidative stress; CSF 8-iso-PGF <sub>2<math>\alpha</math></sub> levels are elevated in a subset of patients with MS	CSF biomarker of oxidative stress, with possible predictive value for therapeutics targeting oxidative pathways
CXCL13	B-cell chemokine elevated in CSF of patients with MS, indicative of humoral responses	CSF biomarker of intrathecal B-cell response; potential biomarker of therapeutic response to rituximab and natalizumab
NFL/NFH	Axonal proteins elevated in CSF as a result of axonal injury	CSF NFH is a possible biomarker of accumulated axonal damage in progressive MS; CSF NFL is a possible biomarker of reduced axonal damage after natalizumab or rituximab
CHI3L1	Chitinase 3-like protein elevated in CSF of patients with CIS who convert to RRMS; expressed by microglia and astrocytes in brains of patients with MS	Prognostic CSF biomarker of conversion from CIS to RRMS; possible biomarker of therapeutic response to natalizumab

8-iso-PGF<sub>2 $\alpha$</sub>  8-iso-prostaglandin F<sub>2 $\alpha$</sub> , *CHI3L1* chitinase 3-like 1, *CIS* clinically isolated syndrome, *CSF* cerebrospinal fluid, *CXCL13* chemokine (C-X-C motif) ligand 13, *IFN* interferon, *NAbs* neutralizing antibodies, *NFH* neurofilament heavy, *NFL* neurofilament light, *RRMS* relapsing–remitting multiple sclerosis

routinely by nursing personnel. Sampling can be carried out in large cohorts of patients, as well as in healthy controls, and can easily be repeated for use in longitudinal studies. While serum biomarkers are suitable for evaluating the peripheral immune targets of various DMAs in MS, they may lack sensitivity in monitoring disease processes in the CNS, particularly with respect to monitoring progressive disease and the effect of therapeutics aimed at neuroprotection and remyelination. On the other hand, CSF is ideally suited to monitoring CNS disease activity because of its close proximity to sites of disease pathology. Discovery and rigorous validation of candidate CSF biomarkers has been limited because CSF collection via lumbar puncture is a relatively invasive procedure, compared with blood collection. In recent years, however, an increasing number of CSF biomarkers have been investigated that potentially reflect key pathological processes underlying disease activity and disease progression in MS [7]. With the increasing rate of discovery and the potential clinical utility of CSF biomarkers, an international effort led by MS researchers has called for standardization of CSF processing, bio-banking, and definition of control samples for comparison between studies [8, 9]. With the understanding that CSF sampling may be necessary during clinical trials testing neuroprotective agents, the majority of patients with MS who have been polled indicated a willingness to undergo lumbar puncture procedures in order to participate [10]. Given the potential of CSF

biomarkers to mirror CNS pathology, it is likely that CSF sampling and biomarker analysis will be incorporated into clinical research studies and into routine clinical care for MS [10, 11].

## 2 Candidate Biomarkers of Therapeutic Response

### 2.1 Neutralizing Antibodies

Detection of neutralizing antibodies (NAbs) to DMAs in MS continues to be one of the more clinically utilized biomarkers in therapeutic decision-making. All protein-based DMAs are potentially immunogenic—in some cases, leading to development of antibodies that neutralize drug activity. IFN $\beta$  preparations are considered a well-tolerated first-line therapy for RRMS, with efficacy in reducing clinical relapses by 30 %. Clinical experience shows that approximately 60 % of patients respond to IFN $\beta$  and are able to control their disease with prolonged IFN $\beta$  therapy. The remaining 40 % are categorized as non-responders [12]. Many initial responders to IFN $\beta$  can develop NAbs to the drug 4–6 months after beginning the therapy, affecting the efficacy of the drug [13]. The incidence of NAb development is dependent on the type of IFN $\beta$ , as well as the route of administration, ranging from 4 % incidence with intramuscular IFN $\beta$ -1a to up to 47 % incidence with subcutaneous IFN $\beta$ -1b [14]. In combination with other

assessments of clinical/MRI disease activity, NAb testing can inform clinical decisions when IFN $\beta$  discontinuation is being considered because of lack of a sufficient therapeutic response.

Although natalizumab is humanized, it is also immunogenic. Like IFN $\beta$  NABs, NABs against natalizumab can also develop early during treatment, within 6 months [15]. Depending on the study, between 4.5 and 14.1 % of natalizumab-treated patients with MS have tested positive for anti-natalizumab antibodies anytime during the treatment, with 1–4.7 % of patients showing transient positivity and 3.5–9.4 % of patients showing persistent positivity [15–17]. In addition, NAB positivity was associated with reduced serum levels of natalizumab, an increased incidence of infusion reactions such as hypersensitivity, and reduced therapeutic efficacy [15, 18]. While NABs against natalizumab can be useful in identifying a failed therapeutic response to natalizumab, better biomarkers are needed to more accurately classify drug non-responders. The necessity of rapid recognition of an inadequate therapeutic response to natalizumab is underscored by the increased risk (1:200) of developing the potentially fatal side effect of progressive multifocal leukoencephalopathy with a longer treatment duration of greater than 24 months [19]. CSF fetuin-A (discussed below) [20] and circulating CD49d expression [21] are emerging as candidate biomarkers for accurate and timely determination of the therapeutic efficacy of natalizumab.

## 2.2 Biomarkers of Disease Activity

The overall clinical management of patients with MS and accurate assessment of therapeutic interventions would be greatly improved by establishment of a universally agreed upon reliable biomarker(s) of disease activity. At present, our clinical parameters for measuring disease activity are rudimentary and depend on relapses or changes in disability scores. Serial brain MRI scans are helpful in monitoring disease activity over time, but patients may worsen clinically without discernible activity on MRI. Furthermore, for spinal cord disease, MRI is even less sensitive in detecting disease activity. Reliable biomarkers reflecting subclinical disease have the potential to serve as surrogate markers of disease activity for future clinical trials, enabling more rapid and cost-effective development of new MS therapies.

Over the past decade, proteomic-based technologies have been used for unbiased CSF biomarker discovery in MS (reviewed in [22]). Though many of the candidate biomarkers have been validated independently, their clinical utility remains unclear. One exception is fetuin-A (alpha-2-HS-glycoprotein), a secreted glycoprotein originally found during a proteomic analysis of CSF from

patients with MS and disease controls [23]. Altered levels of fetuin-A in CSF were associated with early conversion to RRMS [24, 25]. Fetuin-A was also elevated in CSF from subjects with SPMS but not in CSF from subjects with PPMS [23, 26]. Fetuin-A is an abundant serum protein secreted primarily from the liver in the adult [27]. In the CNS, fetuin-A is absent in the normal adult brain but is expressed during fetal brain development and is present at high levels in fetal CSF, suggesting a role for fetuin-A during CNS development [28–30]. In MS, CSF fetuin-A was measured in 100 patients who were clinically categorized as having either active or inactive disease, as defined by a recent relapse, a change in the disability score, or a change in MRI outcomes [20]. Elevation of CSF fetuin-A levels significantly correlated with inflammatory disease activity in patients with MS [20]. Fetuin-A was also elevated in the CNS of mice with experimental autoimmune encephalomyelitis (EAE), a commonly used animal model of MS [20].

A valuable clinical application of CSF biomarkers is their use as surrogate markers for assessing therapeutic efficacy [7]. In that vein, the clinical utility of CSF fetuin-A levels in determining therapeutic response to natalizumab was investigated in a cohort of 77 patients with MS treated with natalizumab longitudinally for 1 year. The decrease in CSF fetuin-A levels in natalizumab-treated patients was highly significant over 6 and 12 months, compared with baseline pre-treatment levels [20]. The decline in CSF fetuin-A levels was even more pronounced in patients who were classified as treatment responders, compared with non-responders, who showed no significant change in fetuin-A levels. Overall, these studies support the clinical application of CSF fetuin-A as an objective and accurate laboratory measure of disease activity and treatment efficacy. These studies also highlight the potential of CSF fetuin-A to be used in a routine clinical setting to support MRI results in the therapeutic decision-making process. Prior to clinical implementation of CSF fetuin-A as a biomarker, validation studies would be required, showing a correlation with disease activity in an independent cohort of patients. In addition, the specificity of CSF fetuin-A for MS in relation to other inflammatory diseases should be defined. Finally, the decrease in CSF fetuin-A levels described in natalizumab-treated patients would have to be extended to therapeutic responses to other DMAs.

Other studies have identified additional CSF biomarkers that are altered in patients with MS treated with DMAs, and include chemokine (C-X-C motif) ligand 13 (CXCL13) [31] and neurofilament [32–34] (both discussed below), as well as hepatocyte growth factor (HGF) [35] and osteopontin [36, 37]. Osteopontin, in particular, has been analyzed extensively in both blood and CSF from patients with MS, as a biomarker of disease activity. Osteopontin is

a pleotropic, pro-inflammatory cytokine secreted by activated immune cells and is expressed abundantly in MS lesions [38]. Osteopontin levels are elevated in plasma from patients with MS compared with controls, and have been shown to correlate with DMA treatment [39, 40]. Subsequent studies, however, have shown that circulating osteopontin levels are not specific enough to differentiate MS disease activity from that of other concurrent disorders; thus, osteopontin is not likely to be useful as a biomarker in a clinical setting [41, 42]. CSF osteopontin levels were also elevated in all subtypes of MS and correlated significantly with clinical severity [36, 43–45], despite a lack of specificity for MS compared with other inflammatory neurological diseases [46]. As a biomarker of therapeutic response, CSF osteopontin levels were reduced in patients with RRMS or SPMS treated with natalizumab [20, 34, 37]. These studies suggested that CSF osteopontin may be used along with other CSF biomarkers, such as fetuin-A and CXCL13, to assess therapeutic effects on intrathecal inflammation. Additional studies analyzing biomarker panels in CSF are warranted, given their potential for use in a clinical trial setting to assess the therapeutic efficacy of natalizumab and other DMAs.

Interestingly, HGF, which has been shown to prevent autoimmunity and contribute to CNS repair, has been inversely correlated with disease activity [35]. CSF HGF may reflect endogenous repair processes that take effect in response to immunomodulation, suggesting its potential utility as a surrogate biomarker for neuroprotection and repair. Although these biomarkers await independent validation in a larger number of patients with a clinically confirmed drug response, they point the way to development of a CSF biomarker panel that would greatly assist in therapeutic decision-making with regard to the efficacy of DMAs. This is especially relevant in patients with progressive MS, where DMA effects on disease activity and intrathecal inflammation are more difficult to assess by MRI [34].

### 2.3 Biomarkers of Oxidative Stress

Normal brain activity is associated with high oxygen consumption relative to that of other tissues; thus, it is highly vulnerable to a buildup of reactive oxygen species. Because of the high lipid content of myelin, increased free radical production as a consequence of oxidative stress can also lead to lipid peroxidation [47]. In MS, inflammation, demyelination, and neurodegeneration can increase the level of metabolic and oxidative stress, which in turn likely contribute to disease progression [3]. Biomarkers indicative of oxidative stress pathway activity would help quantify the impact of oxidative stress on disease progression in MS.

The isoprostane 8-iso-prostaglandin  $F_{2\alpha}$  (8-iso-PGF $_{2\alpha}$ ) is a well-recognized readout of oxidative stress and lipid peroxidation, which is generated by free radical-catalyzed peroxidation of arachidonic acid in membrane phospholipids. Increased levels of 8-iso-PGF $_{2\alpha}$  have been detected in urine and plasma from patients with MS [48, 49]. More recently, CSF levels of 8-iso-PGF $_{2\alpha}$  were tested in 241 patients with MS and were found to be significantly elevated in both RRMS and progressive MS [50]. Interestingly, distinct subsets of patients had normal 8-iso-PGF $_{2\alpha}$  levels (<20 pg/ml), moderately elevated levels (20–80 pg/ml), and highly elevated levels (>100 pg/ml). Highly elevated CSF 8-iso-PGF $_{2\alpha}$  levels were observed in 31 % of patients with SPMS, identifying a subset of patients with progressive MS that exhibited quantifiable evidence of oxidative stress [50]. These findings suggest that oxidative stress may not be a universal phenomenon in all forms of MS but may be a particular manifestation of inflammatory neurodegeneration. With the advent of medications that target oxidative pathways, such as dimethyl fumarate, measurement of CSF isoprostanes may help define a group of patients that would be most likely to benefit from this class of therapeutics. Furthermore, measuring CSF 8-iso-PGF $_{2\alpha}$  levels may help identify other DMAs that may indirectly affect oxidative stress by decreasing inflammation. Although these preliminary observations on CSF 8-iso-PGF $_{2\alpha}$  levels are of interest, a number of additional studies need to be performed to define the specificity and sensitivity of isoprostane measurement. In addition, mechanistic and pathological studies would be needed to validate the use of isoprostanes as a biomarker of oxidative stress in MS.

### 2.4 Biomarkers of B-Cell Involvement in the Central Nervous System

CXCL13 is a potent B-cell chemoattractant, which is emerging as a promising CSF biomarker that is indicative of the humoral immune response in the CNS. Numerous studies have now described elevated levels of CXCL13 in CSF from patients with MS, as well as in CSF from patients with other neuroinflammatory diseases [31, 51–55]. Elevated CSF CXCL13 has been observed in early MS (clinically isolated syndrome [CIS]), where it was associated with an increased risk of conversion to clinically definite MS [52, 56]. CSF CXCL13 was also higher during RRMS, where it correlated with indicators of more severe disease course, such as the relapse rate, HLA genotype, and immunoglobulin (Ig)-G index [52, 55, 57], further supporting the use of CXCL13 as a prognostic biomarker in MS. In addition, the use of CXCL13 as an indicator of intrathecal B-cell responses in MS is supported by its correlation with the number of B cells/plasmablasts, the

IgG index, and the presence and number of oligoclonal bands in the CSF [51–55, 58]. The reproducibility of these data suggests that CXCL13 may indeed be a robust and sensitive indicator of the degree of the humoral response in the CNS.

The correlation between CXCL13 levels and B-cell involvement in the CNS suggests that CXCL13 may have clinical utility in measurement of the therapeutic efficacy of B-cell–targeting therapies. B cells play an important role in MS, which is highlighted by the polyspecific intrathecal B-cell response observed as oligoclonal bands in the CSF and is considered a diagnostic biomarker of MS. More recently, the discovery that clinical progression (along with more severe cortical pathology in the brains of subjects with MS) is associated with ectopic meningeal B-cell follicles further underscores the role of B cells in the disease pathogenesis [59, 60]. Not surprisingly, CXCL13 has been shown to be abundantly expressed within ectopic lymphoid tissue [60]. Rituximab, a B-cell–depleting anti-CD20 antibody therapy (which is currently used off label for treatment of MS [61–63]) has been reported to have some efficacy in disease progression. Importantly, CXCL13, along with chemokine (C-C motif) ligand 19 (CCL19), was shown to be significantly reduced in CSF after rituximab treatment, correlating with reduced B-cell and T-cell numbers [64]. Although baseline CXCL13 or CCL19 levels in a small subset of rituximab-treated patients did not predict therapeutic response [65], the use of CXCL13 and/or CCL19 as a biomarker for use in rituximab therapy remains promising for selection of patients who might benefit from the treatment, and for determination of therapeutic response longitudinally. In terms of other MS therapies, CXCL13 has been reported to be reduced in patients after treatment with natalizumab or methylprednisolone, suggesting that it may have broader utility as a biomarker of therapeutic response [31].

## 2.5 Biomarkers of Axonal Damage

Neurofilaments are major components of the axonal cytoskeleton, which exist as heteropolymers of low (NFL), medium (NFM), and high (NFH) molecular weight protein subunits. As a result of axonal injury, neurofilament proteins are released into the extracellular space [66]; thus, their levels in CSF are thought to reflect the degree of axonal damage in neurodegenerative disease. In patients with MS, CSF levels of both NFH and NFL have been shown to be elevated and were highest during relapses, reflecting acute axonal damage mediated by inflammatory mechanisms in the CNS [67, 68]. In patients with CIS, CSF NFL levels correlated with inflammatory outcomes, such as gadolinium-enhancing lesions, and were predictive of conversion to clinically definite MS and more severe long-term disability outcomes [68, 69]. CSF NFL levels, in

particular, may reflect the level of acute axonal injury in early MS, and may thus have some prognostic value in determining disease outcomes, although these findings require validation in a larger population.

In studies of progressive MS, CSF NFH levels remained elevated and correlated with physical disability and changes in brain volume over 1 year, but they did not correlate with the T2 lesion load, suggesting that NFH levels might be an indicator of ongoing neurodegeneration [67, 70, 71]. Importantly, CSF NFH levels also correlated strongly with age, possibly reflecting underlying age-related neurodegeneration [67]. Nevertheless, in age-corrected samples, the dissociation of NFH levels with many (though not all) inflammatory outcomes that were tested [67] provides encouraging evidence that testing of CSF NFH levels, along with CSF NFL levels, may help quantify the accumulation of axonal damage in patients with MS.

As a potential indicator of axonal loss in MS, studies are now looking at NFL and NFH levels in CSF after administration of DMAs. Natalizumab-treated patients exhibited a 3-fold reduction in CSF NFL levels after 6–12 months, suggesting that effective immunomodulatory therapies are associated with reduced axonal damage [33]. Similarly, patients with progressive MS treated with rituximab, natalizumab, or mitoxantrone also showed a significant reduction in CSF NFL levels after treatment [32, 34]. Unfortunately, these studies were unable to show data correlating CSF NFL levels with the treatment effect. In addition, because rituximab, natalizumab, and mitoxantrone all target immune mechanisms, the observed reduction in NFL levels is presumably only secondary to immunomodulation; thus, treatment effects on axonal damage are only correlative. As new treatments targeting neuroprotection and remyelination come through the pipeline in the next decade, CSF neurofilament levels will likely be a critical readout for therapeutic effects.

Prior to widespread adoption of CSF NFL or NFH testing, issues remain regarding the availability and reproducibility of the tests themselves. Studies have suggested that neurofilament protein instability and potential for aggregate formation may result in test result variability and possible misinterpretation of data [9, 72, 73], although a more recent analysis concluded that neurofilament proteins in CSF are indeed stable [74]. In contrast to NFL, there are no commercially available immunoassays for NFH, requiring significant laboratory setup for its use in the clinical setting. The multicenter effort to validate CSF processing protocols, as well as the NFL immunoassay (UmanDiagnostics NF-light<sup>®</sup>), serves as a template for adoption of other biomarkers like NFH, which will inevitably be used as outcomes in future clinical trials.

Finally, antibodies to NFH and NFL have also been detected in CSF and have been shown in some cases to be

indicative of neurodegeneration in response to a humoral response to axonal proteins [75]. Levels of CSF anti-NFL antibodies detected by antigen microarray are reduced in response to steroid treatment [76]. However, in previous studies, anti-neurofilament autoantibodies have not consistently correlated with specific clinical variables [77–80], perhaps because of the variety of antigens used for neurofilament autoantibody detection.

## 2.6 Prognostic Biomarkers

Chitinase 3-like 1 (CHI3L1) is a chitin-binding protein, which lacks enzymatic activity and is known to play a role in chronic inflammation and tissue injury [81]. Multiple studies have identified elevated CSF CHI3L1 levels in patients with MS as the result of an unbiased proteomic screen of CSF samples [82–84]. In a study of patients with CIS, elevated CSF CHI3L1 levels were associated with a risk of conversion to clinically definite MS [82]. This study suggested that CSF CHI3L1 may have potential use as a prognostic biomarker in MS, although elevated CSF CHI3L1 levels were not specific to MS [82]. More recently, CHI3L1 and chitinase 3-like 2 (CHI3L2) were identified as potential diagnostic biomarkers, since the levels of both were elevated in the CSF of patients with RRMS, compared with controls, and were confirmed as correlating with more rapid conversion from CIS [84]. In patients with MS and optic neuritis, CSF CHI3L1 levels correlated with dissemination in space on MRI, suggesting that CHI3L1 may hold prognostic value for disability progression in MS after relapse [85]. The expression of CHI3L1 in reactive astrocytes in MS and EAE lesions suggests that CSF CHI3L1 levels may be a reflection of astrogliosis [84, 86]. Interestingly, proteomic profiling of CSF before and after natalizumab treatment for 1 year showed that CSF CHI3L1 levels were significantly reduced, suggesting the potential for use of CHI3L1 as a biomarker of therapeutic response [83]. On the basis of these promising early studies, further studies will be needed to determine the prognostic value of CSF CHI3L1 levels, requiring further validation in longitudinal samples from a larger cohort of patients with MS.

## 3 Emerging Biomarker Categories

### 3.1 Transcriptomic Signatures

Gene expression profiling—or “transcriptomics”—of peripheral blood has been used extensively to identify biomarkers for diagnosis, disease activity, and progression of MS [87–89]. To date, conclusions from these studies have been limited by lack of reproducibility and small

sample sizes. The approach is also limited because of heterogeneity of gene expression changes in peripheral blood that is unrelated to MS disease status. More recently, researchers have focused their gene expression profiling on specific immune cell subsets in MS with the hope of increasing the signal to noise ratio [90].

Assessment of the molecular signatures associated with therapeutic responses in MS—specifically, the response to IFN $\beta$ —has been somewhat more productive. In many patients taking IFN $\beta$  therapies, there is no correlation between NAbs and lack of therapeutic response. Thus, one of the biggest challenges in using IFN $\beta$  as a first-line therapy for MS continues to be accurate prediction and assessment of therapeutic response. The search for a biomarker of IFN $\beta$  response has proven even more difficult, since the precise mechanism of action in MS remains unclear. On the basis of the clinical need to predict IFN $\beta$  response in individual patients, numerous studies have attempted to define specific molecular signatures in peripheral blood that differentiate responders from non-responders. Large-scale gene expression profiling has revealed that clinical non-responders exhibit altered expression of IFN-response genes, both at baseline and after IFN $\beta$  treatment [91–93]. These studies have revealed differences in genes related to IFN $\beta$  signaling, such as signal transducer and activator of transcription 1, 91kDa (*STAT1*), as well as genes related to Toll-like receptor 4 signaling in monocytes [94, 95]. In addition, lack of IFN $\beta$  response was found to correlate with more aggressive T helper-17-mediated disease and elevated serum IL17F levels [96]. Unfortunately, the ability of serum IL17F levels to predict IFN $\beta$  response could not be validated in a larger independent cohort of IFN $\beta$ -treated patients with MS [97]. The discrepancy in these studies highlights some of the challenges faced in biomarker validation, including lack of standardized clinical definitions for poor treatment response in individual patients, as well as the complexities surrounding the therapeutic mechanisms of IFN $\beta$ , despite the fact that this was the first treatment to show efficacy in MS.

Recently, more advanced technologies have been applied to the quest for predictive biomarkers of IFN $\beta$  response. Next-generation sequencing (RNA-seq) was recently used to interrogate whole-blood transcriptomes of untreated and IFN $\beta$ -treated patients with MS [98]. A single marker was validated—ribosomal protein S6—which was reduced in IFN $\beta$  responders. Another study profiled microRNA (miRNA) expression changes in response to IFN $\beta$  and found specific downregulation of the miR-29 family of miRNAs [99]. Though encouraging, these studies were plagued by the same challenges in clinical biomarker validation as mentioned above. Until more is understood regarding the mechanism by which IFN $\beta$  benefits certain

patients with MS, the search for a predictive biomarker of therapeutic response may remain a fishing expedition.

### 3.2 Circulating MicroRNAs

MiRNAs are short, single-stranded, non-coding RNA molecules, which modulate gene expression and protein synthesis. They regulate approximately 30 % of genes; thus, they play an important role in many physiological and pathological processes, including those related to autoimmunity and neurodegeneration [100]. Several recent studies have examined the involvement of circulating miRNAs in MS, aiming to uncover important pathological pathways related to MS and to identify potential biomarkers. Initially, a number of miRNA species were found to be differentially expressed in patients with MS compared with controls, and to have the potential for use as diagnostic biomarkers or biomarkers of disease progression. MiRNAs have been profiled from peripheral blood mononuclear cells [101, 102], serum [103], plasma [104, 105], whole blood [106], and CSF [107] from patients with MS, with a variety of results, reflecting the different sampling materials that were used. As miRNA screening evolves, additional studies will need to be carried out with more homogenous patient populations and standardized technologies in order to reproducibly identify specific miRNAs as MS biomarkers.

Recent studies have used miRNA profiling to better understand treatment effects, with the hope of identifying biomarkers of therapeutic response. As mentioned in the previous section, the reduction of miR-29 miRNA was associated with IFN $\beta$  response [99]. In another study, a B-cell miRNA signature was determined from patients with RRMS who were either untreated or treated with natalizumab [101]. Although some differentially expressed miRNAs were found in natalizumab-treated patients (all of whom were responders), the relevance of this finding is unclear, since natalizumab does not target B cells specifically. Using a slightly different approach, another study analyzed pre-selected miRNAs that were previously identified as playing a role in the immune response. In patients with RRMS treated with glatiramer acetate, two miRNAs appeared to be reduced to control levels when compared with those of untreated or IFN $\beta$ -treated patients [108]. Although this study will require validation in glatiramer acetate-treated patients followed longitudinally, it does suggest that therapeutic response may be reflected by restoration of dysregulated miRNAs.

### 3.3 Exosomes/Microvesicles

One of the biggest challenges in identification of biomarkers for pathological mechanisms in the CNS during

MS is the inaccessibility of the diseased tissue. Cerebral biopsies are extremely rare, and imaging techniques are not sensitive enough to detect pathological processes at the cellular level. Many secreted biomolecules, such as cytokines, are readily detected in bodily fluids, including CSF, and can serve as important biomarkers of inflammatory status. However, secreted biomolecules originating from non-circulating CNS cell types, such as neurons, oligodendrocytes, astrocytes, and microglia, are often present in very low concentrations and thus are difficult to detect. In recent years, better understanding of cell-to-cell signaling through secreted microvesicles has suggested that microvesicles may be an important source of biomarkers in many different diseases [109]. Secreted microvesicles, including smaller vesicles referred to as exosomes, are loaded with a cargo of proteins, RNAs, and miRNAs, which are transported to recipient cells, resulting in altered gene expression and protein content. Microvesicles/exosomes have a variety of biological functions, including an active role in intercellular communication in the immune system, where they carry antigens or MHC-peptide complexes, and induce antigen-specific immune responses [110]. In the CNS, microvesicles are thought to play a role in synaptic plasticity, axonal/glial communication, and antigen transfer [111]. Research investigating the role of microvesicles/exosomes in MS is ongoing and aims to identify microvesicle-associated RNA and proteins that reflect ongoing demyelination and neurodegeneration.

Microvesicles are most commonly isolated by ultracentrifugation and are present in most bodily fluids, including CSF. To date, few studies have examined microvesicle populations in CSF during MS disease onset and progression. Over 20 years ago, microvesicles detected in MS CSF were found to originate from injured oligodendrocytes, where they play a possible role in myelin destruction [112]. More recently, CSF microvesicles positive for the myeloid cell marker IB4 were associated with neuroinflammation both in patients with MS and in patients with neuromyelitis optica or other inflammatory diseases [113]. In EAE, myeloid-derived microvesicles have been shown to be capable of promoting neuroinflammation, suggesting that microvesicle shedding may play a pathogenic role, at least in EAE [113]. Interestingly, fingolimod treatment reduced myeloid-derived microvesicles in the CSF of mice with EAE, suggesting that myeloid-derived microvesicles may be a therapeutic biomarker and possibly a therapeutic target of this agent [113].

Despite the exciting potential of exosomes in various clinical applications and as a source of biomarkers, many challenges remain. There is a lack of consistent criteria by which to characterize exosomes, which can vary on the basis of the method of purification, the source material, and the method of biomarker detection [114]. As the field



evolves, the ability to normalize the size and yield of purified microvesicles will be critical for biomarker discovery and validation in diseases such as MS.

### 3.4 Antigen Arrays

The adaptive autoimmune response in MS results in clonal expansion of B cells in the CNS, producing the characteristic oligoclonal immunoglobulin bands that are present in the CSF of patients with MS. Better understanding of the autoantibody repertoire in MS would not only help identify disease-causing antigens and potential therapeutic targets, but would also aid in the discovery of new biomarkers (or biomarker signatures) of disease. The development of microarray-based technologies has allowed for high-throughput analysis of autoantibody reactivity [115]. Analysis of autoantibody signatures in the serum of patients with MS has allowed for discrimination between different stages and pathological processes in MS [116]. More recently, antigen microarrays have been used to identify antibody signatures in CSF [76], allowing for a more specific approach to analyze the intrathecal immune responses that drive disease progression. Patients with RRMS had a CSF-specific antibody response directed against various CNS antigens [76, 117], which was reduced after treatment with methylprednisolone [76]. In addition, use of lipid-based and carbohydrate-based antigen arrays is uncovering additional autoantibody signatures in MS CSF [118–120]. Surprisingly, the CSF antibody signature showed significant heterogeneity between patients, which may be due in part to detection of non-pathogenic autoantibodies as a result of arrays composed of recombinant proteins. Nevertheless, CSF antibody signatures have the potential to be used as biomarkers for diagnostic accuracy, to monitor disease progression, and to aid in decision-making regarding therapy.

## 4 Clinical Use of Biomarkers in the Management of Multiple Sclerosis

The past two decades have seen the emergence of a number of therapeutic options for patients with MS. With rising expectations, complete cessation of disease activity is becoming a desirable goal. Clinical application of reliable biomarkers will likely make this feasible, as it will be possible to assess the effectiveness of a treatment modality objectively even in the absence of clinical deterioration. At present, the clinical use of NAb to IFN $\beta$  as a biomarker is established. In addition, it is likely that with the current validation studies that are ongoing, CSF analysis of fetuin-A and other markers, such as osteopontin, will be used routinely in MS clinical centers. This will be particularly

helpful in determining therapeutic responses in patients with progressive disease, in whom disease activity correlates less well with current clinical measures, in comparison with patients with relapsing disease.

As the reliability of biomarkers becomes validated, their use in clinical trials will greatly reduce the costs and duration of phase III studies. Current trials frequently rely on surrogate markers, such as MRI changes and relapse rates, which require several hundred patients and a minimum study duration of 2–3 years. Use of biomarkers of disease activity that could show significant changes after 6 months of treatment would lead to shorter, less expensive drug trials in RRMS. Furthermore, biomarkers that could accurately reflect disease progression would overcome the real difficulty in assessing outcomes in drug trials in SPMS and PPMS.

## 5 Conclusions

A lack of understanding of the cause of MS, as well as disease heterogeneity, make it unlikely that one single biomarker will satisfy the needs for disease monitoring in MS. The identification of individual biomarker patterns is rapidly evolving into more complex biomarker panels or signatures. In this regard, significant progress has been made since 2009 with respect to biomarker changes in response to therapy [7]. The challenge of biomarker development continues to be the lack of sensitivity and reproducibility. Furthermore, these studies rely on a large number of patients in an environment where research and clinical practice are closely integrated. Despite these limitations, continued progress in biomarker research has led to early-stage clinical application of biomarkers in MS. Optimal treatment of individual patients with MS will ultimately require validated biomarker panels that are capable of predicting and monitoring the efficacy of the growing number of available therapeutic options.

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