Chapter

Biomarkers in Multiple Sclerosis

Valentina Ignatova

Abstract

Clinical, biological, and radiological evidence are currently needed to diagnose MS, but lack of preclinical biomarkers hinders the earliest possible diagnosis and treatment. Conventional biomarkers target immunity, blood-brain barrier disruption, demyelination, and neuronal and axonal damage, as well as mitochondrial activity. An increase of specific brain metabolites with 30–40% is registered before detection of MRI lesions in MS. Potential lipid biomarkers are fatty acids, phospholipids, and oxysterols. The role of proteoforms in the pathogenesis of MS was confirmed. Serum neurofilament light chains (sNfL) are currently being studied as a readily available biomarker for prognosis and response to treatment in MS. The sNfL levels reflect ongoing neuroaxonal damage caused by inflammation, and the sNfL levels predict disease activity over the next few years. The retinal nerve fiber layer (RNFL) thinning is reliable as a biomarker of disability worsening. The neutrophil-to-lymphocyte ratio and CRP are also MS biomarkers. The development of rationally targeted therapeutic agents that allow preventive treatment to stop the disease is also delayed without definite biomarkers.

Keywords: biomarkers, multiple sclerosis, diagnostic, progression, monitoring of immunomodulatory therapy, disease activity

1. Introduction

MS is a chronic disease with autoimmune genesis and social significance, which affects the young persons and manifests clinically with unpredictable relapses and subsequent remissions and/or debilitating progression over time [1]. About 2.5 million people worldwide suffer from MS and women are at least 2-3 times more likely to get the illness than men. Other factors identified in the distribution of the disease include genetics, environment, and ethnic origin [2].

Pathophysiologically, a chronic inflammatory reaction occurs in the CNS, leading to multifocal demyelination of axons in white and gray matter. Axon damage also occurs, leading to neuronal loss and atrophy of the brain and spinal cord [2]. Histopathological studies show that reactive astrocytes in freshly developed plaques release chemokines, which activate microglia and increase the permeability of the blood-brain barrier. This in turn allows the migration of macrophages and T lymphocytes into the brain parenchyma [3]. Therefore, astroglial activation may be an important trigger for the cascade of the immune system, leading to neuronal damage, inflammatory demyelination, and axonal degeneration. On the other hand, damaged astrocytes in chronic lesions are involved in the formation of gliotic scars; therefore,

astroglia may also be involved in the neurodegeneration process along with axonal damage. In fact, neurodegeneration is the main reason for the accumulation of disability and clinical progression of the disease [4].

Diagnosis of MS is often difficult and is currently based on the 2017 revision of the McDonald's criteria, which include clinical neurological examination, the presence of oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF), magnetic resonance imaging (MRI), and the most accurate possible exclusion of diseases related to the differential diagnosis [5]. The main concept in the diagnosis of MS is the coexistence of clinical and imaging indicators showing both spatial distribution (DIS; involvement of different CNS sites) and temporal distribution (DIT; showing chronic disease, e.g., 2 relapses) [6]. Assessment of cerebral atrophy may also be important if it is measured routinely [7].

The disease is categorized into three main phenotypes: relapsing-remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS). Disability and severity of MS are assessed according to the Extended Disability Status Scale (EDSS) [2]. MS shows great heterogeneity in terms of radiological and histopathological findings, clinical course, and progression, as well as in terms of therapeutic response [7, 8]. Therefore, it is very important to identify reliable biomarkers as specific characteristics of the disease that facilitate diagnosis and prognosis and to allow assessment of therapeutic response and risk of side effects [6, 7]. Unfortunately, there is currently no biomarker available to meet the criteria for a surrogate endpoint in MS. It is also clear that biomarkers will play a very important role in MS research and clinical practice in the future.

The purpose of the present work is to analyze the role of the potential biomarkers identified as a result of current research.

2. Definition and nature of biomarkers

The Biomarkers Definitions Working Group gave the following international definition of biomarkers: a characteristic that can be measured objectively and could differentiate the physiological biological phenomena from pathological processes, as well as evaluate the pharmacological reactions to the administered drugs [9]. Biomarker type 0 is considered as a marker for the natural history of the disease and corresponds longitudinally with the known clinical indicators. Biomarker type I perceives the effects of therapeutic procedure including its action mechanism. The surrogate endpoint is a biomarker that is expected as a substitute for a clinically relevant endpoint and serves as a predictor of the therapeutic effect. The clinical endpoint is a clinically relevant measure of how a patient feels, functions, or survives. Evaluation criteria for defining clinical utility of biomarkers include sensitivity/specificity, reliability, evaluation of biomarkers in epidemiological studies or cohorts with natural disease history, evaluation of biomarkers in evidence from clinical trials, evaluation of biomarkers in large multicenter therapeutic studies, evaluation of biomarkers in meta-analyzes, and mathematical modeling of the relationship between a biomarker and a clinical endpoint [10].

The NIH and the FDA have jointly developed a definition for biomarkers, which to be followed both by researchers in their work on obtaining relevant evidence and by practicing specialists to apply biomarkers in healthcare. Different organizations such as Clinical Trials Transformation Initiative and Foundation for National Institutes of Health The Biomarkers Consortium must follow the expansion of this activity. As a result of their joint efforts common definitions have been formulated, which have

gained publicity through the constant updating of the online document "Biomarkers, Endpoints, and other Tools" (BEST) [11].

The ideal biomarker should be a binary value or in other words a characteristic that is detected in persons with a specific disease and is not identified in healthy individuals or in subjects with different diseases or vice versa. If the illness progresses or improves, the biomarker's concentration should be increased or decreased, respectively [7, 12]. Establishing the ideal biomarker should be safe for the subject and it should be easily identified, and recommendatory in noninvasive way. Sensitivity and specificity are other key criteria for biomarkers. Sensitivity describes the proportion of truly positive test results among those who are actually affected by the disease. Specificity, on the other hand, shows the proportion of true negative outcomes among those who are not ill. Since high sensitivity is usually due to a lower specificity and opposite, it is important to find biomarkers that reach a satisfactory balance between the two characteristics. Other significant criteria for the biomarkers are their positive and negative predicted value. They show the proportion of correctly/incorrectly diagnosed patients depending on the positive or negative test result. Last but not least is the transfer of biomarkers from research into clinical practice [13].

3. Requirements for MS biomarkers

The classification of MS-specific biomarkers should be based on a careful assessment of all contributing pathophysiological processes. Based on an analysis of published studies investigating the pathophysiological mechanisms of MS Bielekova and Martin classify the majority of proposed biomarkers in MS in one of the following categories: [10].

I. Immunologic biomarkers:

   1. Cytokines and cytokine receptors

   2. Chemokines and chemokine receptors

   3. Antibodies

   4. Biomarkers, related to complement system

   5. Adhesion molecules

   6. Biomarkers reflecting the processing and presentation of antigens

   7. Other activation biomarkers

   8. Biomarkers associated with cell cycle and apoptosis

II. Markers reflecting immune-associated neuroprotection:

   1. Changes in cellular subpopulations

   2. Functional tests for immunological reactivity

   3. Biomarkers for the state of blood-brain barrier (BBB)

III. Biomarkers for demyelinating lesions

IV. Biomarkers for oxidative stress and excitotoxicity

V. Biomarkers for axonal/neuronal damage

VI. Biomarkers for gliosis

VII. Biomarkers for remyelination and repair

For neurological diseases such as MS, CSF, given its proximity to the CNS, would be the preferred body fluid to look for candidate biomarkers rather than plasma or serum. However, it is clear that CSF sampling is a more invasive procedure with potential risks than plasma sampling. However, the availability of leakage or release of products from different tissues or blood cells in the plasma may correspond with pathological and physiological condition of the specific tissues. Since that plasma is easy to be received in noninvasive way, it can be proposed as a useful fluid for deriving promising diagnostic biomarkers [14].

According to the functional classification provided by the FDA-NIH Biomarker Working Group, molecular biomarkers for MS can be categorized by sensitivity, diagnosis, monitoring, prognosis, safety, and response biomarkers [15] (**Table** 1).

Biomarker	Status	Function	Evidence
IGG OCB	Clinically useful	Diagnostic	Nearly 80% specificity and more than 90% sensitivity for the MS diagnosis. Implemented in 2017 McDonald diagnostic criteria for MS
		Prognostic for conversion	Associated with higher risk for conversion in MS when detected CIS and RIS
IGG index	Clinically useful	Diagnostic	Positive values found in 70–80% of MS patients. Useful as complementary tool, without replacing CSF OCB
		Disease activity	Associated with MRI activity
		Prognostic for conversion	Associated with higher risk of conversion in MS when detected in CIS
		Prognostic for progression	Associated with disease progression
KFLC	Validated	Diagnostic	Useful for MS diagnosis; increased levels detected in MS patients without IGG OCB
		Prognostic for conversion	Associated with higher risk of conversion in MS when detected in CIS
		Prognostic for progression	Associated with disability progression
IGM OCB	Validated	Disease activity	Associated with aggressive disease course
		Prognostic for conversion	Lipid-specific IGM OCB is associated with higher risk of conversion in CIS
		Prognostic for progression	Associated with disability progression and conversion to SPMS
		Treatment response	Lipid-specific IGM OCB predicts a decreased response to IFN beta

Biomarker	Status	Function	Evidence
N-CAM	Validated	Diagnostic	Lower levels detected in MS patients in PPMS compared to RRMS. Indicator of poor remyelination and repair
		Disease activity	Increased levels detected after relapses, especially under steroid treatment, and related to clinical remission
CHI3L1	Validated	Diagnostic	Increased levels in MS and NMO
		Prognostic for conversion	Associated with higher risk of conversion in MS from CIS
		Disease activity	Increased levels in higher clinical and MRI activity
		Treatment response	Increased levels in non-responder patients on INF beta treatment compared to responders
NFs	Validated	Prognostic for conversion	In RIS increased NF-L is independent risk for conversion to CIS and MS, with greater values related to shorter time of conversion
		Disease activity	Double NF-L levels in relapsing patients compared with remitting ones. CSF NF-L levels correlate with NEDA-3, MRI activity, and brain atrophy. Serum NF-L in early phase contributed to predicting the lesion load and brain volume loss over a period of 10 years
		Prognosis for progression	High NF-L is associated with progression in both clinically stable patients and relapsing ones. In CIS patients without ON, CSF NF-L predicts long-term cognitive and physical disability over a period of 9-19 years; higher NF-H

Table 1.
Up-to-date clinically useful and validated MS biomarkers from CSF.

4. Types of biomarkers according to molecular characteristic

4.1 Neurofilaments

Neurofilaments are neuron-specific intermediate filaments formed from etheropolymers of protein subunits with low (neurofilament light [NF-L]) (68 kDa), medium neurofilament medium) (160 kDa) and high (neurofilament heavy [NF-H]) (205 kDa) molecular weight [13]. They are the main components of the cytoskeleton of neurons. Their relative stability and abundance in CNS tissue make them ideal candidates for biomarkers [16]. Levels of neurofilament in biological fluids, particularly CSF, are thought to reflect the degree of axonal damage based on their release into the extracellular space during axonal damage. Neurofilament (NFL) levels are elevated during all stages of MS, especially in relapsing-remitting MS and in progressive MS, while NFL levels decrease to normal during intervention with disease-modifying therapies, suggesting that NFL is associated with various pathological processes involved in MS, reflecting disease activity, disease progression, and treatment efficacy [16]. Interestingly, NF-L and NF-H levels do not always correlate directly with each other, perhaps due to differences in protein stability and sensitivity of the assay. It is thought that NF-L is associated rather with the initial inflammatory stage of MS, as it detects early acute, inflammatory-mediated axonal damage, and correlates weaker with disability progression. On the other hand, NF-H is considered as a marker of neurodegeneration since it highly corresponds with the axonal damage in the course of disease progression [13]. Elevated sNfL levels are prognostic short- and

long-term markers, including recurrence, progression of disability, development of MRI lesions, and loss of brain volume [17].

4.2 Chitinase 3-like proteins

Chitinases represent secreted glycoproteins, united in a family, which bind and hydrolyze chitin. Chitinase I (CHIT1) or chitotriosidase, Chitinase 3-like-1 (CHqI3L1), and Chitinase 3-like-2 CHI3L2) are proteins, homologous to chitinases, which bind with chitin, but do not have the capacity to hydrolyze it. In brain tissue in MS, CHI3L1 (also known as YKL-40) and CHI3L2 are expressed in astrocytes in white matter plaques and in normal-looking white matter, and CHI3L1 is also expressed in microglia in MS lesions. Validation in larger cohorts will be required before they can be used as part of the general clinical practice of MS [13].

4.3 Biomarkers of innate immunity

Due to expansion of understanding of the involvement of microglia and macrophages in MS, CNS biomarkers for innate immune activation are needed to be established for evaluation of the course of the disease and efficacy of the immunomodulating therapies. The detection of soluble cell surface biomarkers in CSF could determine the immune phenotype of intrathecal inflammation in MS. Biomarkers derived from the myeloid line such as soluble CD163 (sCD163) and sCD14 are secreted by monocytes and are elevated only in CSF of MS patients. sCD1 correlates weakly with the absolute number of monocytes in CSF, suggesting that the sCD14/monocyte ratio could be used as a marker for activation of microglia. Several studies suggest that sCD163 may be a biomarker of macrophage activity because of its good correlation with monocyte count in CSF of MS persons. Quantification of intrathecal sCD production revealed an increased CSF/serum ratio of sCD163 in persons with RRMS and PPMS, in parallel with other biomarkers of inflammation and neurodegeneration, including elevated NF-L in CSF. The trigger receptor expressed on myeloid cells 2 (TREM-2) is found at high levels in CNS microglia, where it may play a role in weakening the immune response. Soluble TREM-2 increased in CSF in MS patients and decreased after natalizumab treatment [13]. Immunoglobulin (Ig) M and IgG antibodies revealed as OCBs in CSF are considered to reflect the antigen-driven pathophysiology in MS, albeit the certain antigens are still unclear. Intrathecal OCBs, in particular IgG, are a hallmark of MS and are the most commonly applied diagnostic biomarkers in MS, although it is not specific to the disease [13].

Azzolini et al. found a significant positive correlation between IL-9 and TREM-2 CSF levels. In EAE and MS IL-9 is associated with anti-inflammatory action and neuroprotection. IL-9 reduces the activation of macrophages and microglia, inhibits the release of pro-inflammatory molecules, and promotes the anti-inflammatory phenotype [18]. The correlation between GFAP and sTREM-2 and the levels of different inflammatory cytokines is consistent with the cross-link between CSF inflammation and the activation of microglia and astroglia in MS [19].

4.4 Circulating microRNA (miRNAs)

MicroRNA (miRNAs) are a class of small noncoding RNAs consisting of 17–25 nucleotides, whose main role is gene regulation by mediating mRNA degradation, as well as by regulating transcription and translation. miRNAs form up to 1% of

the human genome [20]. Circulating miRNAs, usually packaged in microvesicles or exosomes, are relatively stable. They are found in most biofluids, such as CSF, serum, plasma, and whole blood and peripheral blood mononuclear cells (PBMCs). miRNAs are detected through multiple methods such as quantitative PCR, miRNA array analysis, small noncoding RNA cloning, or next-generation sequencing. Dysregulation of miRNAs may play an important role in the underlying mechanisms of MS and potentially serve as a reference for measuring disease progression [13].

4.5 Proteoma

Based on analysis of protein spots of interest seven differentially expressed proteins in CSF samples from RRMS-patients compared to subjects with other inflammatory diseases of the CNS were identified, as determined by 2D-PAGE, respectively Alpha-1-antichymotrypsin, prostaglandin D synthase (PGDS), retinol-binding protein-4 (Rbp4), transthyretin (TTR), apolipoprotein E (ApoE), and gelsolin and angiotensinogen [21]. The most striking change in the CSF proteome in RRMS is the oligomerization of TTR in high molecular weight species (conformers) in about 70% of the analyzed samples. Proteomic studies have shown a decrease in alpha-1-antichymotrypsin in the CSF of patients with RRMS compared with samples collected from patients with other inflammatory diseases of the CNS. This is supported by the results obtained in the validation of studies using ELISA in both sexes [14, 21].

4.6 Metabolomic

Metabolomics is a promising technique that studies small molecules (<1500 Da) in various biological matrices, including cells, biofluids such as serum, plasma, cerebrospinal fluid (CSF), urine, feces, tissues, and exhaled gases. Metabolomics has gained notoriety in recent years for its usefulness in identifying potential biomarkers of MS and providing insight into the pathogenesis of the disease. A growing number of studies show that metabolomics is a promising tool for the diagnosis and prognosis of MS [22].

4.7 Kappa free light chains (KFLC) in CSF

Kappa free light chains (KFLC) in the cerebrospinal fluid (CSF) are promising biomarkers for multiple sclerosis (MS), especially the kappa (K) index.

Martins et al. determine KFLC in CSF and serum samples of patients with MS, clinically/radiologically isolated syndrome (N = 39), and controls (N = 152; inflammatory and noninflammatory neurological diseases). The researchers found higher KFLC parameters in the MS group and the K index performed best among them (AUC 0.92). At a limit of 7.25, it showed better sensitivity (85% vs. 77%) but less specificity (88% vs. 91%) than OCBs. The effectiveness of the IgG index was lower (AUC 0.83). A K index threshold of 2.55 (97% sensitivity) would reduce OCB testing by 52% in the study population. The proposed threshold of 7.25 may help diagnose MS and identify some false-negative cases from OCB studies [23].

4.8 CNS endothelial-derived extracellular vesicles (EEVs)

Mazzucco et al. conducted the first study in which CNS EEVs or EVs derived from BBB were identified in human circulation. The authors develop a new method for identifying EVs derived from CNS endothelial cells by detecting multiple cell-specific

markers on EVs isolated from the patient's plasma by flow cytometry. Using this method, the researchers identified three different populations of CNS-EEV including CNS-EEV31, CNS-EEV105, and CNS-EEV144. The scientists found that CNS-EEV concentrations were higher in patients with RRMS with active disease than in HC, stable in patients with RRMS who did not receive disease-modifying therapies (DMT), stable in patients with RRMS who were not receiving natalizumab, and stable in patients with RRMS receiving ocrelizumab [24].

5. Types of biomarkers according to clinical characteristics of MS

5.1 Diagnostic biomarkers for MS

Biomarkers that are suitable for the diagnosis of MS should be able to distinguish MS patients from healthy people or from those with other diseases [7].

A 30–40% increase in specific metabolites (e.g. choline) was detected by proton magnetic resonance spectroscopy in the brain prior to MRI detection of lesions in normal-looking white matter [25]. Decreases in N-acetylaspartate have been found in the brain areas of MS patients and correlated with impairment in which conventional MRI images failed to show a correlation [26, 27]. The results of Ferreira et al. show for the first time that serum phospholipid in MS is significantly different from that of healthy controls and that it may be suitable as biomarkers for clinical applications for MS [27, 28].

CRP is a nonspecific reagent in the acute phase, as it is influenced by several factors, such as infections, inflammation, smoking, and body mass index. DMTs generating lymphopenia can cause higher NLR [29].

Momtazmanesh et al. found significantly higher levels of NFL in the CSF of patients with CIS compared to healthy persons. GFAP levels are remarkably higher in the CSF of MS patients compared to controls. In general, CSF t-tau levels are higher in MS patients with moderate significance. Both CHI3L1 and S100B levels are significantly higher in the CSF of MS patients compared to controls [30].

OCBs were introduced in 1983 as a diagnostic criterion for MS and thus represent the first biomarker of this disease [31, 32]. Since OCBs, meanwhile, have not been used for diagnostics according to McDonald's 2010 criteria, they are again part of the diagnostic algorithm in the 2017 update [33]. CSF IgG OCB is found in almost 90% of patients with MS and in nearly 70% of patients with CIS [34]. Of all the possible models, type 2 is detected when at least two IgG bands are present in the CSF but not in the serum, suggesting intrathecal IgG synthesis and thus inflammatory CNS disease [35].

Immunoglobulin (Ig) G index indicates the ratio of IgG in CSF/serum compared to CSF/serum reference protein albumin. Albumin ratio, i.e., the albumin in CSF/the albumin in serum, is a measure of impaired blood-CSF barrier function in MS. The IgG index is applied as a marker for intrathecal synthesis of immunoglobulins. An IgG index >0.7 is an indicator of an increased intrathecal B-cell response and thus indicates the presence of MS [36]. About 70% of MS patients have an elevated IgG index.

Several studies have reported an increased concentration of free light chains in the CSF of patients with MS [37]. The KFLC index corresponds positively with the IgG index, which is a measure of intrathecal synthesis [38], using a cut-off value of 5. KFLS shows greater sensitivity (more than 96% vs. almost 50% for IgG index) for the detection of OCB (IgG) in CSF and diagnosis of MS and in regard to the negative prognosis it has comparable specificity. According to consensus report from 1994 on the role of CSF in MS diagnosis, the intrathecal Ig-synthesis against viruses, such as

measles, rubella, and varicella zoster, is used as a complementary diagnostic exam in MS [39]. Such kind of local humoral response, known as measles-rubella-varicella-zoster (MRZ) response (MRZR), is registered in about 94% of persons with MS in case of at least one intrathecal virus-specific response is found, and the anti-measles response is the most common [40].

5.2 Biomarkers for MS-progression

5.2.1 Biomarkers for conversion from CIS to MS

Neuronal and glial biomarkers may be useful in determining the risk of conversion to MS in patients with CIS or RIS [31]. In patients with RIS, elevated CSF levels NF-L > 619 ng/L have been shown to be an independent risk factor for conversion to CIS and MS [41]. CHI3L1 levels in CSF correlate with the time of conversion from CIS to MS. However, the correlation did not remain significant for patients when followed for more than 5 years [42]. Other studies with a follow-up period of less than 3 years found that CHI3L1 levels in CSF were not a predictor of conversion in patients with CIS. No correlation was found between the baseline levels of the other markers (t-tau, GFAP, and S100B) and the conversion time from CIS to MS [42].

The results from actual research show that detection of CSF OCB in children with RIS is associated with increased risk of developing pediatric MS and also improves the specificity of MRI criteria in this population [43]. Another study on 75 RIS patients confirmed that CSF OCB was an independent risk factor for conversion from RIS to CIS and to MS, which happened for a shorter time [44]. In patients with CIS, the identification of CSF lipid-specific IgM OCB is associated with an increased MRI lesion load and brain atrophy at the first clinical event with an aggressive course of the disease. The load on periventricular lesions in the first years of the disease is also associated with the formation of intrathecal IgM synthesis in patients with CIS, so it is assumed that IgM plays an active role in the development of demyelinating lesions [45]. In another study by Ferraro et al., the identification of CSF IgM OCB in patients with CIS predicted another recurrence within 1 year [46]. The results of a blinded multicenter study involving 52 neurological patients and 13 centers confirmed the reproducibility of the test [47]. OCBs in CIS patients also predict a more aggressive course of the disease and correlate with brain atrophy, lesion load, and elevated CSF levels of CXCL13, a chemokine that directs B cell migration [13].

In a study by Comabella and colleagues, CSF CHI3L1 levels were further correlated with shorter latency conversion times and with the progression of disability during follow-up and radiological activity of the disease [48]. High levels of glial markers for activation of YKL-40 and GFAP are associated with earlier progression to EDSS 3 and that high levels of YKL-40 are also associated with earlier progression to EDSS 6. Martínez et al. also reported higher levels of YKL-40 in patients with CIS with a reduced time to conversion to CDMS, which supports the results of a previous study [49]. However, the prognostic value of YKL-40 is lost when the conversion time is extended by more than 5 years. These findings further suggest that glial activation may play a key role in the progression of MS [13, 42].

5.2.2 Markers of disease progression

GFAP and sTREM-2 have been studied in MS as useful tools for monitoring disease progression. Serum and CSF concentrations of GFAP have also been associated with

clinical impairment and radiological activity [50]. In patients with progressive MS, serum GFAP concentrations are related to age and EDSS, as well as to neurofilament light levels (NF-L) [19, 51]. Increased expression of pro-inflammatory molecules, including IL-1β, IL-2, IL-6, and IL-8, has been associated with higher disease prospective activity, impairment, and neurodegeneration in MS [52, 53].

Guzel et al. [54] found that both CRP and NLR had discriminatory capacity for patients with EDSS > 5 versus EDSS ≤5.36. Demirci et al. [55] concluded that NLR may be a potential predictor of disability progression, and Bisgaard et al. categorizes NLR as an additional marker [56] No significant difference was found between NFL CSF levels in RRMS (N = 752) compared to PMS (N = 462) patients based on a meta-analysis summarizing several studies [31].

In a study of 29 MS patients who were followed for 5–16 years, the presence of CSF IgM OCB was strongly associated with conversion to SPMS and achieving a higher EDSS score [57, 58]. In other studies, serum GFAP levels were also associated with higher EDSS scores but also with longer disease duration and progressive course [42, 59]. Earlier studies have also found associations between miRNAs expression and MS damage or disease progression.

Higher NfL are associated with a higher subsequent rate of whole-brain atrophy, and recent inflammatory activity (new/increasing T2 lesions), as well as T2LV, is associated with higher NfL [60]. Clinically significant prognostic value of NF-H was also recently demonstrated in a cohort of 51 patients followed for an average of 15 years [13, 17].

Regarding the diagnosis of primary progressive MS (PPMS), the presence of CSF OCB is one of the mandatory criteria [33] and its role has been confirmed over time in successive revisions following the Poser criteria [61].

5.3 Biomarkers as indicators for the efficacy of the DMT

The therapeutic benefit of some DMTs, such as interferon beta (IFNβ) and natalizumab, often weakens due to neutralizing antibodies production. These serum antibodies are routinely tested during certain periods and are used as biomarkers for the effect of treatment. The myxovirus resistance protein (MxA) is another valuable biomarker of the IFNβ response frequently used in clinical practice.

CSF NF-L was reduced in patients after switching from IFN or glatiramer acetate to rituximab, which correlates with traditional NMR measurements for inflammatory activity, further supporting CSF NF-L as a measure of disease activity. NfL has shown utility as a biomarker for treatment with fingolimod, siponimod, natalizumab, and ocrelizumab in PMS cohorts [13].

Natalizumab has been associated with progressive multifocal leukoencephalopathy (PML) caused by reactivation of the JC virus in the CNS. The risk of PML is monitored by prospective serum testing of JCV antibodies. Currently, the use of a "PML risk stratification test" that measures the level of anti-JCV antibodies through an ELISA-based test in patients receiving natalizumab is helpful. Altered levels of miRNAs in PBMCs are normalized by autologous hematopoietic stem cell transplantation and natalizumab. Regarding the risks of natalizumab, several miRNAs are possible biomarkers for the development of PML in patients receiving natalizumab. Fingolimod treatment decreased miR 150 plasma levels and did not affect cerebrospinal fluid (CSF) levels, while natalizumab treatment increased miR-150 plasma levels and decreased CSF levels [13].

NF-L concentrations in CSF have been shown to reduce during the second year of the immunosuppressive therapy in patients with active progressive MS and after switching from first-line therapies to fingolimod in those with RRMS. In addition, CSF NF-L has shown the advantage of better therapeutic biomarker after 12 months of NTZ treatment in subjects with RRMS, compared to NF-H, [62]. However, the potential role of CSF NF-L as a biomarker for response to treatment is severely limited by the invasiveness of performing serial lumbar punctures. Conversely, serial NF-L serum scores would be an easier-to-detect marker and a reliable indicator of NF-L CSF levels [63]. The serum levels of NF-L correlated positively with clinical and radiological activity in MS at baseline and during follow-up, trend to decrease at the 6 months of IMD administration and reached stable values below 8 pg/ml in those subjects who maintained NEDA-3. In addition, persons who expressed clinical and radiological activity of the disease during observation period also showed elevated serum NF-L levels up to 5 months before relapses.

There is not sufficient evidence of possible interactions between DMD and CSF IgM OCB. Patients with RRMS on treatment with IFN-β showed reduced therapeutic response depending on CSF lipid-specific IgM OCB, who experienced a mild reduction of the relapse rate and increased likelihood of reaching deteriorated EDSS. NTZ has been shown to decrease serum IgM and IgG concentrations after 2 years of treatment onset in a time-dependent way [64].

Some studies have examined variations in matrix metalloproteinase (MMP) levels in patients with DMD. Significant reductions in serum MMP-9 mRNA in patients with RRMS below IFN-β have been observed after 12 months of follow-up by Galboiz and colleagues [65] and confirmed by other studies [66]. It is worth noting that a significant elevation of TIMP-1 levels was observed in the group of respondents compared to nonrespondents [67]. A possible therapeutic effect to NTZ treatment has also been studied. Balasa and colleagues found a significant reduction of MMP-9 in the serum after 8 months of treatment onset and a positive correlation between the biomarker concentration and the disease activity [68], but this finding has not been affirmed by other research [69]. Decreased baseline MMP-9 levels were found in patients treated with NTZ in patients who developed progressive multifocal leukoencephalopathy compared to those who did not. [70].

In patients with CIS, in parallel with the assessment of the risk of conversion, it is important to choose the adequate treatment decisions preferably supported by biomarkers that could predict the future course of the disease. For example, biomarkers associated with axonal damage or oligodendroglial waste could facilitate the recognition of subjects who need aggressive and early treatment approaches to suppress the disease progression and long-term disability [13].

5.4 Markers of MS activity

CRP and NLR as biomarkers of disease activity in MS. NLRs appear to reflect better systemic inflammation than specific neutrophil and lymphocyte counts alone. NLR is calculated as the ratio of the number of neutrophils to lymphocytes, which makes it a simple, fast, nonspecific, and inexpensive way to detect increased systemic inflammation. NLR as a biomarker comes from observations showing that systemic inflammation regularly leads to neutrophilia and lymphocytopenia [29].

Nitric oxide metabolites. Due to the role of oxidative stress in the pathogenesis of MS, nitrates and nitrites have been studied as biomarkers of disease activity [71].

Interferon-beta (IFN-β) has shown remarkable inhibition of inducible expression of NO synthase in astrocytes [72–74]. Significantly higher levels of nitrites and nitrates were found in patients with relapse than in remission and patients treated with steroids in the previous 1–2 months [74, 75]. Accordingly, NO metabolites predict disease activity with 71% specificity and 66% susceptibility [76].

Osteopontin. Osteopontin (OPN) is closely linked to the immune system. In its soluble form, it is secreted by macrophages and activated leukocytes and also interacts with them, reducing the inducible form of NO synthase, and stimulating inflammatory process. In its intracellular form, OPN is expressed by dendritic cells and promotes the differentiation of Th17 and Treg [77]. OPN is probably facilitating increased regulation of Th1 and Th17 cytokines, mostly IFN-γ and IL-17 [78, 79]. A specific subset of Th1 cells, particularly those occurring in CSF during relapses, are thought to produce OPN, high levels of IFN-γ, and matrix metalloproteinase-9 (MMP-9) after polyclonal stimulation, playing a pathogenetic role [80, 81].

C-X-C motif ligand 13. The C-X-C motif ligand 13 (CXCL13), also known as a chemokine that attracts B cells (BCA-1), is a protein that promotes the chemotaxis of mature B lymphocytes by interacting with its CXCR5 receptor [79]. In fact, CXCL13 has been found to be overexpressed in active MS lesions and in intrameningeal B-cell follicles of chronic white matter lesions, maintaining humoral autoimmunity and disease activity [82, 83]. In a study by Khademi et al. CSF CXCL13 was found to be significantly higher in infectious neurological diseases and MS [84].

MMP-9. During inflammation, many molecules are able to activate MMPs, including reactive oxygen species and TNF-α and IL-17 via NF-κB [85]. It has been suggested that MMPs may also act in MS by digesting myelin basic protein (MBP), in addition to promoting leukocyte leakage into postcapillary venules [86].

Myelin basic protein. It has long been known that MBP is a potential biomarker of disease activity for MS, as it shows acute CNS myelin damage, although it is not disease-specific. Several studies have found elevated levels of MBP in CSF in MS patients temporarily associated with relapses [87] and detectable up to 5–6 weeks later [88]. Accordingly, patients with RRMS with disease activity showed higher values than progressive MS and stable patients [89]. MBP concentrations in CSF are also higher when polysymptomatic and severe relapses occur, which correlates with EDSS score and MRI activity and decreases after treatment with corticosteroids [90].

Neuronal cell adhesion molecule (N-CAM). The adhesion molecule of neuronal cells (N-CAM) is considered a marker for recovery and remyelination and is expressed mainly in the CNS [91].

5.5 Biomarkers for MS relapses

Patients with recurrent MS have higher levels of CSF NFL than patients in remission. No significant difference in GFAP CSF levels was found between patients in relapse and remission. The difference in CSF t-tau levels between patients with relapse and remission was not significant [30].

The results of Martínez et al. are consistent with previous studies showing higher NFL levels during relapse [30, 41]. The authors confirm the conclusion of a previous study by Malmeström et al. that NFL levels decrease further 60 days after the onset of relapse [92]. Conversely, MCP-1 levels increase in the stable phases of the disease, indicating that this marker may reflect an anti-inflammatory effect [93].

In a group of patients with active recurrent and progressive MS, Thebault et al. showed that increased sNfL at baseline and also longitudinal elevation of sNfL

from previously low baseline values predict relapse manifestations over a 12-month follow-up period. Increased baseline sNfL rates are also corresponding with subsequent gadolinium-enhanced lesions during disease activity and with deterioration of disability. sGFAP is associated with upcoming MRI activity only, but not with other parameters [17].

In patients with milder relapses, treated with drugs on first-line, the sNfL levels are more stable than in severe relapsed subjects. In MS patients with more active course of the disease, increased sNfL was observed 5 months before appearance of new crisis and almost 80% of the increased sNfL (>3 SD) were corresponding with clinical and MRI activity of the disease. Although these group-level observations are important evidence that dynamic change in sNfL is appropriate, utility at the individual patient level is limited [17].

The results of Martínez, 2015 are consistent with previous studies showing higher levels of NFL during relapse [41]. Researchers confirm previous findings that NFL decreases further after 60 days of relapse [92]. This model has not been observed for other biomarkers.

5.6 Biomarkers for neuronal and glial damage in the differentiation of MS subtypes

GFAP alone has been shown to be a useful biomarker for differentiating different MS subtypes. Patients with PMS had higher GFAP levels than RRMS. No significant difference in S100B CSF levels was found between patients with RRMS and SPMS [30]. While in RRMS the movement of adaptive immune cells from the periphery to the CNS is the main pathological mechanism, in PMS the players of innate immunity, including astrocytes and microglia, play a more important role. Molecular biomarkers of reactive astrogliosis show promising results in the differentiation of RRMS and PMS. This may be one of the reasons for the higher levels of GFAP, which reflect astrogliosis, in patients with PMS compared to RRMS. Serum GFAP levels are also higher in patients with SPMS compared to RRMS.

No significant difference was found between t-tau levels in CSF in RRMS compared to PMS. No significant difference was found between the CSF levels of CHI3L1 in RRMS compared to PMS [30]. Metabolic serum metabolic profiling may reveal reliable biomarkers for distinguishing between RRMS, SPMS, and PPMS.10. Metabolic profiling of CSF is currently being developed, but all of these studies require further validation before clinical use [13].

5.7 Association of biomarkers for neuronal and glial disorders with age and sex

Recent meta-analysis has shown that CSF NFL levels are positively correlated with age in HC, but they do not have or have a negative correlation with age in MS. Abnormal changes during the course of MS affecting CSF NFL levels are considered as main feature, differentiating MS patients form HC [30].

A meta-regression analysis showed a negative correlation between the percentage of women and the magnitude of the effect of comparing CSF NFL levels among MS patients. Gender may be a determinant of the CSF levels of neuronal and glial biomarkers of damage. Higher CSF levels of CHI3L1 and t-tau have been found in men suffering from MS. A recent meta-analysis found higher levels of CSF NFL in men in the HC and MS groups. However, in patients with PMS, CSF NFL levels are moderately higher in women. Finally, in addition to CSF levels of biomarkers for neuronal

and glial damage, their blood level may also be a practical biomarker in MS. CSF and blood levels of these biomarkers may be affected by DMT and they can potentially be used to monitor the response to treatment.

To date, only GFAP has shown a significant correlation with age, with higher levels found in the elderly [42].

5.8 Biomarkers for cognitive impairment in MS

Cognitive impairment (CI) is a common and disabling symptom in MS. Axonal damage may contribute to the development of CI in the early stages. However, there are currently no biomarkers available to monitor CI in MS patients. Virgilio E et al. in their study aimed to investigate the correlation of axonal biomarkers of CSF, in particular: light chain neurofilaments (NFL), Tau, and beta-amyloid protein (Abeta) in patients with MS with CI at diagnosis. The researchers included 62 newly diagnosed patients with MS and cognition was assessed using the BICAMS battery. CSF levels of NFL, Abeta, and Tau were determined by ELISA. No differences were found in demographic, clinical, and MRI characteristics (with exception of the lower educational level) in persons with CI.

The patients with CI, who accounted for 45.1%, did not differ in demographic, clinical, and MRI parameters (with exception of lower educational level), but showed more severe neurodegeneration, based on higher mean CSF Tau protein (162.1 ± 52.96 pg/ml vs. 132.2 ± 63 pg/ml p: 0.03). No significant differences were reported for Abeta and NFL. A correlation between the number of impaired tests and Tau levels was significant (r: 0.32 p: 0.01). Tau is increased, especially in persons with delayed data rate (IPS) (p: 0.006) and linear regression analysis, subtyping EDSS, MRI, and MS, confirms Tau as a weak predictor of IPS and cognitive impairment. CI has significant impact on the quality of life of MS persons and should be sought even at diagnosis. Biomarkers of axonal damage, in particular Tau, appear to reflect cognitive impairment at the early stages of the disease [94].

In a longitudinal trial on 22 IFNβ-1a- and riluzole-treated patients and 20 IFNβ-1a- and placebo-treated persons with MS at an early stage, the serum NF-L concentrations were evaluated over a 24-month period. The NF-L levels correlated positively with EDSS deterioration, Gd + lesions, and cerebral atrophy. In addition, elevated serum NF-L levels correlated with poorer results in neuropsychological tests that evaluated visual-spatial orientation, recollection, and verbal and nonverbal episodic learning [95]. Similar results on the relationship between serum NF-L levels and CI at the early stages of MS associated with increasing EDSS have been confirmed by other studies. Although the serum NF-L levels correlated with EDSS in patients with PMS, they failed to correspond with EDSS deterioration in the previous year and during a mean follow-up of 27 months. In particular, serum NF-L was elevated in all of the patients with PMS, including those who did not show increasing in EDSS or deepening of the disability [96].

6. Discussion

Many systematic biological approaches such as genomics, epigenomics, and proteomics have been used to expand the knowledge in MS, helping to extract valuable information on the pathogenesis of the disease. Despite this progress, there remains a need for additional tools to understand the exact etiopathogenesis of MS.

There is also a significant unmet need for diagnostic and prognostic biomarkers in MS, especially in progressive forms of the disease [22].

In MS, the potential biomarkers are classified on the basis of their ability to establish the diagnosis, predict the outcomes, and assess the response to treatment. Essentially, the biomarkers for MS need to be able to identify individuals who are vulnerable for receptivity to the disease or at high risk of severe attacks in case of confirmed diagnosis and to predict which individuals are likely to respond to certain treatments. Based on these considerations, many published candidate biomarkers have emerged, although most of them are correlative and have yet to be shown to have significant prognostic potential for the disease. In addition, some biomarkers are common markers of inflammation and, therefore, have no specificity for MS. Nevertheless, they have been shown to be important in elucidating the mechanism of disease, progression, and susceptibility, despite their inability to become practical clinical biomarkers [6].

CSF is a unique source of potential biomarkers for MS, although it requires some invasiveness to collect them. Currently, only diagnostic biomarkers for CSF are used in clinical practice, although hundreds of molecules have been validated as indicating disease activity and prognostic biomarkers. IgG OCBs maintain an important role as a validated diagnostic biomarker and are considered an alternative MRI tool that can replace the spread over time based on the 2017 revision of the McDonald's criteria. They also have a predictive role for conversion from CIS to MS when found in patients with first demyelinating event. NF-L has been shown to be a valuable biomarker that indicates disease activity in MS. The ability to measure NF-L in the serum at different time points makes it suitable for monitoring the response to treatment. The KFLC index was established as a more sensitive but less specific diagnostic biomarker than IgG OCB. It is a potential first-line assessment in patients with suspected MS and minimizes the need for IgG OCB analysis. The KFLC index is a prognostic biomarker for CIS conversion, but the lack of a universal threshold is still a limitation. IgM OCBs have a good potential as a predictive biomarker because of their association with aggressive course of the disease, a higher risk of conversion from CIS to MS, progression of disability, and conversion from RRMS to SPMS. Several biomarkers of the disease activity appear promising, although they require additional validation. Elevated levels of NO metabolites, OPN, MBP, MMP-9, N-CAM, CXCL13, and CHI3L1 were found to be closely correlated with relapses [30, 41]. The role of biomarkers in monitoring the effect of applied BMI is extremely valuable.

Based on the review presented, we can conclude that there are several biomarkers with a degree of relevance in the clinical environment. However, no biomarker is effective in determining diagnosis and prognosis and in terms of sensitivity and specificity. MRI and OCB are currently important in the diagnosis of MS. However, recent studies have shown that the MRZ or NfL reaction is already or may be useful in the future, respectively [6, 32].

The development of biomarkers is comparable to the development of drugs, and independent validation must be demonstrated in large cohorts after a positive pilot test. If biomarker tests are to be used to stimulate patient care, understanding and carefully evaluating these concepts is essential, as "a bad biomarker test is as bad as a bad medicine" [97]. The validation process is often lengthy and usually takes between 5 and 15 years [98]. For this reason, the enrichment of the repertoire of biomarkers for MS has been slow so far.

Biomarkers currently used in clinical practice to diagnose MS include glycoproteins, chemokines, IgG and IgM antibodies, and cellular surface markers of inflammation. As

a step toward a better understanding of the mechanisms of neurodegeneration in MS, recent studies have found new correlations between neurofilaments and other biomarkers of disease activity. CSF NF-L was found to be inversely related to serum vitamin D levels in a group of 153 MS patients [26]. This study suggests that normal or high normal vitamin D levels are not only associated with reduced inflammatory activity in MS but can also protect against axonal damage. It has also been found that axonal damage, measured by neurofilaments, correlates with mitochondrial dysfunction (CSF lactate) and CNS autoimmunity and inhibition of remyelination (CSF lipocalin 2), thus potentially expanding the repertoire of CSF current marker biomarkers. Activity in MS [13].

However, the course of MS disease is very variable and the diversity in the phenotype of the disease is not well related to these biomarkers. Thus, it is imperative to identify new specific biomarkers that can help differentiate clinical phenotypes of MS, predict disease progression, and provide correlation with disability [2].

In addition, biomarkers are needed that reflect the ongoing neurodegeneration, demyelination, and remyelination of gray and white matter, microgliosis, astrogliosis, and oxidative stress, which contribute to the overall activity of the disease. The need is particularly important for progressive MS (SPMS and PPMS), where biomarkers are lacking that can objectively assess the mechanisms of the disease that contribute to neurological deterioration.

Future research is needed to further investigate the clinical use of neuronal and glial biomarkers in MS. More studies are indispensable to shed light on the importance of these markers in differentiating between different phenotypes of MS and the specific course of the disease. Establishing cut-off values for different biomarkers in diagnosing MS and determining its prognosis can be useful [30].

7. Conclusion

Biomarkers are crucial for the emergence of scientific discoveries, for the development of adequate pharmacological products and for quality healthcare for the individual and the population as a whole. The emergence of accurate and reliable biomarkers of CSF, along with the development of safe and effective intrathecal therapies, will make CSF analysis a routine part of optimal clinical management of MS. Peripheral blood collections are less invasive and easier to obtain than CSF collections. Blood biomarkers capable of detecting disease activity in MS and distinguishing different disease phenotypes may be useful in personalized treatment of MS with disease-modifying drugs and predict treatment response. An approach to the development of a biomarker that includes a common regulatory science across multiple disciplines is needed to ensure that evidence-based rational development of biomarkers maintains a pace with scientific and clinical needs.

Conflict of interest

No.

Notes/Thanks/Other declarations

NA.

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Author details

Valentina Ignatova
Clinic of Neurology, National Cardiology Hospital, Sofia, Bulgaria

*Address all correspondence to: valyaig@abv.bg

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