Molecular biomarkers in multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a highly heterogenous disease regarding radiological, pathological, and clinical characteristics and therapeutic response, including both the efficacy and safety profile of treatments. Accordingly, there is a high demand for biomarkers that sensitively and specifically apprehend the distinctive aspects of the MS heterogeneity, and that can aid in better understanding of the disease diagnosis, prognosis, prediction of the treatment response, and, finally, in the development of new treatments. Currently, clinical characteristics (e.g., relapse rate and disease progression) and magnetic resonance imaging play the most important role in the clinical classification of MS and assessment of its course. Molecular biomarkers (e.g., immunoglobulin G (IgG) oligoclonal bands, IgG index, anti-aquaporin-4 antibodies, neutralizing antibodies against interferon-beta and natalizumab, anti-varicella zoster virus and anti-John Cunningham (JC) virus antibodies) complement these markers excellently. This review provides an overview of exploratory, validated and clinically useful molecular biomarkers in MS which are used for prediction, diagnosis, disease activity and treatment response.

Keywords: multiple sclerosis, molecular biomarker, neurofilament, cerebrospinal fluid, diagnosis, treatment response

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Introduction

Multiple sclerosis (MS) is an autoimmune neurological disease featured by chronic inflammation of the central nervous system (CNS), resulting in a range of physical, or even psychiatric symptoms (1). The etiology of MS is not fully understood; it is generally accepted that MS arises from a combination of genetic susceptibility, epigenetic events, and various environmental factors such as chemical and microbiological agents, smoking and diet (2). MS is a highly heterogenous disease regarding radiological, pathological, and clinical characteristics and therapeutic response, including both the efficacy and safety profile of the treatment. Accordingly, there is a high demand for biomarkers that sensitively and specifically apprehend the distinctive aspects of the MS heterogeneity, and that can aid in better understanding of the disease diagnosis, prognosis, prediction of the treatment response and finally in the development of new treatments (3). Currently, clinical characteristics (e.g. relapse rate and disease progression) and magnetic resonance imaging (MRI) play the most important role in the clinical classification of MS and assessment of its course, with biomarkers complementing these markers excellently.

This review provides an overview of clinically useful, validated and promising exploratory molecular biomarkers in MS which are used for prediction, diagnosis, disease activity and treatment response regarding the efficacy and safety of the treatment.

Biomarkers in MS: definition, importance, and classification

Biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention” (4). According to the World Health Organization (WHO), a good biomarker involves “almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction”. Comparably to drug development, the establishment of a new biomarker in the clinical practice is a lengthy and costly process, which consists mostly of the discovery of a biomarker and its validation. This process can take 5 to 15 years, as an independent validation of a biomarker has to be exhibited in large cohorts of patients after its discovery in positive small-size studies (5).

According to the strength of evidence, molecular biomarkers for MS can be categorized into exploratory, validated and clinically useful biomarkers (3, 6). Exploratory biomarkers for MS represent the biomolecules proposed as candidate biomarkers; most of the biomarkers have been revealed by so-called omics techniques, such as proteomics and genomics, in addition to research in microRNA (3, 7, 8). A newly discovered biomarker has to be validated/reproduced across different patient populations in independent studies. This process is critical for movement from bench to bedside (3) and is often time-consuming and demanding. Sometimes highly promising biomarkers for MS do not get the necessary confirmation in independent studies, e.g. cleaved cystatin C in the CSF (9), CSF soluble Nogo-A protein (10), serum soluble HLA-G (11), tested
as diagnostic biomarkers for MS, and serum IL17F, tested as a response biomarker for interferon-beta treatment (12). A usual problem in assessing the validity of a biomarker is insufficient specificity of a biomarker: for instance, myelin basic protein (MBP) can also be detected in some other neurological diseases (13), whereas levels of matrix metalloproteinase 9 (MMP9) and osteopontin can be altered in other autoimmune diseases in addition to MS (14, 15). The second group of biomarkers in MS comprises validated biomarkers, which show a higher degree of association with MS pathology. These biomarkers have been tested in different studies with a high degree of agreement in the findings (at least three studies), or have been tested with an independent clinical replication (3). Finally, the third group of molecular biomarkers consists of several biomolecules that are already integrated into routine clinical practice (Table I).

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**Abbreviations:** CSF = cerebrospinal fluid; NMOSD = neuromyelitis optica spectrum disorder; VZV = varicella zoster virus; JC virus = John Cunningham virus; PBMCs = peripheral blood mononuclear cells.
According to another classification of molecular biomarkers in MS (whether they can predict, diagnose, correlate with disease activity and response to treatment), biomarkers can be predictive, diagnostic, disease activity, and treatment-response biomarkers. Some of these biomarkers belong to more than one group. A good example is the light subunit of neurofilaments: it can serve as a prognostic factor of conversion from clinically isolated syndrome (CIS) to MS (16, 17), as well as a biomarker of disease activity (18-20) and treatment response.

**An ideal biomarker**

An ideal biomarker in MS should be binary, meaning it is detectable only in patients with MS, but not in healthy individuals or those with other pathological conditions, and its concentration elevates or reduces when the disease exacerbates or improves, respectively (3). Depending on the class of a biomarker for MS, an ideal biomarker should possess some specific additional properties. For diagnostic biomarkers, a high predictive power is desirable. For disease activity biomarkers, they should specifically be connected to MS pathological processes, especially neurodegeneration. For treatment-response, the biomarker should completely apprehend the treatment effects on clinical response. Other general criteria for a good biomarker in MS (5, 6) are the following:

1. It should be highly sensitive and specific;
2. It should be easily measured and safe for a patient (preferably non-invasive);
3. The analytical method for the biomarker detection should be highly accurate, robust, and reproducible;
4. It should be cost-effective;
5. It should be clinically useful, in other words, clinical decisions could be made based on the biomarkers and patients could benefit from their use.

Molecular biomarkers of MS can be measured in bodily fluids, such as urine, blood, or cerebrospinal fluid (CSF). Although urine analysis is the least invasive for sample collection, it does not accurately reflect MS pathology, due to its anatomic distance from the CNS. Biomarkers measured in blood samples are minimally invasive and can be routinely collected at multiple different time points; in addition, large quantities can be examined (5, 21). However, these biomarkers likely mirror peripheral immunity, and may only indirectly reflect immunity processes in the CNS (21). Additional disadvantages of blood biomarkers can also be significant diurnal variabilities and low concentrations of many biomolecules, and their propensity to undergo processes of degradation and being concomitantly observed in other diseases. Due to its proximity to the CNS, CSF represents the gold standard matrix for measurement of many neurological diseases, including MS. However, the collection of CSF is associated with many drawbacks, including difficulty of measuring at different timepoints, exposure to invasive lumbar puncture procedures and the fact that only small amounts can be obtained (5, 21). Nevertheless, the incidence of the unwanted effects is now significantly reduced by the use of atraumatic needles of 24 gauge or greater (21, 22).
There are several types of biomolecules which can serve as biomarkers: proteins, micro- and messenger ribonucleic acids (miRNA and mRNA), as well as deoxyribonucleic acid (DNA). Currently clinically used molecular biomarkers are only proteins, mostly antibodies. In comparison with RNA or protein biomarkers, DNA biomarkers are more reproducible; less demanding for sampling, handling, storing and measuring; more cost-effective; and can be assessed at any timepoint (3, 23). In comparison with other biomolecules, RNA and protein biomarkers are more convenient for the monitoring of treatment response, and surrogate endpoints, due to their quantitative nature (3, 23).

**Predictive biomarkers**

Predictive biomarkers should aid in the identification of individuals who are at high risk of developing MS. These biomarkers should ideally be assessed in individuals without neurological symptoms, mostly in first-degree relatives of patients with MS. Currently, there are no such biomarkers in routine clinical practice, but there are some promising validated biomarkers which are expected to be clinically useful in the near future. These are antibodies to Epstein-Barr virus nuclear antigens (anti-EBNA) measured in serum (24, 25). Recently, two Swedish population-based case-control studies, consisting of 5,316 cases and 5,431 matched controls, have shown that high levels of anti-EBNA and infectious mononucleosis history act synergistically to increase MS risk (26).

**Diagnostic biomarkers**

Considering that treating MS early and effectively is the best manner of restraining permanent damage to the CNS, speeding up the diagnosis of MS with improved accuracy is a principal goal. Diagnostic biomarkers are needed to help in distinguishing MS patients from healthy individuals or individuals with other neurological diseases. In combination with clinical and radiological diagnostic criteria, these biomarkers can be useful in improving the sensitivity and specificity of diagnosis (CIS, relapsing-remitting MS, RRMS and progressive MS, PMS).

**Clinically useful biomarkers**

Biomarkers which are already used in clinical praxis are immunoglobulin g (IgG) oligoclonal bands (OCBs), and IgG index in CSF for diagnosis of MS and anti-aquaporin-4 (AQP4) antibodies in CSF and serum for a differential diagnosis between MS and neuromyelitis optica spectrum disorder (NOSD).

**Oligoclonal bands and IgG Index**

IgG antibodies in CSF originate from clonally expanded B cells from CSF (27). Their production can be detected as OCBs using electrophoresis/isolectric focusing. Another indicator of IgG antibodies production in CSF is the IgG index, which can be calculated by the CSF/serum quotient of IgG to the CSF/serum quotient of the reference
protein albumin formulae (28-30). Sensitivity of detection of OCBs in CSF is over 95%, when the method of detection is isoelectric focusing on agarose gels followed by immunoblotting immunofixation, which is accepted as a gold standard (31-33). This sensitivity was confirmed by a meta-analysis of 49 studies (34).

IgG OCBs were included in the diagnosis of MS as the first biomarker in 1983 in the Poser criteria (35). The status of OCBs has changed overtime in McDonald criteria versions 2001-2017; today, the existence of OCBs in CSF, while not demonstrable in serum, is used as a part of procedure for MS diagnosis in patients who had been diagnosed with CIS previously (36-39). The presence of OCBs in CSF has been confirmed in over 95% patients with MS (40). Still, IgG OCBs are not specific for MS, as they are also observed in other inflammatory disorders. To improve the diagnostic precision, it has been recommended to use the analysis of oligoclonal bands in CSF with other neurological analyses (MRI, occurrence of clinical attacks) (41). In addition to MS diagnosis, OCBs can also be used as a prognostic marker for the conversion from CIS to MS, as the study with pediatric CIS patients showed that OCBs improved the specificity of MRI criteria (41).

Along with OCBs detection, increased level of IgG index (>0.7) in CSF provides an evidence for an ongoing antigen-driven humoral immune response in the CNS, which supports the diagnosis of MS. An increased level of the IgG index in CSF is detected in approximately 70% of patients with MS (42). Although an increased IgG index infrequently occurs in MS patients without OCB, the IgG index is one of the accepted molecular diagnostic biomarkers of MS (29).

**Anti-aquaporin-4 antibodies**

AQP4 is a water channel protein widely expressed by astrocytes in the CNS. AQP4-IgG antibodies are used as a highly specific and sensitive serum biomarker for neuromyelitis optica (NMO). AQP4-IgG can be detected by enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence, cell-based assays, or flow cytometry. AQP4-IgG can be found in approximately 75% of NMOSD patients, while it is almost absent in patients with MS. Therefore, in clinical practice, they are used for establishment of a differential diagnosis between NMODS and MS (3, 29, 43-45).

**Validated diagnostic biomarkers**

Important validated diagnostic biomarkers are IgM OCBs, chitinase and chitinase 3-like proteins, complement components and measles, rubella, and varicella zoster (MRZ) reaction.

**IgM OCBs**

IgM OCBs seem to be present in 40% of MS patients (46). Similarly to IgG OCBs, their presence seems to be predictive of conversion from CIS to clinical MS, but also to predict a clinically more aggressive disease course (47). Elevated IgM antibodies in CSF
along with neurofilaments corresponded to a high severity score on MRI lesion, as well as thinning of the retinal fibers (48).

**Chitinase and chitinase 3-like proteins**

Chitinases or chitotriosidases, are glycoproteins that hydrolyze chitin; chitinase 3-like-1 (CHI3L1) and chitinase 3-like-2 (CHI3L2) are similar to chitinase, as they bind to chitin but lack the ability to hydrolyze. CHI3L1 and CHI3L2 are also known to assist in cell trafficking across the blood brain barrier (BBB). These biomolecules are expressed by astrocytes within the white matter plaques, while CHI3L1 is also expressed by microglia in MS lesions (49).

CHI3L1 seems to be a promising prognostic factor of conversion from CIS to MS. Increased CSF concentrations of CHI3L1 have been shown to be predictive of conversion from CIS or optic neuritis to MS (50). Potentially, it could also serve as a biomarker of disease activity. However, a correlation between CHI3L1 expression and MRI imaging is still vague, as 3 studies have reported a correlation between CHI3L1 and the number of gadolinium enhancing (Gd+) lesions (51-53), whereas one has not found a relationship between these two parameters (54).

CHI3L2 seems to be a promising biomarker for differentiation between RRMS and PMS; lower CSF CHI3L2 levels were detected in PMS than in RRMS patients (55). Accordingly, CSF CHI3L1/CHI3L2 ratio accurately discriminated PMS from RRMS. However, these results have to be validated in other studies.

**Complement components**

Complement system consists of around 30 different proteins and is a key part of the innate immune system. Numerous studies have demonstrated an important role of complement system in MS pathogenesis (56). Complement components and activation products in serum and CSF have been proposed as potential biomarkers for diagnosis of MS subtypes and differential diagnosis between NMOSD and MS (57, 58). Complement factor H, which is a main regulator of the formation and functions of complement factors C3 and C5, has been found to be the most promising; it has been validated in several studies. Ingram et al. have found that levels of factor H (FH), C1 inhibitor (C1inh), C3, C4, C4a were significantly higher, whereas the level of C9 was lower in MS patients than in matched controls (59). Additionally, FH has been proposed to be a useful marker of MS disease activity, as its levels in serum were markedly higher in progressive disease compared to controls and relapsing patients (60). Moreover, FH, C1s, C1inh and C5 levels were lower in patients with MS compared with NMOSD patients. A combination of complement biomarkers C1inh and terminal complement complex (TCC) was successful in distinguishing NMOSD from MS. Thus, the combination of complement biomarkers was proposed to be useful for making a differential diagnosis between NMOSD and MS (61). Further studies are needed to elucidate a possible relationship between complement profiling and differential diagnosis of MS.
**MRZ reaction**

There is a polyspecific B-cell immune response to neurotropic viruses, mostly to measles, rubella, and varicella zoster virus, in patients with MS. MRZ reaction (MRZR) is described as a positive intrathecal response to at least two of these three viral agents and was found to be 97% specific to MS. It was claimed that positive MRZR improved the diagnosis of MS (62). In another study, MRZR was found to be able to predict the conversion of patients with CIS to MS (63).

**Exploratory diagnostic biomarkers**

The role of miRNAs, short single-stranded segments of RNA, has been studied in MS patients. It has been found that high miR-150 and low miR-219 levels in CSF may have a potential to distinguish MS from other demyelinating diseases (64).

Recent studies have also shown that insulin-like growth factor-binding protein 7 (IGFBP7), somatostatin (SST) (65), soluble isoform of the interferon-β (IFN-β) receptor (sIFNAR2) (64), brain-derived neurotrophic factor (BDNF) (66), and chemokine C-X-C motif chemokine-13 (CXCL13) (67) levels can be useful in diagnosis of MS.

Additional prospective exploratory diagnostic are anti - myelin oligodendrocyte glycoprotein (MOG) antibodies and neural cell adhesion molecule (NCAM).

**Anti-MOG antibodies**

MOG is a CNS-specific myelin protein expressed on myelin sheaths and membranes of oligodendrocytes. MOG represents a potential target for demyelinating diseases (68, 69). Anti-MOG antibodies have been detected in the CSF of patients with several demyelinating diseases, such as bilateral optic neuritis, myelitis encephalitis, transverse myelitis, brainstem encephalitis, subgroup of pediatric patients with acute disseminated encephalomyelitis (ADEM), and MOG-IgG-associated encephalomyelitis (MOG-EM) (29, 70, 71). Anti-MOG antibodies are rarely detected in adult patients with MS and NMOSD; they are predominantly observed in pediatric patients, as well as in patients with severe optic neuritis, transverse myelitis and brainstem attacks, and patients exhibiting high disease activity in spite of treatment with disease-modifying therapies (DMT) (72). Therefore, anti-MOG antibodies may be more suitable for a differential diagnosis between MS or NMOSD and other anti-MOG antibodies-associated demyelinating diseases (73). Hence, anti-MOG antibodies testing should be considered in MS or NMOSD patients with atypical features (74).

Some studies support the value of anti-myelin antibodies in predicting the conversion from CIS to MS; the positive patients relapse in shorter time intervals than the negative patients (75). In another study, Lim et al. have analyzed forty-seven CIS patients with detectable anti-MOG and anti-MBP antibodies. The authors have not found any relationship between the antibody status and MS diagnosis confirmed by either McDonald or Poser criteria (76).
Further studies are needed to confirm the usefulness of this biomarker in diagnosis of MS.

*The Neural Cell Adhesion Molecule (NCAM)*

NCAM is a glycoprotein constructed from domains of the immunoglobulin superfamily and is located in the membrane of neuronal and glial cells. NCAM plays an integral role in the remyelination process, neuronal growth, and repair mechanisms in the CNS (77, 78). A sensitive ELISA assay for measurement of NCAM level in the CSF has been developed and validated. NCAM level has been measured in a healthy control group and in patients with certain neurological diseases, such as MS, Alzheimer's disease, encephalitis, and cognitive impairment. Lower NCAM values were measured in patients with MS in comparison to controls (79). Similarly, NCAM levels were reduced (p < 0.01) in CSF of MS patients in comparison to healthy controls (80). More studies are needed to clarify this unexplained relationship between reduced levels of NCAM and MS.

**Disease activity biomarkers**

Disease activity biomarkers should aid in differentiation between RRMS and PMS; additionally, they could also help in distinguishing benign from aggressive MS disease courses. In future, different biomolecules, such as markers of inflammation and oxidative stress could be employed as biomarkers of RR phases of MS, whereas markers of glial dysfunction, axonal damage and remyelination could serve as potential biomarkers of progressive and neurodegenerative phases of the disease (6).

**Validated biomarkers**

The most prospective validated biomarkers for disease activity are neurofilaments.

**Neurofilaments**

Neurofilaments are cytoskeletal components of neurons that are particularly abundant in axons. They belong to the intermediate filaments family with triplet subunits according to the molecular weight: e.g. neurofilament light (NFL), neurofilament medium (NFM) and neurofilament heavy chain (NFH) (81).

During the axonal or neuronal damage, neurofilament proteins are released into the CSF and blood, where they can be measured (82). Therefore, neurofilaments offer great potential as biomarkers of neurodegeneration and axonal injury. NF-L has been shown as particularly useful, due to its stability and consistency across studies in different patient populations. This biomolecule could serve as a biomarker for several purposes, including: i). diagnosis (specifically, for prognosis of conversion from CIS to MS) (16, 17); ii). evaluation of disease activity, as it correlates with MRI activity, and brain atrophy rate (18-20) and iii) therapeutic response. Several studies have already shown a decrease in the amount of NFL in CSF of MS patients following treatment with natalizumab (83, 84). In another study, individual NFL variation over time was followed in MS patients treated with alemtuzumab (85). Before the treatment, sNFL level increased about a month prior
to the first clinical symptoms, whereas the level decreased after the treatment. Patients with higher activity of the disease and higher sNFL values required alemtuzumab retreatment.

**Exploratory biomarkers**

Eventual exploratory disease activity biomarkers are those of a subset of myeloid cells and certain cytokines important for the pathophysiology of MS.

**Biomarkers of a subset of myeloid cells**

MS lesions are infiltrated by macrophages and monocytes, which act as the main drivers of the pro-inflammatory response in the CNS (86). CD163 is a monocyte/macrophage-specific membrane protein and serves a receptor for haptoglobin-hemoglobin complexes (87). Its soluble form sCD163 can be found in the CSF and blood; sCD163 CSF/serum ratio was found to be elevated in RRMS and PPMS, along with other biomarkers like NFL (88). Higher levels were especially found in PPMS patients.

**Cytokines**

Increased activity of T cells in MS patients results in elevated levels of cytokines like interleukin (IL)-1β, IL-4, IL-6, IL-12, and IL-13, IFN-γ, granulocyte macrophage colony-stimulating factor (GM-CSF), and IL-17, which can be useful biomarkers in assessing the level of immune activity (89, 90). However, these cytokines are not specific for MS, but are also characteristic for other inflammatory and autoimmune diseases.

**Biomarkers for monitoring therapy response**

Due to the progressive elucidation of the MS pathophysiology, a number of DMTs with specific mechanisms of action are now available. However, not all patients respond equally to the treatment (91). To be able to treat each patient with the individually optimized MS treatment at the right time, it is necessary to have biomarkers for predicting the therapeutic response and monitoring its effectiveness. These markers can be also seen as prognostic markers for (poor) response to specific DMTs.

**Clinically useful biomarkers**

In clinical praxis, neutralizing antibodies against interferon beta (IFNβ) and natalizumab are already used.

**Neutralizing antibodies against interferon beta (IFNβ)**

IFNβ is the most commonly prescribed DMT in MS (92). Surprisingly, up to 47% of treated patients are unresponsive to IFNβ therapy, due to generation of neutralizing anti-IFNβ antibodies (93). The incidence of neutralizing antibodies following treatment with IFNβ for MS varies substantially with dose, frequency of dosing and type of product (94). Based on the results from phase III clinical trials, a wide range of incidence of
neutralizing antibodies has been observed: from 2.1–22% following intramuscular application of IFNβ-1a, 12.5–25% after subcutaneous application of IFNβ-1a, and 27.8–47% after subcutaneous application IFNβ-1b (95). These antibodies usually develop during the first 2 years of treatment. Neutralizing antibodies prevent IFNβ from effective binding or activating its receptor, therefore they block its biologic effects and inhibit its therapeutic effects, as seen through an increase in annual relapse rate, and MRI activity, as well as disability progression (96). Together with clinical markers, antibody titres are utilized for the evaluation of IFNβ clinical efficacy and guidance on continuation of IFNβ treatment (97). For patients with persistently high antibody titres, cessation of IFNβ treatment is recommended, whereas for patients with an absence of antibodies, continuation of the treatment is advised. Nonetheless, for patients with low to moderate antibodies titres, additional information for a proper clinical decision is needed. Usually, myxovirus resistance protein A (MxA) bioactivity measurement is performed; this assay measures MxA mRNA in vivo after IFNβ application, and quantifies the IFNβ biological response (98). A good correlation between MxA mRNA concentrations and relapse rates has been found (99).

Neutralizing antibodies against natalizumab
Natalizumab is a monoclonal antibody which is highly effective in the treatment of MS. It is a humanized monoclonal antibody directed against α4 integrin subunit of very late activation antigen-4 (VLA-4). Blocking of VLA-4 results in inhibition of lymphocytes trafficking from the blood into the CNS, thereby in attenuation of the CNS inflammation (100, 101). Treatment with natalizumab is also related to generation of neutralizing antibodies, which have detrimental effects on natalizumab treatment response. A positive correlation between the level of neutralizing antibodies and Gd+lesions on MRI was found (102, 103). During first 90 days of treatment with natalizumab, neutralizing antibodies were found among 6% of patients (104-106). Still, it is recommended to determine the level of neutralizing antibodies within 3 to 4 months after the therapy onset, as antibodies are usually formed within first six months of treatment (107, 108). Neutralizing antibodies can also act as a biomarker for therapeutic adverse effect, as they are associated with the infusion-related adverse effects (109).

Validated biomarkers
CXC motif chemokine-13
CXCL13 is a potent B cell chemoattractant; it has a significant role in the recruitment of B cells into the CNS in MS. A relationship between high levels of CXCL13 and high disease activity was previously reported (110). Additionally, patients receiving natalizumab therapy had lower CXCL13 values than patients treated with IFN-β (111). Another study also observed a reduction in CXCL13 levels after conversion from IFN-β, glatiramer acetate, or teriflunomide to fingolimod (112). Therefore, CXCL13 can serve as a biomarker of therapeutic response to different DMTs, especially fingolimod. Moreover, increased levels of CXCL13 at the time of CIS were associated with a worse
disease prognosis and severe disability (113) and along with other biomarkers, such as IgG and IgM OCBs, predicted conversion of CIS patients to MS patients (47). Therefore, CXCL13 could also be employed as a prognostic factor of conversion from CIS to MS. Finally, as previously mentioned, CXCL13 belongs also to the group of exploratory diagnostic MS biomarker.

**Exploratory biomarker**

A novel biomarker of microglia activation which could be a useful biomarker for therapeutic response is the triggering receptor expressed on myeloid cells 2 (TREM-2) (114, 115). TREM-2 levels increase after treatment with natalizumab and mitoxantrone; however, the mechanism is still not understood (116). Therefore, further research is warranted to confirm these findings.

**Biomarkers for monitoring therapeutic adverse effects**

In addition to clinical response, adverse effects are a decisive criterion for the success of a therapy. Molecular biomarkers can be an important tool for predicting and monitoring adverse effects. For some biomarkers, further long-term studies in the large groups of MS patients are still needed before their implementation into clinical practice.

**Biomarkers in the clinical practice**

Important biomarkers for monitoring therapeutic adverse effects of several DMTs which are already in clinical praxis are anti-John Cunningham (JC) virus and anti-Varicella-zoster virus (VZV) antibodies.

**Anti-John Cunningham virus antibodies**

JC virus is a small, non-enveloped, double stranded DNA virus belonging to *Polyomaviridae* family that infects only humans. JC virus can trigger progressive multifocal leukoencephalopathy (PML), a rare demyelinating disease in the CNS (117, 118). It has been shown that JC virus can become reactivated in the CNS, after living latently in other organs, and lead to infection of oligodendrocytes and astrocytes resulting in encephalopathy (119). The status of anti-JCV antibodies in plasma or serum is important in assessing the risk of PML in MS patients, where certain DMTs (especially natalizumab) may be implicated. Although infection by JC virus is a prerequisite for PML, the mechanism by which natalizumab can react with JCV in the CNS is not clear (120). PML tends to occur at least 24 months after initiating treatment with natalizumab (121). Prevalence of anti-JCV antibodies is approximately 57%, according to the largest study executed in 7724 MS patients from 10 countries (122). Prevalence seems to increase with age and to be lower in females compared to males.

**Anti-varicella zoster virus antibodies**

VZV is a neurotropic herpesvirus that is commonly acquired in childhood, when it causes a mild disease, with malaise and itchy rash for a few days. However, when
acquired in adult age, especially in immunocompromised individuals, it can represent a significant health risk.

Antibodies against VZV are a biomarker for adverse effects of various DMT used in RRMS. Recently, it has been shown that there is a good correlation between the antibody level and the cellular VZV response (123). To avoid VZV reactivation during the course of therapy, the anti-VZV antibody titers should be determined in serum before starting treatment with fingolimod, alemtuzumab, and cladribine in patients who have previously not had chickenpox disease or have not been vaccinated (124, 125). In cladribine therapy, herpes prophylaxis should be considered if the lymphocyte counts drop below 200/μl for the duration of grade 4 lymphopenia (126).

Exploratory biomarkers

L-selectin (CD62L) is an adhesion molecule on the cell surface of lymphocytes. The proportion of CD62L-expressing CD4+ T cells in peripheral mononuclear blood cells is a promising biomarker candidate for the assessment of PML risk in natalizumab therapy (127), as a correlation between the CD62L values and the JCV serostatus and JCV index has been found (128). A low CD62L proportion increased the risk of developing PML by a factor of 55. However, another study with 21 PML patients treated with natalizumab and 104 control group patients treated with natalizumab showed no correlation between CD62L level and PML risk (129). Therefore, further studies are necessary to validate CD62L as a biomarker for therapeutic adverse effects of DMTs.

Conclusion

Molecular biomarkers can aid in making a personalized decision from MS diagnosis towards therapy. An ideal biomarker should be easily measured; moreover, it should be highly sensitive and specific, cost-effective, robust, and reproducible in different laboratories across different patient populations. Currently, there are several biomarkers already implemented into the clinical practice: oligoclonal bands and the IgG index, anti-AQP4 antibodies, neutralizing antibodies against IFN-β and natalizumab, as well as anti-JCV and anti-VZV antibodies. There are some promising biomarkers validated in several studies, such as NFL, CXCL13 and CHI3L1, which could be used for different purposes (diagnosis, conversion from CIS to MS, and monitoring of therapeutic response). Still, there are many biomarkers which need more stringent validation in long-term studies with larger cohorts of patients. These studies will hopefully allow quicker movement from bench to bedside.
References


**Molekularni biomarkeri u multiploj sklerozi**

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**Kratak sadržaj**

Multipla skleroza (MS) je veoma heterogena bolest u pogledu radioloških, patoloških i kliničkih karakteristika i terapijskog odgovora, uključujući i efikasnost i bezbednosni profil tretmana. Shodno tome, postoji velika potražnja za biomarkerima koji osjetljivo i specifično reflektuju specifične aspekte heterogenosti MS, i koji mogu pomoći u boljem razumevanju dijagnoze bolesti, prognoze, predviđanja odgovora na lečenje, kao i u razvoju novih tretmana. Trenutno, kliničke karakteristike (npr. stopa relapsa i progresija bolesti) i snimanje magnetnom rezonomcom igraju najvažniju ulogu u kliničkoj klasifikaciji i proceni toka MS. Molekularni biomarkeri (npr. oligoklonalne imunoglobulin G (IgG) trake, IgG indeks, anti-akvaporin-4 antitela, anti-interferon-beta i anti-natalizumab neutrališuća antitela, anti-varicella zoster virus i anti-John Cunningham (JC) antitela) odlično dopunjuju ove markere. U ovom preglednom radu je dat kratak rezime validiranih, klinički korisnih molekularnih biomarkera, kao i biomarkera u fazi istraživanja u MS koji se mogu koristiti za predikciju, dijagnozu, određivanje aktivnosti bolesti i terapijskog odgovora u pogledu efikasnosti i bezbednosti lečenja.

**Ključne reči:** multipla skleroza, molekularni biomarker, neurofilament, cerebrospinalna tečnost, dijagnoza, terapijski odgovor