Molecular biomarkers in multiple sclerosis

**Danica Michaličková*, Hatice Kübra Öztürk, Debanjan Das,
Syed Osama Bukhari, Ondřej Slanař**

Institute of Pharmacology, First Faculty of Medicine & General University Hospital,
Charles University, Albertov 4, 12800, Prague, Czech Republic

*Corresponding author: Danica Michaličková, e-mail: marrtta@gmail.com

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

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).

Table I   Molecular biomarkers currently in clinical use
Tabela I   Molekularni biomarkeri koji su trenutno u kliničkoj upotrebi

Biomarker	Matrix for detection	Biomarker categorization
IgG oligoclonal bands	CSF, serum	Diagnostic
IgG index	CSF, serum	Diagnostic
Anti-aquaporin-4 antibodies	CSF, serum	Diagnostic (differential diagnosis between MS and NMOSD)
Neutralizing antibodies against interferon-beta	Serum, PBMCs	Interferon-beta response biomarker
Neutralizing antibodies against natalizumab	Serum	Natalizumab-response biomarker
Anti-VZV antibodies	Serum, plasma	Fingolimod-response biomarker for adverse effects
Anti-JC virus antibodies	Serum, plasma	Natalizumab-response biomarker for adverse effects

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/isoelectric 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 *Polymaviridae* 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

1. Van Den Hoogen WJ, Laman JD,T Hart BA. Modulation of multiple sclerosis and its animal model experimental autoimmune encephalomyelitis by food and gut microbiota. Front Immunol. 2017;8:1081.

2. Grigoriadis N, Pesch V. A basic overview of multiple sclerosis immunopathology. Eur J Neurol. 2015;22(S2):3-13.

3. Comabella M, Montalban X. Body fluid biomarkers in multiple sclerosis. Lancet Neurol. 2014;13(1):113-126.

4. Group BDW, Atkinson Jr AJ, Colburn WA, Degruttola VG, Demets DL, Downing GJet al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharm Therap. 2001;69(3):89-95.

5. Ziemssen T, Akgün K,Brück W. Molecular biomarkers in multiple sclerosis. J Neuroinflammation. 2019;16(1):1-11.

6. Paul A, Comabella M,Gandhi R. Biomarkers in multiple sclerosis. Cold Spring Harbor perspectives in medicine. 2019;9(3):a029058.

7. Ottervald J, Franzén B, Nilsson K, Andersson LI, Khademi M, Eriksson Bet al. Multiple sclerosis: Identification and clinical evaluation of novel CSF biomarkers. J Proteom. 2010;73(6):1117-1132.

8. Guerau-De-Arellano M, Alder H, Ozer HG, Lovett-Racke A,Racke MK. miRNA profiling for biomarker discovery in multiple sclerosis: from microarray to deep sequencing. J Neuroimmunol. 2012;248(1-2):32-39.

9. Del Boccio P, Pieragostino D, Lugaresi A, Di Ioia M, Pavone B, Travaglini Det al. Cleavage of cystatin C is not associated with multiple sclerosis. Ann Neurol. 2007;62(2):201-204.

10. Lindsey JW, Crawford MP,Hatfield LM. Soluble Nogo-A in CSF is not a useful biomarker for multiple sclerosis. Neurology. 2008;71(1):35-37.

11. Waschbisch A, Sandbrink R, Hartung H-P, Kappos L, Schwab S, Pohl Cet al. Evaluation of soluble HLA-G as a biomarker for multiple sclerosis. Neurology. 2011;77(6):596-598.

12. Bushnell S, Zhao Z, Stebbins C, Cadavid D, Buko A, Whalley Eet al. Serum IL-17F does not predict poor response to IM IFNβ-1a in relapsing-remitting MS. Neurology. 2012;79(6):531-537.

13. Whitaker JN, Williams PH, Layton BA, Mcfarland HF, Stone LA, Smith Met al. Correlation of clinical features and findings on cranial magnetic resonance imaging with urinary myelin basic protein-like material in patients with multiple sclerosis. Ann Neurol. 1994;35(5):577-585.

14. Lund SA, Giachelli CM,Scatena M. The role of osteopontin in inflammatory processes. J Cell Commun Signal. 2009;3(3):311-322.

15. Ram M, Sherer Y,Shoenfeld Y. Matrix metalloproteinase-9 and autoimmune diseases. J Clin Immunol. 2006;26(4):299-307.

16. Disanto G, Adiutori R, Dobson R, Martinelli V, Dalla Costa G, Runia Tet al. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. J Neurol Neurosurg Psychiatry. 2016;87(2):126-129.

17. Martínez MaM, Olsson B, Bau L, Matas E, Calvo ÁC, Andreasson Uet al. Glial and neuronal markers in cerebrospinal fluid predict progression in multiple sclerosis. Mult Scler J. 2015;21(5):550-561.

18. Arrambide G, Espejo C, Eixarch H, Villar LM, Alvarez-Cermeño JC, Picón Cet al. Neurofilament light chain level is a weak risk factor for the development of MS. Neurology. 2016;87(11):1076-1084.

19. Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, Naegelin Yet al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain. 2018;141(8):2382-2391.

20. Kuhle J, Nourbakhsh B, Grant D, Morant S, Barro C, Yaldizli Öet al. Serum neurofilament is associated with progression of brain atrophy and disability in early MS. Neurology. 2017;88(9):826-831.

21. Harris VK, Tuddenham JF,Sadiq SA. Biomarkers of multiple sclerosis: current findings. Degenerative neurological and neuromuscular disease. 2017;7:19.

22. Gafson AR,Giovannoni G. Towards the incorporation of lumbar puncture into clinical trials for multiple sclerosis. Mult Scler J. 2012;18(10):1509-1511.

23. Ziegler A, Koch A, Krockenberger K,Großhennig A. Personalized medicine using DNA biomarkers: a review. Hum Genet. 2012;131(10):1627-1638.

24. Sundström P, Juto P, Wadell G, Hallmans G, Svenningsson A, Nyström Let al. An altered immune response to Epstein-Barr virus in multiple sclerosis: a prospective study. Neurology. 2004;62(12):2277-2282.

25. De Jager P, Simon K, Munger K, Rioux J, Hafler D,Ascherio A. Integrating risk factors: HLA-DRB1* 1501 and Epstein–Barr virus in multiple sclerosis. Neurology. 2008;70(13 Part 2):1113-1118.

26. Hedström AK, Huang J, Michel A, Butt J, Brenner N, Hillert Jet al. High levels of Epstein–Barr virus nuclear antigen-1-specific antibodies and infectious mononucleosis act both independently and synergistically to increase multiple sclerosis risk. Front Neurol. 2020;10:1368.

27. Monson NL, Brezinschek H-P, Brezinschek RI, Mobley A, Vaughan GK, Frohman EMet al. Receptor revision and atypical mutational characteristics in clonally expanded B cells from the cerebrospinal fluid of recently diagnosed multiple sclerosis patients. J Neuroimmunol. 2005;158(1):170-181.

28. Lefvert AK,Link H. IgG production within the central nervous system: a critical review of proposed formulae. Ann Neurol. 1985;17(1):13-20.

29. Ziemssen T, Akgün K,Brück W. Molecular biomarkers in multiple sclerosis. J Neuroinflammation. 2019;16(1):272-272.

30. Holmøy T. The Discovery of Oligoclonal Bands: A 50-Year Anniversary. Eur Neurol. 2009;62(5):311-315.

31. Freedman MS, Thompson EJ, Deisenhammer F, Giovannoni G, Grimsley G, Keir Get al. Recommended standard of cerebrospinal fluid analysis in the diagnosis of multiple sclerosis: a consensus statement. Arch Neurol. 2005;62(6):865-70.

32. Keir G, Luxton RW,Thompson EJ. Isoelectric focusing of cerebrospinal fluid immunoglobulin G: an annotated update. Ann Clin Biochem. 1990;27 (Pt 5):436-43.

33. Ohman S, Ernerudh J, Forsberg P, Henriksson A, Von Schenck H,Vrethem M. Comparison of seven formulae and isoelectrofocusing for determination of intrathecally produced IgG in neurological diseases. Ann Clin Biochem. 1992;29 (Pt 4):405-10.

34. Petzold A. Intrathecal oligoclonal IgG synthesis in multiple sclerosis. J Neuroimmunol. 2013;262(1):1-10.

35. Poser CM, Paty DW, Scheinberg L, Mcdonald WI, Davis FA, Ebers GCet al. New diagnostic criteria for multiple sclerosis: Guidelines for research protocols. Ann Neurol. 1983;13(3):227-231.

36. Mcdonald WI, Compston A, Edan G, Goodkin D, Hartung H-P, Lublin FDet al. Recommended diagnostic criteria for multiple sclerosis: Guidelines from the international panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001;50(1):121-127.

37. Polman CH, Reingold SC, Edan G, Filippi M, Hartung H-P, Kappos Let al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58(6):840-846.

38. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi Get al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018;17(2):162-173.

39. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi Met al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011;69(2):292-302.

40. Link H,Huang Y-M. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J Neuroimmunol. 2006;180(1-2):17-28.

41. Makhani N, Lebrun C, Siva A, Narula S, Wassmer E, Brassat Det al. Oligoclonal bands increase the specificity of MRI criteria to predict multiple sclerosis in children with radiologically isolated syndrome. Mult Scler J Exp Transl Clin. 2019;5(1):2055217319836664-2055217319836664.

42. Ziemssen T,Ziemssen F. The role of the humoral immune system in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). Autoimmun Rev. 2005;4(7):460-467.

43. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ,Weinshenker BG. The spectrum of neuromyelitis optica. Lancet Neurol. 2007;6(9):805-815.

44. Flanagan EP, Cabre P, Weinshenker BG, Sauver JS, Jacobson DJ, Majed Met al. Epidemiology of aquaporin-4 autoimmunity and neuromyelitis optica spectrum. Ann Neurol. 2016;79(5):775-783.

45. Waters PJ, Pittock SJ, Bennett JL, Jarius S, Weinshenker BG,Wingerchuk DM. Evaluation of aquaporin-4 antibody assays. Clin Exp Neuroimmunol. 2014;5(3):290-303.

46. Thangarajh M, Gomez-Rial J, Hedstrom AK, Hillert J, Alvarez-Cermeno JC, Masterman Tet al. Lipid-specific immunoglobulin M in CSF predicts adverse long-term outcome in multiple sclerosis. Mult Scler. 2008;14(9):1208-13.

47. De Biasi S, Simone AM, Bianchini E, Lo Tartaro D, Pecorini S, Nasi Met al. Mitochondrial functionality and metabolism in T cells from progressive multiple sclerosis patients. Eur J Immunol. 2019;49(12):2204-2221.

48. Sadaba MC, Tzartos J, Paino C, Garcia-Villanueva M, Alvarez-Cermeno JC, Villar LMet al. Axonal and oligodendrocyte-localized IgM and IgG deposits in MS lesions. J Neuroimmunol. 2012;247(1-2):86-94.

49. Hinsinger G, Galeotti N, Nabholz N, Urbach S, Rigau V, Demattei Cet al. Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. Mult Scler. 2015;21(10):1251-61.

50. Borras E, Canto E, Choi M, Maria Villar L, Alvarez-Cermeno JC, Chiva Cet al. Protein-Based Classifier to Predict Conversion from Clinically Isolated Syndrome to Multiple Sclerosis. Mol Cell Proteomics. 2016;15(1):318-28.

51. Burman J, Raininko R, Blennow K, Zetterberg H, Axelsson M,Malmeström C. YKL-40 is a CSF biomarker of intrathecal inflammation in secondary progressive multiple sclerosis. J Neuroimmunol. 2016;292:52-57.

52. Canto E, Tintore M, Villar LM, Costa C, Nurtdinov R, Alvarez-Cermeno JCet al. Chitinase 3-like 1: prognostic biomarker in clinically isolated syndromes. Brain. 2015;138(4):918-931.

53. Correale J,Fiol M. Chitinase effects on immune cell response in neuromyelitis optica and multiple sclerosis. Mult Scler J. 2011;17(5):521-531.

54. Modvig S, Degn M, Horwitz H, Cramer SP, Larsson HB, Wanscher Bet al. Relationship between cerebrospinal fluid biomarkers for inflammation, demyelination and neurodegeneration in acute optic neuritis. PloS one. 2013;8(10):e77163.

55. Hinsinger G, Galéotti N, Nabholz N, Urbach S, Rigau V, Demattei Cet al. Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. Mult Scler J. 2015;21(10):1251-1261.

56. Ingram G, Hakobyan S, Robertson N, Morgan BP. Complement in multiple sclerosis: its role in disease and potential as a biomarker. Clin Exp Immunol. 2009;155(2):128-139.

57. Abarca-Zabalía J, García MI, Lozano Ros A, Marín-Jiménez I, Martínez-Ginés ML, López-Cauce B et al. Differential Expression of SMAD Genes and S1PR1 on Circulating CD4+ T Cells in Multiple Sclerosis and Crohn's Disease. Int J Mol Sci. 2020;21(2):1-14.

58. Tatomir A, Talpos-Caia A, Anselmo F, Kruszewski AM, Boodhoo D, Rus Vet al. The complement system as a biomarker of disease activity and response to treatment in multiple sclerosis. Immunol Res. 2017;65(6):1103-1109.

59. Ingram G, Hakobyan S, Hirst CL, Harris CL, Loveless S, Mitchell JPet al. Systemic complement profiling in multiple sclerosis as a biomarker of disease state. Mult Scler. 2012;18(10):1401-11.

60. Ingram G, Hakobyan S, Hirst CL, Harris CL, Pickersgill TP, Cossburn MDet al. Complement regulator factor H as a serum biomarker of multiple sclerosis disease state. Brain. 2010;133(6):1602-1611.

61. Hakobyan S, Luppe S, Evans DR, Harding K, Loveless S, Robertson NPet al. Plasma complement biomarkers distinguish multiple sclerosis and neuromyelitis optica spectrum disorder. Mult Scler. 2017;23(7):946-955.

62. Jarius S, Eichhorn P, Franciotta D, Petereit HF, Akman-Demir G, Wick Met al. The MRZ reaction as a highly specific marker of multiple sclerosis: re-evaluation and structured review of the literature. J Neurol. 2017;264(3):453-466.

63. Brettschneider J, Tumani H, Kiechle U, Muche R, Richards G, Lehmensiek Vet al. IgG antibodies against measles, rubella, and varicella zoster virus predict conversion to multiple sclerosis in clinically isolated syndrome. PLoS One. 2009;4(11):e7638.

64. Órpez-Zafra T, Pavía J, Hurtado-Guerrero I, Pinto-Medel MJ, Rodriguez Bada JL, Urbaneja Pet al. Decreased soluble IFN-β receptor (sIFNAR2) in multiple sclerosis patients: A potential serum diagnostic biomarker. Mult Scler. 2017;23(7):937-945.

65. Shi Y, Ding Y, Li G, Wang L, Osman RA, Sun Jet al. Discovery of Novel Biomarkers for Diagnosing and Predicting the Progression of Multiple Sclerosis Using TMT-Based Quantitative Proteomics. Front Immunol. 2021;12:700031.

66. Islas-Hernandez A, Aguilar-Talamantes HS, Bertado-Cortes B, Mejia-Delcastillo GJ, Carrera-Pineda R, Cuevas-Garcia CFet al. BDNF and Tau as biomarkers of severity in multiple sclerosis. Biomark Med. 2018;12(7):717-726.

67. Khademi M, Kockum I, Andersson ML, Iacobaeus E, Brundin L, Sellebjerg Fet al. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult Scler. 2011;17(3):335-43.

68. Amor S,Giovannoni G. Antibodies to myelin oligodendrocyte glycoprotein as a biomarker in multiple sclerosis — are we there yet? Multiple Sclerosis Journal. 2007;13(9):1083-1085.

69. Lalive PH, Menge T, Delarasse C, Della Gaspera B, Pham-Dinh D, Villoslada Pet al. Antibodies to native myelin oligodendrocyte glycoprotein are serologic markers of early inflammation in multiple sclerosis. Proc Natl Acad Sci U S A. 2006;103(7):2280-5.

70. Deisenhammer F, Zetterberg H, Fitzner B,Zettl UK. The Cerebrospinal Fluid in Multiple Sclerosis. Front Immunol. 2019;10:726-726.

71. Jarius S, Paul F, Aktas O, Asgari N, Dale RC, De Seze Jet al. MOG encephalomyelitis: international recommendations on diagnosis and antibody testing. J Neuroinflammation. 2018;15(1):134.

72. Spadaro M, Gerdes LA, Krumbholz M, Ertl-Wagner B, Thaler FS, Schuh Eet al. Autoantibodies to MOG in a distinct subgroup of adult multiple sclerosis. Neurol.: Neuroimmunol. NeuroInflammation. 2016;3(5).

73. Höftberger R,Lassmann H, Chapter 19 - Inflammatory demyelinating diseases of the central nervous system, in Handbook of Clinical Neurology, G.G. Kovacs and I. Alafuzoff, Editors. 2018, Elsevier. p. 263-283.

74. Papp V, Langkilde AR, Blinkenberg M, Schreiber K, Jensen PEH,Sellebjerg F. Clinical utility of anti-MOG antibody testing in a Danish cohort. Mult Scler Relat Disord. 2018;26:61-67.

75. Tomassini V, De Giglio L, Reindl M, Russo P, Pestalozza I, Pantano Pet al. Anti-myelin antibodies predict the clinical outcome after a first episode suggestive of MS. Mult Scler. 2007;13(9):1086-94.

76. Abzalimov RR, Kaplan DA, Easterling ML,Kaltashov IA. Protein conformations can be probed in top-down HDX MS experiments utilizing electron transfer dissociation of protein ions without hydrogen scrambling. J Am Soc Mass Spectrom. 2009;20(8):1514-1517.

77. Massaro AR, De Pascalis D, Carnevale A,Carbone G. The neural cell adhesion molecule (NCAM) present in the cerebrospinal fluid of multiple sclerosis patients is unsialylated. Eur Rev Med Pharmacol Sci. 2009;13(5):397-9.

78. Massaro AR. The role of NCAM in remyelination. Neurological Sciences. 2002;22(6):429-435.

79. Gnanapavan S, Grant D, Illes-Toth E, Lakdawala N, Keir G,Giovannoni G. Neural cell adhesion molecule-description of a CSF ELISA method and evidence of reduced levels in selected neurological disorders. J Neuroimmunol. 2010;225(1-2):118-22.

80. Strekalova H, Buhmann C, Kleene R, Eggers C, Saffell J, Hemperly J et al. Elevated levels of neural recognition molecule L1 in the cerebrospinal fluid of patients with Alzheimer disease and other dementia syndromes. Neurobiol Aging. 2006;27(1):1-9.

81. Yuan A, Rao MV,Nixon RA. Neurofilaments at a glance. J Cell Sci. 2012;125(14):3257-3263.

82. Karlsson Je RL, Haglid Kg. Quantitative and qualitative alterations of neuronal and glial intermediate filaments in rat nervous system after exposure to 2,5-hexanedione. J Neurochem. 1991;5(Oct):1437-1444.

83. Novakova L ZH, Sundström P, Axelsson M, Khademi M, Gunnarsson M, Malmeström C, Svenningsson a, Olsson T, Piehl F, Blennow K, Lycke J. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. 2017;Nov 28(89(22)):2230-2237.

84. Amor S VDSB, Bosca I, Raffel J, Gnanapavan S, Watchorn J, Kuhle J, Giovannoni G, Baker D, Malaspina a, Puentes F. Neurofilament light antibodies in serum reflect response to natalizumab treatment in multiple sclerosis. Mult Scler. 2014;Sept(20(10)):1355-1362.

85. Akgün K KN, Haase R, Proschmann U, Kitzler Hh, Reichmann H, Ziemssen T. Profiling individual clinical responses by high-frequency serum neurofilament assessment in MS. Neurol Neuroimmunol Neuroinflamm. 2019;May(6(3)):e555.

86. Van Horssen J, Schreibelt G, Drexhage J, Hazes T, Dijkstra C, Van Der Valk Pet al. Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression. Free Radic Biol Med. 2008;45(12):1729-1737.

87. Graversen JH, Madsen M,Moestrup SK. CD163: a signal receptor scavenging haptoglobin–hemoglobin complexes from plasma. Int J Biochem Cell Biol. 2002;34(4):309-314.

88. Stilund M, Reuschlein AK, Christensen T, Moller HJ, Rasmussen PV,Petersen T. Soluble CD163 as a marker of macrophage activity in newly diagnosed patients with multiple sclerosis. PLoS One. 2014;9(6):e98588.

89. Chwojnicki K, Iwaszkiewicz-Grześ D, Jankowska A, Zieliński M, Łowiec P, Gliwiński M et al. Administration of CD4 + CD25 high CD127 - FoxP3 + Regulatory T Cells for Relapsing-Remitting Multiple Sclerosis: A Phase 1 Study. BioDrugs. 2021;35(1):47-60.

90. Pette M, Fujita K, Kitze B, Whitaker JN, Albert E, Kappos Let al. Myelin basic protein-specific T lymphocyte lines from MS patients and healthy individuals. Neurology. 1990;40(11):1770-6.

91. Gafson A, Craner MJ,Matthews PM. Personalised medicine for multiple sclerosis care. Mult Scler J. 2017;23(3):362-369.

92. Nikfar S RR, Abdollahi M. A meta-analysis of the efficacy and tolerability of interferon-β in multiple sclerosis, overall and by drug and disease type. Clin Ther. 2010;2010 Oct;32(11):1871-88.

93. Rudick RA, Lee JC, Simon J, Ransohoff RM,Fisher E. Defining interferon β response status in multiple sclerosis patients. Ann Neurol. 2004;56(4):548-555.

94. Bachelet D, Hässler S, Mbogning C, Link J, Ryner M, Ramanujam Ret al. Occurrence of anti-drug antibodies against interferon-beta and natalizumab in multiple sclerosis: a collaborative cohort analysis. PloS one. 2016;11(11):e0162752.

95. Dunn N, Fogdell-Hahn A, Hillert J,Spelman T. Long-Term Consequences of High Titer Neutralizing Antibodies to Interferon-β in Multiple Sclerosis. Front Immunol. 2020;11.

96. Deisenhammer F, Schellekens, H. & Bertolotto. Measurement of neutralizing antibodies to interferon beta in patients with multiple sclerosis. J Neurol. 2004;251:ii31–ii39.

97. Polman CH, Bertolotto A, Deisenhammer F, Giovannoni G, Hartung H-P, Hemmer Bet al. Recommendations for clinical use of data on neutralising antibodies to interferon-beta therapy in multiple sclerosis. Lancet Neurol. 2010;9(7):740-750.

98. Bertolotto A, Gilli F, Sala A, Audano L, Castello A, Magliola Uet al. Evaluation of bioavailability of three types of IFNβ in multiple sclerosis patients by a new quantitative-competitive-PCR method for MxA quantification. J Immunol Methods. 2001;256(1-2):141-152.

99. Malucchi S, Gilli F, Caldano M, Marnetto F, Valentino P, Granieri Let al. Predictive markers for response to interferon therapy in patients with multiple sclerosis. Neurology. 2008;70(13 Part 2):1119-1127.

100. Yednock T.A. CC, Fritz L.C., Sanchez-Madrid F., Steinmant L. Prevention of experimental autoimmune encephalomyelitis by antibodies against α4β1 integrin. Nature. 1992;356:63-66.

101. Mattoscio M NR, Sormani Mp. Hematopoietic mobilization: Potential biomarker of response to natalizumab in multiple sclerosis. Neurology. 2015;84(14):1473-1482.

102. Vennegoor A RT, Mrijbis E, Seewann a, Uitdehaag Bm, Balk Lj. Clinical relevance of serum natalizumab concentration and anti-natalizumab antibodies in multiple sclerosis. Mult Scler J. 2012;19:593-600.

103. Sehr T, Proschmann U, Thomas K, Marggraf M, Straube E, Reichmann Het al. New insights into the pharmacokinetics and pharmacodynamics of natalizumab treatment for patients with multiple sclerosis, obtained from clinical and in vitro studies. J Neuroinflammation. 2016;13(1):1-11.

104. Hegen H AM, Deisenhammer F. Predictors of Response to Multiple Sclerosis Therapeutics in Individual Patients. Drugs. 2016;76:1421-1445.

105. Sehr T PU, Thomas K, Marggraf M, Straube E, Reichmann H. New insights into the pharmacokinetics and pharmacodynamics of natalizumab treatment for patients with multiple sclerosis, obtained from clinical and in vitro studies. J Neuroinflammation. 2016;13:164.

106. Kaufmann M HR, Proschmann U, Ziemssen T, AkgüN K. Real-World Lab Data in Natalizumab Treated Multiple Sclerosis Patients Up to 6 Years Long- Term Follow Up. Front Neurol. 2018;9:1071.

107. Deisenhammer F JM, Lauren a, Sj Din a, Ryner M, Fogdell-Hahn A. Prediction of natalizumab anti-drug antibodies persistency. Multiple Scler Houndmills Basingstoke Engl. 2018;1352458517753721.

108. Link J RR, Auer M, Ryner M, H Ssler S, Bachelet D. Clinical practice of analysis of anti-drug antibodies against interferon beta and natalizumab in multiple sclerosis patients in Europe: A descriptive study of test results. PLoS One. 2017;12:e0170395.

109. Calabresi P GG, Confavreux C, Galetta S, Havrdova E, Hutchinson,M. The incidence and significance of anti-natalizumab antibodies: Results from AFFIRM and SENTINEL. Neurology. 2007;69(1):391-400.

110. Khademi M KI, Andersson Ml, Iacobaeus E, Brundin L, Sellebjerg F, Hillert J, Piehl F, Olsson T. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult Scler. 2011;17(3):335-343.

111. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, Malmeström Cet al. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. J Neurochem. 2017;141(2):296-304.

112. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, Malmeström Cet al. Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis. Mult Scler J. 2017;23(1):62-71.

113. Uher T, Horakova D, Tyblova M, Zeman D, Krasulova E, Mrazova Ket al. Increased albumin quotient (QAlb) in patients after first clinical event suggestive of multiple sclerosis is associated with development of brain atrophy and greater disability 48 months later. Mult Scler. 2016;22(6):770-81.

114. Klesney-Tait J, Turnbull IR,Colonna M. The TREM receptor family and signal integration. Nat Immunol. 2006;7(12):1266-73.

115. Sessa G, Podini P, Mariani M, Meroni A, Spreafico R, Sinigaglia Fet al. Distribution and signaling of TREM2/DAP12, the receptor system mutated in human polycystic lipomembraneous osteodysplasia with sclerosing leukoencephalopathy dementia. Eur J Neurosci. 2004;20(10):2617-28.

116. Öhrfelt A, Axelsson M, Malmeström C, Novakova L, Heslegrave A, Blennow Ket al. Soluble TREM-2 in cerebrospinal fluid from patients with multiple sclerosis treated with natalizumab or mitoxantrone. Mult Scler J. 2016;22(12):1587-1595.

117. A. Bellizzi CN, E. Anzivino, D. Rodio, D. Fioriti, M. Mischitelli, F. Chiarini, V. Pietropaolo. Human polyomavirus JC reactivation and pathogenetic mechanisms of progressive multifocal leukoencephalopathy and cancer in the era of monoclonal antibody therapies. J Neurovirol. 2012;18:1-11.

118. C.S. Tan YC, R.P. Viscidi, R.P. Kinkel, M.C. Stein, I.J. Koralnik. Discrepant findings in immune responses to JC virus in patients receiving natalizumab. Lancet Neurol. 2010;9:565-566.

119. Mcgavern DB,Kang SS. Illuminating viral infections in the nervous system. Nat Rev Immunol. 2011;11(5):318-329.

120. Gorelik L LM, Bixler S, Crossman M, Schlain B, Simon K, Pace a, Cheung a, Chen Ll, Berman M, Zein F, Wilson E, Yednock T, Sandrock a, Goelz Se, Subramanyam M. Anti-JC virus antibodies: implications for PML risk stratification. Ann Neurol. 2010;68(3):295-303.

121. Antoniol C,Stankoff B. Immunological Markers for PML Prediction in MS Patients Treated with Natalizumab. Front Immunol. 2014;5:668.

122. Bozic C, Subramanyam M, Richman S, Plavina T, Zhang A,Ticho B. Anti-JC virus (JCV) antibody prevalence in the JCV Epidemiology in MS (JEMS) trial. Eur J Neurol. 2014;21(2):299-304.

123. Matko S AK, Tonn T, Ziemssen T, Odendahl M. Antigen-shift in varicella-zoster virus-specific T-cell immunity over the course of Fingolimod-treatment in relapse-remitting multiple sclerosis patients. Mult Scler Relat Disord. 2020;Feb(38):101859.

124. Arvin Am WJ, Kappos L, Morris Mi, Reder at, Tornatore C, Gershon a, Gershon M, Levin Mj, Bezuidenhoudt M, Putzki N. Varicella-zoster virus infections in patients treated with fingolimod: risk assessment and consensus recommendations for management. JAMA Neurol. 2015;72(1)(Jan):31-39.

125. Ziemssen T TK. Alemtuzumab in the long-term treatment of relapsing-remitting multiple sclerosis: an update on the clinical trial evidence and data from the real world. Ther Adv Neurol Disord. 2017;10(10)(Oct):343-359.

126. Cook S LT, Comi G, Montalban X, Giovannoni G, Nolting a, Hicking C, Galazka a, Sylvester E. Safety of cladribine tablets in the treatment of patients with multiple sclerosis: An integrated analysis. Mult Scler Relat Disord. 2019;Apr(29):157-167.

127. Schwab N S-HT, Posevitz V, Breuer J, Göbel K, Windhagen S, Brochet B, Vermersch P, Lebrun-Frenay C, Posevitz-Fejfár a, Capra R, Imberti L, Straeten V, Haas J, Wildemann B, Havla J, Kümpfel T, Meinl I, Niessen K, Goelz S, Kleinschnitz C, Warnke C, Buck D, Gold R, Kieseier Bc, Meuth Sg, Foley J, Chan a, Brassat D, Wiendl H. L-selectin is a possible biomarker for individual PML risk in natalizumab-treated MS patients. Neurology. 2013;Sep 3(81(10)):865-871.

128. Schwab N S-HT, Pignolet B, Spadaro M, Görlich D, Meinl I, Windhagen S, Tackenberg B, Breuer J, Cantó E, Kümpfel T, Hohlfeld R, Siffrin V, Luessi F, Posevitz-Fejfár a, Montalban X, Meuth Sg, Zipp F, Gold R, Du Pasquier Ra, Kleinschnitz C, Jacobi a, Comabella M, Bertolotto a, Brassat D, Wiendl H. PML risk stratification using anti-JCV antibody index and L-selectin. Mult Scler. 2016;July(22(8)):1048-1060.

129. Schwab N S-HT, Wiendl H. CD62L is not a reliable biomarker for predicting PML risk in natalizumab-treated R-MS patients. Schwab N, Schneider-Hohendorf T, Wiendl H. 2016;30(Aug):958-9.

Molekularni biomarkeri u multiploj sklerozi

**Danica Michaličková*, Hatice Kübra Öztürk, Debanjan Das,
Syed Osama Bukhari, Ondřej Slanař**

Institute of Pharmacology, First Faculty of Medicine & General University Hospital,
Charles University, Albertov 4, 12800, Prague, Czech Republic

*Autor za korespondenciju: Danica Michaličková, e-mail: marrtta@gmail.com

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 osetljivo i specifično reflektuju spcifične 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 rezonancom 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