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REVIEW

Blood Biomarkers as Outcome Measures in Inflammatory Neurologic Diseases

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Abstract Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system. Only a few biomarkers are available in MS clinical practice, such as cerebrospinal fluid oligoclonal bands and immunoglobulin index, serum anti-aquaporin 4 antibodies, and serum anti-John Cunningham virus antibodies. Thus, there is a significant unmet need for biomarkers to assess prognosis, response to therapy, or potential treatment complications. Here we describe emerging biomarkers that are in development, focusing on those from peripheral blood. There are several limitations in the process of discovery and validation of a good biomarker, such as the pathophysiological complexity of MS and the technical difficulties in globally standardizing methods for sampling, processing, and conserving biological specimens. In spite of these limitations, ongoing international collaborations allow the exploration of many interesting molecules and markers to validate diagnostic, prognostic, and therapeutic-response biomarkers.

Keywords Multiple sclerosis · Autoimmune demyelination · Biomarkers · Disease progression · Therapeutic response

Introduction

Inflammatory neurologic diseases encompass infectious, postinfectious, autoimmune, and vasculitic diseases. Even neurodegenerative neurologic diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis have an inflammatory component. For the purpose of this review, we will focus on multiple sclerosis (MS), an autoimmune demyelinating disorder of the central nervous system (CNS). Clinically, the MS course may be relapsing-remitting (RRMS) or progressive and is called secondary progressive (SPMS) when it follows a RRMS onset, or primary progressive MS (PPMS) when it is progressive from onset [1]. The earliest clinical stage of the disease is detectable after a single episode of neurological dysfunction [clinically isolated syndrome (CIS)]. MS is considered a predominantly T-cell-mediated autoimmune disorder, although B cells have an important contribution in disease pathogenesis [2]. Myelin-specific $CD4^+$ T-helper (Th) cells type 1 and Th17 cells, as well as $CD8^+$ T cells enter the CNS where they encounter their cognate ligand and initiate an immune response leading to recruitment of other cells B cells, macrophages, and natural killer cells [3–5], resulting in tissue damage and neurologic dysfunction [6]. Alterations in apoptosis of autoreactive immune cells have also been described, leading to persistence of these proinflammatory cells [7, 8]. Pathologically, inflammation, demyelination, and axonal loss are observed [9–11], and although MS is classically described as a white matter disease, the gray matter is significantly involved [12–14]. Biomarkers may be used to support the diagnosis, and identify potential converters from CIS to MS so treatment could be initiated early. As more effective but potentially more toxic therapies become available we need biomarkers that predict disease severity and response to treatment so we can select the appropriate therapy. We also need biomarkers for predicting risk of adverse events.

There are some biomarkers already in clinical use for MS; cerebrospinal fluid (CSF) oligoclonal bands and immunoglobulin production have been in use for many years to support the diagnosis and may help predict conversion of CIS to MS [15]. Levels of anti-aquaporin 4 antibodies in the serum are used to differentiate MS from neuromyelitis optica (NMO) [16], while

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serum anti-John Cunningham virus (JCV) antibodies are useful in risk stratification during natalizumab therapy [17].

The pathophysiology of MS involves 3 principal compartments: 1) the peripheral blood, where immune processes are initiated in the relapsing-remitting phase; 2) the blood–brain barrier (BBB), which becomes overpermeable to autoreactive immune cells entering the CNS; 3) the CNS, where acute lesions indicate sites of inflammation and neural damage, leading to the manifestation of symptoms and disability. These compartments can be evaluated via blood tests, CSF studies, CNS imaging, and sampling of tissue through biopsies. Peripheral blood sampling and neuroimaging techniques are the most feasible and innocuous methods for repeated testing in MS, but the latter lack pathological specificity and are relatively expensive.

In this review we will focus on emerging blood biomarkers that have shown reproducible results and may be closer to clinical implementation (Table 1). We included emerging blood biomarkers validated in 2 independent cohorts of patients, or recent data on known biomarkers proposed to answer different clinical questions. For biomarkers to become clinically applicable they have to go through discovery, verification, clinical validation, then multicenter validation before being available for implementation. Proximity to clinical application was assessed based on the completion phase of this process. Given the significant heterogeneity in the research on blood biomarkers, and the fact that many validation studies investigated simultaneously blood and CSF concentrations of the same biomarkers, some data CSF biomarker data are considered as worth mentioning.

Description of an "Ideal" Biomarker

A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes, or pharmacological responses to a therapeutic intervention" [18, 19]. An ideal biomarker should be precise, reliable, and differ between healthy controls and patients with MS. It should be easily studied in a body fluid that is practical to obtain and measurable with affordable laboratory procedures. It should be involved in the disease pathogenesis, and correlate with clinical disease activity and disability progression. Ideally, it should have a high sensitivity in detecting relevant disease activity, conserving a high specificity as well. It is even better if it correlates with radiologic disease activity markers, such as magnetic resonance imaging (MRI) findings. In the case of a biomarker of inflammation, it should undergo rapid normalization under therapy in responders but no normalization under therapy in nonresponders.

Many of these characteristics of an ideal biomarker depend on the relationship between the pathogenesis of MS and the biological sample tested. The majority of MS lesions are usually located in the periventricular white matter of the brain and superficial regions of the spinal cord, being anatomically close to the CSF space [20]. Because brain biopsies are typically not feasible in these cases, CSF is used for measurements of soluble markers and cell populations by flow cytometry analysis, polymerase chain reaction studies, and cell functional analyses. However, CSF collection is relatively invasive, being usually collected during the diagnostic phase of MS [19].

Biomarkers measurable in the peripheral blood through noninvasive methods are of significant clinical importance in MS. The main drawback is that much of the disease pathology occurs in the CNS, which is separated from the periphery by the BBB. Therefore, events associated with CNS lesions may not be easily detectable in peripheral blood. Furthermore, there might be significant variations in many of the soluble markers, owing to circadian fluctuations, systemic infections, degradation in the liver, or excretion through the kidney. However, blood biomarkers provide information about immune triggers in MS and some of the effects of disease-modifying drugs [19–21]. Notably, the majority of protein content of the CSF is blood-derived, while the rest is derived from the brain or produced intrathecally [20].

Biomarkers to Support the Diagnosis of MS and MS Subtypes

The diagnosis of RRMS requires evidence of dissemination in time and space, as well as absence of any other explanation of the clinical findings (usually called MS mimickers). In this respect there have been significant advances in the diagnostic criteria merging clinical and radiologic features to diagnose confidently RRMS [1, 22]. However, progressive forms of MS could be difficult to diagnose early on, and having a good biomarker to support the diagnosis would be very useful.

Epidermal and Hepatocyte Growth Factors, CCL4, and CCL11

Tejera et al. [23] analyzed a set of 30 different plasma cytokines, chemokines, and growth factors in blood of 129 patients with MS with different clinical forms (RRMS, SPMS, and PPMS) and 53 healthy controls, across 2 independent cohorts, using Luminex xMAP technology. They showed that different MS forms are associated with distinct profiles of circulating plasma protein biomarkers, with distinct signatures being composed of chemokines and growth/angiogenic factors, and proposed the evaluation of a set of 4 circulating biomarkers (hepatocyte growth factor, eotaxin/CCL11, epidermal growth factor, and macrophage inflammatory protein-1β/CCL4) as a tool in the diagnosis and more

personalized therapeutic targeting of patients with MS [23].

Noncoding microRNAs

In another recent study 3 microRNAs (miRNAs) were differentially expressed in the serum of patients with RRMS compared with patients with PPMS, and were validated in 2 other independent small cohorts within the study, and were useful to differentiate RRMS from PPMS [24]. Notably miR-223 has been implicated in the regulation of CNS inflammasomes [25], and miR-15b has been described as a promoter for neurogenesis [26].

Other noncoding miRNAs in peripheral blood, such as miR-20a-5p [27, 28] and miR-22-5p [28, 29], which are involved in T-cell regulation, could be promising novel biomarkers to identify CIS converters and support early MS diagnosis. However, further replication in larger cohorts is still needed.

Anti-myelin Oligodendrocyte Antibodies

Anti-myelin oligodendrocyte (MOG) antibodies have been studied as an early predictor of the subsequent course of demyelination in children, showing that its presence is suggestive of acute disseminated encephalomyelitis rather than MS in the pediatric population (using a serum dilution of 1:160 as a cut-off for positivity) [30]. However, the usefulness of anti-MOG antibodies in the adult population is not as clear. In a study of adults with NMO and suspected limited forms of MS (optic neuritis and myelitis), MOG antibody titers through full-length MOG cell-based assay could not help in differentiating between the different clinical phenotypes, including monophasic and relapsing diseases. Furthermore, low MOG antibody titers were not always associated with a monophasic course or better outcome, and persistence of antibodies for several years was reported in patients with clinical symptom resurgence [31]. Moreover, the presence of MOG IgG in the serum of patients with NMO has been described, with inconsistent data regarding the coexistence of aquaporin 4 IgG and MOG IgG in these patients. As MOG IgG has been consistently absent in MS, it still can be a promising biomarker to help in differentiating between MS and aquaporin 4 IgG-negative NMO, which is a common clinical scenario [32–34].

Antigen Array Signatures

Quintana et al. [35] studied IgG antigen arrays signatures based on low-affinity autoantibody patterns. Informative patterns emerged from autoantibodies that bound peptides of myelin molecules and heat shock proteins, proteins and lipids that were detectable at 1:10, but not at higher dilutions (low affinity). They showed that SPMS samples have an immune reactivity closer to that observed in patients with PPMS. Later they also showed a significantly higher number of antibodies in pediatric MS compared with other neurologic disorders, attributing this to the phenomenon of epitope spreading [36]. In the same study they evaluated the performance of IgG antigen array reactivity classifiers in discriminating a monophasic acute demyelinating episode from MS at the time of an acute demyelinating attack, with an area under the curve of 0.872 [36]. The use of antigen arrays has not yet been replicated and has not shown usefulness in early diagnosis.

Neurofilaments and Other Neurodegenerative Biomarkers

Neurodegenerative biomarkers usually consist of neuron-specific proteins released following axonal damage. Several studies have shown elevated CSF levels of neurofilaments in MS [37–39]. In CIS, serum and CSF levels of neurofilament light subunit (NfL) were not associated with fast conversion to clinically definite MS (CDMS), but were significantly higher in CIS compared with healthy controls [40]. The axonal Tau protein was found to be upregulated in patients with RRMS and those with PPMS [41–46]. Furthermore, CSF levels of Tau tended to be highest in the early stage of the disease [43, 47]. However, these markers are not specific to MS as they are upregulated in many other degenerative, ischemic, and infectious CNS disorders.

Anti-KIR4.1 Channel Antibodies

Some authors have reported a high prevalence of antibodies directed against the glial inwardly rectifying potassium channel KIR4.1 (anti-KIR4.1) in serum of adults with MS and almost 50 % of children with MS [48, 49]. However, 2 other studies using a comparable enzyme-linked immunosorbent assay technique could not reproduce these findings [50, 51]. Levels of anti-KIR4.1 antibodies could not differentiate between MS and NMO, although higher levels of these antibodies were found in patients with MS during relapses [52]. Thus, more studies are needed to validate anti-KIR4.1 antibody levels as a biomarker in MS.

Serum 24-Hydroxycholesterol

There is recent interest in 24-hydroxycholesterol as a biomarker of MS, as modestly decreased levels of this lipid in serum have been reported in MS—more so in patients with PPMS and older patients with RRMS than in healthy controls [53–55], but further replication studies are needed.

Table 1 Established and emerging blood biomarkers for different intended uses in multiple sclerosis (MS)

Intended clinical use	MS diagnosis	Conversion from CIS to definite MS	Distinction between NMO and MS	Progression from RRMS to SPMS	Predictor of disease activity	Predictors of therapeutic efficacy or AEs
Established	IgG OCBs [15]	IgG OCBs [15]	Anti-aquaporin 4 Abs [16]			Anti-IFN Abs [109–113];
	CSF IgG [15]					Anti-natalizumab Abs [121, 122]
						Anti-JCV Abs [132–134]
Emerging	Epidermal and hepatocyte growth factors, CCL4, and CCL11 [23]	Anti-MOG Abs [56]	Anti-MOG Abs [31–34]	Fas and MIF [66]	Fas, FasL [79–86]	miR-26a-5p [108–110]
	Anti-MOG Abs [30, 57]	Anti-MBP Abs [56]		Anti-Nf Abs [69–71]	sPECAM-1 [88]	TRAIL [128, 129]
	miR-223 [25]	CHI3L1 [58, 59]		NfL [37, 68]	sP-selectin and sE-selectin [88]	L-selectin [135, 137]
	miR-15b [26]	CHI3L2 [59]		NfH [73–76]	NfL [40]	
	NfL [40]	CXCL13 [61–65]		CHI3L1 [58, 72, 78]	MMP-9 [89, 90]	
	Tau [41–46]	25-OH vitamin D [15, 66]		CHI3L2 [59, 78]	NO [91–93]	
	Anti-Kir4.1 Abs [48, 49]				Neurotrophins [93–95]	
	24-OHC [53–55]				miR-92a-1 and miR-454 [99, 100]	

CIS = clinically isolated syndrome; NMO = neuromyelitis optica; RRMS = relapsing-remitting MS; SPMS = secondary progressive MS; AE = adverse effect; OCB = oligoclonal band; Abs = antibodies; IFN = interferon; CSF = cerebrospinal fluid; JCV = John Cunningham virus; MOG = myelin oligodendrocyte; MIF = macrophage migration inhibitory factor; FasL = Fas ligand; miR = microRNA; MBP = myelin basic protein; Nf = neurofilament; sPECAM-1 = soluble platelet endothelial cell adhesion molecule 1; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand; CHI3L1 = chitinase 3-like-1; Nfl = neurofilament light chain; CHI3L2 = chitinase 3-like-2; Nfh = neurofilament heavy chain; CXCL = chemokine (C-X-C motif) ligand 13; MMP = matrix metalloproteinase; 25-OH = 25-hydroxy; NO = nitric oxide; 24-OHC = 24-hydroxycholesterol

Biomarkers to Predict the Conversion from CIS to Definite MS

Serum Anti-MOG and Antimyelin Basic Protein Antibodies

There is general agreement that early initiation of treatment after CIS could delay conversion to definite MS and mitigate future disability, so biomarkers that allow identification of patients at the highest and lowest risk of further attacks and disability are needed to personalize the treatment plan. In an initial study, Berger et al. [56] showed that the presence of serum IgM anti-MOG and anti-myelin basic protein antibodies could predict the risk of conversion from CIS to clinically definite MS. However, further investigations revealed that anti-MOG antibodies were only detectable in cases of acute disseminated encephalomyelitis [30, 57].

Chitinase 3-Like-1 and Chitinase 3-Like-2 Proteins

Chitinase 3-like-1 (CHI3L1) is a member of the family of chitinases and chitinase-like proteins containing a highly conserved glyco-18 domain as common feature. For these proteins, chitin is the only documented substrate. CHI3L1 can bind chitin but lacks chitinolytic activity. In the CNS, CHI3L1 expression has been mainly observed in astrocytes of monkeys and humans with lentiviral encephalitis, and patients with brain infarcts. Increased circulating levels of CHI3L1 have been reported in a wide variety of heterogeneous conditions characterized by chronic inflammation such as rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematous, asthma, and sarcoidosis; nonetheless, its mechanism of action remains poorly understood, beyond a suggestion that CHI3L1 may be a tissue remodeling factor. In patients with CIS, increased CSF CHI3L1 levels was a risk factor for conversion to definite MS, independent of strong predictors of conversion to MS such as brain MRI abnormalities and the presence of IgG oligoclonal bands [58]. Furthermore, patients with CIS with high CSF CHI3L1 had a shorter time to definite MS [58]. Interestingly, patients with CIS with higher serum levels of CHI3L1 and chitinase 3-like-1 (CHI3L2) have a higher conversion rate to definite MS, and converted much faster as well [59].

Chemokines and Their Receptors: C-X-C Motif Ligand 13

Chemokines and their receptors play an important role in the recruitment of autoreactive immune cells from the periphery to the CNS and are detectable in MS plaques [60]. Among these molecules, chemokine (C-X-C motif) ligand (CXCL)13 was found to be upregulated in patients with active MS. Furthermore, elevated levels of CXCL13 also predict CIS conversion to clinically definite MS [61–65].

25-OH Vitamin D

Among several studies that suggested a relationship between vitamin D deficiency and risk of MS, a group studying 100 patients with CIS showed that those with very low (below the tenth percentile) and low (below the twenty-fifth percentile) 25-hydroxy (OH) vitamin D levels in serum were at higher risk of conversion to definite MS after a median follow-up of 7.7 years [66].

A large multicenter study of > 1000 CIS cases, with a median follow up of 4.3 years, showed that lower 25-OH vitamin D levels in serum were associated with conversion to definite MS in a univariate analysis, but this association was mitigated at the multivariate level when controlling for the presence of oligoclonal bands in the CSF, number of T2 lesions on MRI, and age [15].

Biomarkers of Disease Activity

Serum Apoptotic Molecules and Cytokines

In a study to identify biomarkers of disease activity and progression in MS, Hagman et al. [67] analyzed the serum profiles of cytokines, chemokines, and apoptotic molecules in CIS, RRMS, PPMS, SPMS, and healthy controls (72 patients with MS, 17 with CIS, and 21 healthy controls). They correlated their levels with clinical and MRI findings acquired over a 1-year follow-up. They found increased levels of the apoptotic serum Fas (sFas) molecule in patients with MS with worsening Expanded Disability Status Scale (EDSS) score and accumulation of hypointense lesions on MRI. In these patients, the levels of macrophage migration-inhibitory factor were higher than in clinically stable patients. The authors suggested that sFas and migration-inhibitory factor can be candidate biomarkers of disability progression due to neurodegeneration. They also found that increased levels of serum tumor necrosis factor (TNF)-α and CCL2 seemed to reflect MS-related inflammatory responses, especially in PPMS, but stated that their role as biomarkers of clinical disease activity needs to be evaluated in a long-term study involving a larger cohort [67].

Neurofilaments and Anti-neurofilament Antibodies

Neurofilaments are important axonal cytoskeletal proteins where the 68-kDa NfL forms the core of the neurofilament, while the 190- to 210-kDa heavy neurofilaments (NfH) are located more peripherally. NF in the serum and CSF were suggested to correlate with damage to axons and disease activity in MS [37, 68].

Anti-neurofilament antibodies have also been suggested to be markers of tissue damage. Anti-neurofilament antibodies

are detected both in serum and CSF of patients with MS and they are shown to correlate with brain parenchymal fraction, T2 and T2 lesion load [69, 70]. In another study, serum anti-neurofilament antibodies were significantly elevated in PPMS [71].

Lately, CSF NfL levels showed some prognostic value in MS, as the levels at diagnosis correlated with MS severity score, and patients with NfL levels above the median had 5-fold increased odds of severe MS, including conversion to SPMS at 8 to 20 years after disease onset [72]. In another cohort of patients with CIS, NfH levels in the CSF correlated with physical disability and brain volume loss over 1 year [73]. In another cohort of patients with RRMS followed up for a median of 14 years, CSF NfL levels at diagnosis correlated with the MS Severity Score in the long term, while cases with high NfL levels (>386 ng/l) were more likely to convert into SPMS than those with low levels (<60 ng/l) [74]. In a cohort of patients with progressive MS, NfH was a predictor of continuing disability, and NfL was a predictor of EDSS annual increase [75]. There is currently a new promising sensitive immunoassay for quantification of NfL in serum [76]. Serum NfL levels were reported to predict recovery after an episode of myelitis in RRMS [72, 77]. Moreover, patients with CIS had higher levels of serum NfL than controls, which was also associated with the number of T2-hyperintense and gadolinium-enhancing lesions, as well as with increased disability [40].

Although the data on neurofilament proteins as biomarkers show promise, their prognostic value in individual patients needs a more extensive validation through prospective cohort studies.

CHI3L1 and CHI3L2 Proteins

In a prospective study CSF CHI3L1 level was a strong predictor of disability progression and, in fact, it was the only significant independent risk factor associated with the development of disability in multivariate Cox regression models [58]. CSF CHI3L1 levels above the 170 ng/ml cut-off were conferring, as a unique predictor, a 4-fold increased risk for the development of disability. High CSF CHI3L1 levels were associated with earlier disability progression (5-year difference in median time to reach EDSS 3.0 *vs* patients with low-protein values) with a sensitivity > 70 % [58]. In another study, CSF CHI3L1 levels were associated with brain MRI abnormalities at baseline and disability progression during follow-up [78]. Moreover, other authors reported that higher serum and CSF CHI3L1 and CHI3L2 were found in progressive MS than in RRMS and CIS [59].

Proapoptotic Molecules: Fas and Fas Ligand

Several studies demonstrated downregulation of proapoptotic molecules in active MS, indicating abnormalities in the apoptotic cell death of lymphocytes in MS [79–81]. Increased mRNA expression of Fas and Fas ligand has been regularly reported in peripheral blood mononuclear cells in RRMS, but the data on the sFas have been inconsistent [82–86].

Adhesion Molecules: Soluble Platelet Endothelial Cell Adhesion Molecule 1, sP-Selectin, and sE-Selectin

Migration of immune cells into the CNS is mediated by adhesion molecules, normally expressed at very low levels on vascular endothelial cells but increase after cytokine stimulation in MS [87]. There are soluble forms of adhesion molecules as well, released from endothelial cells, immune cells, and platelets. The soluble adhesion molecules soluble platelet endothelial cell adhesion molecule 1, sP-selectin, and sE-selectin have been shown to be upregulated in patients with RRMS when compared with PPMS. The levels of these molecules were also found to be upregulated during MS exacerbations, suggesting their potential as biomarkers for disease activity [88]. Larger longitudinal studies are needed to confirm the clinical usefulness of these markers.

Metalloproteases and Nitric Oxide

Matrix metalloproteinases have been shown to contribute to the inflammatory injury to the BBB and CNS myelin. An elevated level of matrix metalloproteinase 9 was associated with active gadolinium-enhancing lesions on MRI in patients with MS [89, 90]. Similarly, free radicals such as nitric oxide (NO) that contribute to the neurodegenerative cascade in the CNS through oligodendrocyte injury, axonal degeneration, and impairment of nerve conduction were increased in some acute demyelinating lesions [11]. Elevated levels of NO metabolites nitrite and nitrate are associated with disease activity [91, 92]. NO synthase was also increased in patients with MS [93].

Neurotrohpins

The expression of some neurotrophins that can stimulate regeneration and promote repair has been described in MS lesions [94]. Low levels of brain-derived neurotrophic factor have been reported in blood and CSF [95, 96]. The levels of brain-derived neurotrophic factor, neural cell adhesion molecule, and ciliary neurotrophic factor in the CSF of patients with MS have been associated with disease activity [96, 97].

NfL

CSF levels of NfL are elevated in all stages of MS, without initial marked differences between RRMS and progressive MS. However, in RRMS the concentration of NfL is CSF and serum is consistently higher in patients who are in clinical exacerbation or who have gadolinium-enhancing lesions on MRI, while in progressive MS, NfL levels are elevated, irrespective of MRI activity, but this information still needs to be replicated in larger cohorts [40].

RNA Profiles: "MSa and MSb" Gene Signatures

In a transcriptomics study, an RNA profile (from peripheral mononuclear cells) identified 2 subsets of patients with MS differing in disease activity. An increased expression of genes involved in the "T-cell receptor" and "B-cell receptor" signaling pathways was found in a subset of patients called "MSa" as compared with their counterpart, the "MSb". The MSa signature included genes found in the nuclear factor of activated T cells, integrin-linked kinase, phosphatidylinositol 3-kinase, and epidermal growth factor. The MSa and MSb gene signatures were associated with disease outcome, with the MSb patients being 40 % less likely to have a relapse. None of the available clinical and paraclinical data was different between the 2 MS subsets after correcting for testing of multiple hypotheses, except for a difference in disease duration at the time of sampling in patients treated with glatiramer acetate [98].

Noncoding miRNAs

Several noncoding RNAs in serum were also explored as biomarkers of disease activity in MS. A recent study reported that elevated levels of miR-92a-1 and miR-454 correlated with increasing disease severity disability [99, 100].

Cholesterol

Some researchers reported in patients with MS a relationship between disease progression and elevated serum levels of triglycerides, low-density lipoprotein, and total cholesterol, while high-density lipoprotein levels correlated with lower lesion volume load on MRI [101]. Furthermore, others showed a positive correlation between serum LDL levels and the number of active white matter lesions in patients with CIS [102]. However, the normal biological variability of the lipid profile in serum makes it difficult to validate these molecules as biomarkers in MS [103].

Tryptophan Metabolism and the Kynurenine Pathway

Alterations in tryptophan metabolism through the kynurenine pathway have been reported in patients with MS and other neurologic disorders [104, 105]. Some researchers examined the transcription of the tryptophan-depleting enzyme indoleamine 2,3 dioxygenase (IDO) in sera of stable patients with MS, patients during an acute MS relapse before and after treatment with corticosteroids, and healthy controls. IDO expression was increased during a relapse (before treatment with corticosteroids) compared with stable patients with MS. After treatment with glucocorticoids, clinical improvement occurred along with a significant reduction in IDO gene expression and IDO catalytic activity [106].

25-OH Vitamin D

Among other studies, an analysis of > 450 patients with CIS followed up to 5 years in 1 of the Betaferon/Betaseron trials showed that higher serum 25-OH vitamin D predicted less disease activity. They suggested that a 20 ng/ml increment in the mean serum vitamin D levels within the first year predicted a 57 % lower rate of new active lesions, 57 % lower relapse rate, 25 % lower increase in yearly T2 lesion volume, and 0.41 % lower loss in yearly brain volume [107]. Another group explored the data of 65 patients of the phase II CIS trial of atorvastatin (STAyCIS) and reported that each 25 nmol/l higher 25-OH vitamin D level was associated with 7.8 ml higher gray matter volume (95 % confidence interval 1.0–14.6), suggesting a potential impact on neurodegeneration and disability progression [108].

Biomarkers of Therapeutic Response

Anti-Interferon Neutralizing Antibodies

It has been established in clinical practice that anti-interferon (IFN)-β antibodies herald a lack of therapeutic effect of interferons in RRMS, although this biomarker identifies only a group of nonresponders to MS treatments [109]. Interestingly several human leukocyte antigen class II alleles and short nucleotide polymorphisms have been associated with anti-IFN-β neutralizing antibody titers [110, 111], although these data still need to be validated for use at the individual level. A recent prospective European MS cohort suggested that an early increase in binding antibody titers could reliably predict the development of anti-IFN-β neutralizing antibodies; moreover, the authors reported CXCL10 as a promising predictor of neutralizing antibody-associated IFN-β response attenuation [112]. Furthermore, some patients develop such neutralizing antibodies only transiently, making the judgment of their future response to IFN debatable.

However, in a post hoc retrospective analysis of the BENEFIT trial it was shown that in early MS, early high levels of anti-IFN-β1b neutralizing antibodies predicted a high rate of persistence of the antibodies later on, suggesting an early differentiation of persistently neutralizing antibody-positive patients from transiently positive patients [113].

Concentrations of Interleukin-17 in Serum

There has been mounting evidence for involvement of interleukin (IL)-17 in the pathogenesis of MS [114, 115]. High concentrations of IL-17F before initiation of therapy were reported to be associated with lack of response to IFN-β [116]. However, in a better-powered study, levels of IL-17F measured at baseline and at 6 months after initiation of treatment did not correlate with clinical or radiologic failure on treatment after 2 years [117]. Only extremely high levels of IL-17F (>200 pg/ml), which were found in few patients (4.4 %), were associated with nonresponsiveness to IFN-β treatment.

Noncoding miRNAs

Levels of miRNAs in the blood also might be potential biomarkers of response to IFN-β [118, 119]. A prospective study showed that changes in miR-26a-5p concentrations in serum could serve as biomarkers of the effects of IFN-β therapy, and could have a good predictive value in identifying responders at the individual patient level [120].

Anti-natalizumab Antibodies

Several groups reported the clinical relevance of persisting antibodies against natalizumab [121, 122]. These antibodies are formed early during treatment, persist in around 6 % of patients, and are related to a decrease in treatment efficacy and adverse reactions to natalizumab. However, any new disease activity would generally be obvious clinically and radiologically in this group of patients, as relapses and new active or enhancing lesions rarely occur during such treatment in daily practice; therefore, there might be a limited role for anti-natalizumab antibodies as a biomarker in clinical practice [123].

Serum TNF-Related Apoptosis-Inducing Ligand

TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily expressed in soluble and membrane bound forms from lymphocytes and monocytes in an activation-dependent manner [124]. It has been shown that soluble TRAIL inhibits proliferation of activated T cells [125, 126], and inhibition of TRAIL outside the CNS worsened experimental autoimmune encephalomyelitis [127]. Increased expression of TRAIL mRNA has been reported in peripheral blood mononuclear cellss of patients with RRMS treated with IFN-β who responded to treatment. Based on these observations it has been suggested that TRAIL could be used as a biomarker reflective of response to treatment with IFN-β in MS [128, 129].

B-Cell Activating Factor

As a member of the TNF family, B-cell activating factor (BAFF) is a major survival factor for B cells, which, classically, has been postulated to be involved in the development of many autoimmune and inflammatory conditions [130]. One prospective study of 170 patients with RRMS and 49 healthy controls, with a mean follow-up of 2.3 years, reported that plasma BAFF levels were significantly higher in stable patients with MS compared with controls. Nevertheless, stable patients with MS had significantly higher serum BAFF levels than patients with recent preceding relapses. Interestingly, treatment with IFN-β but not glatiramer acetate raised BAFF levels, and treatment of the relapses with high-dose intravenous steroids did not significantly change plasma BAFF levels in 65 % of patients [130]. Notably, a previous study in 73 patients with RRMS, 8 patients with melanoma treated with IFN-α, and 26 healthy controls, showed that IFN-β significantly increases serum BAFF levels [131].

Biomarkers to Predict the Risk of Adverse Effects of Disease-Modifying Therapy

Anti-JCV Antibodies in Serum

During treatment with natalizumab, the presence of anti-JCV antibodies in blood is associated with the development of progressive multifocal leukoencephalopathy (PML) [132, 133]. JCV antibody serum index enables stratification of the risk of PML in JCV-seropositive patients [134], as is currently used in clinical practice. In patients without immunosuppressant use prior to natalizumab, a low anti-JCV antibody index confers a low risk of PML. Increasing titers of anti-JCV antibodies, exposure to a previous immunosuppressant, and prolonged use of natalizumab increase dramatically the risk of PML, especially after 2 years of treatment. Therefore, anti-JCV antibodies in serum currently have an established role as a biomarker for risk stratification in patients treated with natalizumab.

L-Selectin-Expressing $CD4^+$ T Cells in Peripheral Blood

Aiming to advance the ability to predict PML risk during treatment with natalizumab, the results of a study using flow cytometry in peripheral mononuclear blood cells suggested that the frequency of L-selectin-expressing $CD4^+$ T cells was

lower in patients who had received long-term treatment with natalizumab than in those patients not exposed to natalizumab, or healthy controls [135]. Furthermore, among a subgroup of patients who developed PML, a significant 9-fold decrease in the number of L-selectin-expressing $CD4^+$ T cells was found in their blood samples taken before development of PML. Through a validation study in an independent cohort of patients, the same group reported that a low frequency of L-selectin-expressing $CD4^+$ T cells in patients treated with natalizumab increased a patient's relative risk of PML 55-fold [136]. However, recent data from a cohort of patients treated with natalizumab indicated that L-selectin is not a useful biomarker of PML risk [137]. Future research would have to clarify the usefulness of such a biomarker to predict the risk of PML in patients treated with natalizumab.

Discussion

MS biomarker research is an area of intense interest, as is evident by the number of publications on the subject. In this review we focused specifically on peripheral blood as an easily accessible tissue that is amenable to repeat sampling. A large number of interesting molecules and markers are emerging that have not been discussed in this review as we focused on the few biomarkers that have been replicated in more than 1 study.

Clinical implementation is clearly the goal of biomarker research, but several steps have to occur between discovery and implementation: namely, the finding must be verified, replicated, and then clinically validated. Technical aspects related to the methods of collection, measurement, and quality controls have to be instituted. Ideally, the techniques should be easily standardizable and affordable. Recently, there have been efforts to standardize the tools necessary for biomarker research and creating guidelines for biobanking [138–140] and quality controls [International Society for Biological and Environmental Repositories, http://www.isber.org]. The creation of these guidelines and multicenter collaborations will pave the way for more rapid implementation of biomarkers for MS and other inflammatory neurologic diseases.

Important limitations for the development of biomarkers include the critical need for quantitative, standardized outcome measures for MS disease activity and progression, and validated definitions of treatment response. Using only clinical evidence of disease activity is clearly insensitive as we know from MRI studies that inflammation can be present in the absence of clinical signs. Future studies may need to include MRI to distinguish groups with active *versus* stable disease. Multicenter collaborative efforts and the use of well-characterized cohorts that include banked longitudinal blood and other bio-fluid samples would go a long way towards advancing the field.

Advances in understanding the pathophysiologic mechanisms of MS are helping to identify novel candidate biomarkers. However, technological advances (e.g., proteomics, molecular profiling, immunophenotyping, and microarray gene and antigen analysis) allow simultaneous testing of multiple biomarkers. Implementation of international exchange of biological samples for the purpose of cross-validation and the use bioinformatics may lead to the developments of biomarkers panels that could be clinically useful.

Conclusion

MS is a complex disease where the primary area of pathology is not easily accessible, thus requiring investigators to sample surrogate material such as the CSF or the blood. But, in spite of these limitations, some biomarkers are emerging that may have value for diagnosis, predicting disease progression, and therapeutic response. Validation and replication studies are still needed, but ongoing collaborations will help move the field forward.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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