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Narrative Review

Molecular Biomarkers in Multiple Sclerosis

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune inflammatory-neurodegenerative disease of the central nervous system (CNS) characterized by significant inter- and intra-individual different presentations. Using the clinical and imaging biomarkers is currently not able to predict the severity of disease. However, molecular biomarkers which are easily detectable come from the aspects of immunology and neurobiology due to the causal immunopathogenesis and can excellently predict other disease characteristics. Only a few molecular biomarkers have so far been routinely assessed in clinical practice as the assessment of their sensitivity, specificity, and measurement take a long time. In this review, we shed a light on the characteristics that an ideal MS biomarker should have and also the problems of introducing new biomarkers. Furthermore, clinically associated and well-established biomarkers from the blood and cerebrospinal fluid (CSF) are described which are practical for MS diagnosis and prognosis as well as for the evaluation of therapy response and complications.

Keywords: Multiple sclerosis, Molecular biomarker, Pathogenesis, Immune system, Blood.

Introduction

Multiple sclerosis (MS) is a chronic autoimmune neurological disorder presenting with inflammatory demyelination and neurodegeneration in the central nervous system (CNS) of young adults (1). The disease characterized by a great heterogeneity with respect to radiological and histopathological variations, clinical manifestations and progression, as well as response to treatment (2). It is therefore very essential to define specific characteristics of the disease that improve diagnosis and prognosis and allow an evaluation of the therapeutic response and risk of complications (3). Currently, the lesion load in the CNS is assessed by magnetic resonance imaging (MRI) as well as clinical features, e.g., relapse rate and severity of the disease, play the most pivotal role (4). However, although it is possible to measure and standardize these characteristics in larger sample size of patients, it is not possible until now in individual patients. Molecular biomarkers, on the other hand, are easily measureable and can excellently improve diagnostic accuracy of MRI and clinical symptoms. Biomarkers for MS extracted from the areas of immunology and neurobiology due to the assessment of immunopathogenesis of disease (5). Although the efficacy of molecular biomarkers has been increasingly investigated in recent years, their establishment is a lengthy process, so that only a few biomarkers have so far been approved in clinical practice. However, the number of possible biomarkers at different stages of evaluation is promising. This study reviewed the features that a promising MS biomarker should show and the problems of approving new biomarkers. Furthermore, clinically relevant and potential biomarkers from the blood and cerebrospinal fluid (CSF) are introduced which are effective for

prediction of MS diagnosis and prognosis as well as for the evaluation of response to treatment and complications.

What Are the Characteristics of an Ideal Biomarker for MS?

A biomarker is known as a characteristic that can be objectively quantified and assessed and serves as an marker of normal biological processes, pathological processes or pharmacological mechanisms following treatment (6). Ideally, this is a binary system, in other words a feature that is present in subjects with a certain disease and is absent in healthy subjects or subjects with another disease or vice versa. If the severity of disease increase or decrease, the level of the biomarker should increase or decrease accordingly. Another feature of an ideal biomarker is that it has no side effects for the patient and as easy to measure as possible, in the best case it is a non-invasive procedure. The technique of measurement should be highly accurate reproducible, and cost-effective and at the same time be done fast and simply. Moreover, the result of the measurement of the biomarker should be insensitive to systematic conditions such as sample collection, processing, and storage in the laboratory (7). In addition to the common clinical manifestations of a disease, imaging biomarkers are often assessed with the aid of imaging techniques. In this regard, MRI provides information on the size, number, duration, and development of lesions in the CNS and has a significant role in diagnosis and assessment of response to the treatment (8). In the future, brain atrophy could also play an important role if its evaluation becomes possible in MS patients. There are different types of molecular biomarkers including deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins. DNA as a molecular marker is less dependent to operator as well as an easier and less expensive measurement. In contrast, RNA and proteins are measurable biomarkers that are appropriate for monitoring disease specific processes. Regarding diagnosis of MS, all approved molecular biomarkers are currently proteins and most of them are antibodies (9). For the measurement of molecular biomarkers, a sample must be taken from the blood or CSF of patient. Because blood collection is known as a less invasive procedure, the validation of new molecular biomarkers should examine whether serum or plasma detection is as appropriate as CSF detection.

Major Issues in Improvement of Biomarkers

The above-mentioned properties of an optimal biomarker have to be considered and some additional problems have to be solved, which are presented below. Sensitivity and specificity are two important parts of biomarkers. Sensitivity is defined as the proportion of true positive test results among those who are actually affected by the disease. Specificity, on the other hand, is known as proportion of true negative results among those who are not patient (10). Since high sensitivity is commonly at the expense of low specificity and vice versa, it is of great importance to find biomarkers that have a logical balance of both properties. Other important characteristics of a promising biomarker are the positive and negative predictive value. These describe the proportion of patients with a positive or negative test result who are correctly diagnosed. Different analytical approaches are often used for the measurement of molecular biomarkers. However, the use of different measurement techniques can lead to different findings and thus severely limit the informative value of the biomarker. Research on interleukin (IL)-21 as a possible biomarker to predict the risk of secondary autoimmunity following treatment with alemtuzumab shows that even the exchange of individual components using the same measurement technique can change the results (11). In this case, the use of other enzyme-linked immunosorbent assay (ELISA) kits for measurement the concentration of IL-21 in the serum no longer revealed a predictive association. Therefore, the development of molecular biomarkers needs validation with different assessment techniques. Initial evaluation of new biomarkers usually take place in small sample sizes, followed by improvements of the biomarker candidates in large, independent cohort studies. However, this re-evaluation of the results in large populations is not always promising. Biomarker development is in some part comparable with drug development as independent validation has to be approved in large cohorts after positive pilot study. If biomarker tests are going to be used to improve patient care, than an increasing of knowledge and careful evaluation of these concepts are necessary, because “A Bad Biomarker Test Is as Bad as a Bad Drug”. Due to the problems presented above in establishing new biomarkers, accurate validation of possible candidates is necessary (12). In this regard, the accuracy of the detection procedure should be assessed and the validity of the findings in large

sample size of patients should be approved. Therefore, the expansion of the list of approved biomarkers for MS has so far been slow. Molecular biomarkers can improve the efficacy of MRI and clinical evaluations in different stages of MS disease. These include diagnosis and prediction of prognosis as well as response to treatments which affect progression of the disease and also the occurrence of complications.

Potential Biomarkers for Diagnosis of MS

Biomarkers that are appropriate for MS diagnosis should make it possible to discriminate between patients with MS and healthy subjects or those with other underlying diseases.

Oligoclonal Bands

Oligoclonal bands are bands of immunoglobulins that are detected when patient's blood serum and CSF are assessed in parallel. They are produced by immunoglobulin G (IgG) and M (IgM) created by plasma cells in the CNS (13). The existence of these immunoglobulins within the CSF, but not within the plasma, is a strong indicator of intrathecal antibody production and, interestingly, is detected in nearly all patients with diagnosis of clinically definitive MS. Intrathecal antibodies are commonly synthesized by plasma cells (terminally differentiated B cells), and hence higher activity of B cells in the pathogenesis of MS has long been suggested (14). In more than 95% of MS subjects, OCB are found in the CSF, but mostly not in plasma. However, OCB are not MS specific and can also be detected in other inflammatory CNS disorders. If other diagnoses are excluded though, OCB suggest the diagnosis of MS. They were already found in 1983 as a diagnostic indicator in MS and thus known as the first biomarker of this disease (14, 15). After OCB have meanwhile not been measured for diagnosis according to the McDonald criteria, they are now again assessed as a part of the diagnostic algorithm in the updated version of 2017 (16). This shift to substitute of a positive CSF finding for dissemination in time rather than to substitute for dissemination in space is a practical one, but it increases the responsibility of clinical neurologists to evaluate CSF biomarker (17). Patients with typical clinical manifestations, typical lesions, and with other diagnoses reasonably excluded most probably have multiple sclerosis. Approving the presence of OCB will show supporting evidence of the neuroinflammatory nature of the disease without having to demonstrate for dissemination in time to occur (17). OCB are thus known as an established biomarker with importance for MS diagnosis.

IgG Index

The immunoglobulin (Ig) G index is characterized by the ratio of the CSF/serum quotient of IgG to the CSF/serum quotient of the reference protein albumin (18). The albumin quotient, albumin in CSF/albumin in serum, is assessed as a measure of blood-CSF barrier damage in MS (19). IgG index is measured as a marker of intrathecal synthesis of immunoglobulins. A value of IgG index > 0.7 is an marker of an increased intrathecal B cell activity and thus suggests the diagnosis of MS (19). About 70% of MS patients show an increased IgG index. Therefore, the sensitivity of this index is lower than that of the OCB (20). Moreover, a disrupted IgG index rarely exists in MS patients without OCB. Nevertheless, the IgG index is one of the approved biomarkers of MS diagnosis and is regularly evaluated in the course of CSF diagnostics.

Measles, Rubella, Varicella-zoster Reaction

If antibodies against the neurotrophic viruses, measles virus, rubella virus, and varicella-zoster virus (VZV), are found in the CSF, this shows a poly-specific intrathecal B cell response. Therefore, the detection of measles, rubella, varicella-zoster (MRZ) reaction is one of the suggested measures in cases of suspected MS (21). Brettschneider and colleagues also revealed that an MRZ reaction is significantly more commonly measurable in patients with a conversion from clinically isolated syndrome (CIS) to MS than in patients who do not show clinically definite MS. This result is in line with the notion that immunological changes associated with B cell activation and intrathecal IgG production occur early on in the development of MS (22, 23). In MS, the intrathecal MRZ humoral reaction seems to show the enhanced B cell-promoting environment.

Anti-aquaporin-4 Antibodies

Aquaporin-4 (AQP-4) is a water channel protein synthesized in the CNS by astrocytes which plays a pivotal role in the regulation of water homeostasis in the CNS (24). Antibodies against this channel protein are measurable in about 75% of patients with neuromyelitis optica spectrum disorder (NMOSD), but not in MS patients (25). This makes anti-aquaporin-4 antibodies more specific for diagnosis of NMOSD. It is the first clinically approved

molecular biomarker that makes differentiation between various inflammatory demyelinating diseases of the CNS more possible for neurologists. Measurement of anti-aquaporin-4 antibodies is usually carried out in serum in patients suspected of having NMOSD. Different measurement techniques are available: immunofluorescence, ELISA, flow cytometry, and cell-based assays. Cell-based methods are characterized by particularly high specificity and sensitivity and are therefore suggested for the detection of anti-aquaporin-4 antibodies (26, 27).

Anti-MOG Antibodies

MOG is a myelin protein produced exclusively on the surface of myelin sheaths and membranes of oligodendrocytes and is known as a possible target molecule for the autoimmune attacks in demyelinating diseases (28). Unlike initially stated, anti-MOG antibodies are not appropriate for the diagnosis or prognosis of MS, but rather for differential diagnosis. Using cell-based methods, it was revealed that anti- MOG antibodies are detected in a subgroup of pediatric patients with acute disseminated encephalomyelitis (ADEM), patients with clinical symptoms of NMOSD, and patients with bilateral optic neuritis (29). In classical MS, however, high anti-MOG antibody levels are rare, with the rate of seropositive MS patients being highest in the pediatric patient group. In a recent research, it has been shown that the prevalence of anti-MOG antibodies was 38.7% in patients with an initial clinical event under 10 years of age, whereas only 4.3% of patients with onset of the disease in adulthood (> 18 years of age) were seropositive (30). Comparison of this clearly distinct cohort with AQP-4+ NMO and MS suggests that MOG+ CNS demyelinating disease shows a distinct novel disease entity. So far, anti-MOG antibodies are not commonly be used as biomarkers in clinical practice despite these new findings (31).

Antinuclear Antibodies

Antinuclear antibodies (ANA) are tissue non-specific autoantibodies against parts of the cell nucleus, the level of which is measured in the plasma (32). According to the guidelines of the German Neurological Society, the ANA test is a necessary laboratory test for evaluation of differential diagnosis (33). A persistently high level suggests collagenoses such as systemic lupus erythematosus (SLE) (34). However, in a recent study, Becker and colleagues reported conflicting results that whether a positive ANA test without clinical manifestation of connective tissue disease is useful and concluded that testing without suspicion should be well noted (35). They also reported that the antibodies against double-stranded DNA (dsDNA), which are also diagnostic for SLE, should only be measured after a positive ANA test finding. In the German guideline, however, the measurement of anti-dsDNA antibodies is also one of the necessary laboratory tests for evaluation of differential diagnosis (36, 37).

Biomarkers for Prediction of MS Prognosis

Biomarkers for MS prognosis can represent information on the course of disease severity and indicate conversion to another types of MS, for example from CIS to relapsing-remitting MS (RRMS) or from RRMS to secondary progressive MS (SPMS) (38).

Oligoclonal Bands

The measurement of oligoclonal IgG bands in CSF is correlated with a conversion from CIS to MS and can therefore be used as a biomarker for MS prognosis. For example, a study of Tintore and colleagues with 1015 patients revealed that oligoclonal IgG bands are associated with the increased risk of clinically confirmed MS and progression of disability independently of other assessed factors (39). Furthermore, in an investigation of Kuhle and colleagues, oligoclonal IgG bands showed to be the strongest prognostic factor for conversion from CIS to MS, along with the lesion load and the age at the onset of the symptoms (40). A recent research also showed a prognostic importance of oligoclonal IgG bands in the conversion of radiologically isolated syndrome (RIS) to CIS (41). OCB can also be synthesized from the production of IgM in the CNS. In some studies oligoclonal IgM bands have been correlated with an increased risk of conversion from CIS to MS and with a progressive course of the disease (42, 43). However, there are also studies that show no association between oligoclonal IgM bands and the MS prognosis (44). Therefore, the efficiency of oligo-IgM as prognostic biomarker remains to be approved by further studies.

Chitinase-3-like-1

The protein chitinase-3-like-1 is a glycosidase produced by monocytes, microglia, and activated astrocytes (45). The main role of chitinase-3-like-1

(CHI3L1) in the CNS is unclear; however, its role in inflammatory lesions suggests that it may be a significant component of the astrocytic response to change CNS inflammation (46). It is commonly found in the CSF. Cantó and colleagues reported in a multicenter longitudinal cohort investigation with 813 participants that the CHI3L1 concentration is an independent risk factor for the conversion from CIS to MS. High CHI3L1 levels were also correlated with faster course of disability (47). Although CHI3L1 is not yet clinically approved, it is a possible candidate as a biomarker of prediction of MS prognosis and response to treatment (45).

Neurofilaments

Neurofilaments (NF) are known as neuronal cytoskeletal proteins with a light (NFL), an intermediate (NFM), and a heavy (NFH) chain (48). They change the diameter of axons and are involved in axonal transfer. If axonal or neuronal damage occurs, NF are released and can be measured in the CSF and blood. For measurement in blood, an ultra-sensitive technique known as single molecule arrays (SIMOA) has been developed only recently, for the first time makes it possible to detect NFL in serum (49). Compared to measurement using ELISA or electrochemiluminescence (ECL) based assays, SIMOA is known by > 25 times higher analytical sensitivity (50). NFL are also highly stable and insensitive to the common storage conditions, which increases the efficacy of the measurement methods. According to a study by Disanto and colleagues, MS patients have increased NFL levels compared to the control group, with a strong correlation of values assessed simultaneously in CSF and serum (51). Serum NFL levels also associate with MRI activity, severity of disability, and level of brain atrophy (52). Moreover, NFL is also useful as a prognostic biomarker for the conversion from CIS to MS (53, 54). A recent investigation also reported a prognostic importance of serum NFL in the conversion from RIS to CIS (55). Taken together, the measurement of serum NFL concentration, which does not necessarily need a lumbar puncture but can be now detected in the blood, seems to associate with many clinical and magnetic tomographic features of MS. A future improvement as a prognostic biomarker in clinical practice is therefore possible. While NFL detection in serum is a well-established biomarker of neuroaxonal damage in MS, there are convincing data on astroglial markers in serum as glial fibrillary acid protein (GFAP) (56).

Biomarkers for Assessment of Response to Treatment

With regard to the progressive course of the MS pathophysiology, a wide variety of disease-modifying treatments with specific mechanisms of action were developed. However, not all MS patients respond equally to these treatments. In order to treat each patient with a specific treatment at the right time, it is necessary to use biomarkers for prediction of the response of the patient to treatment and monitoring its effectiveness.

Neutralizing Antibodies Against Interferon-β

Neutralizing antibodies can be produced in response to the treatment of patient with mostly protein drugs and prevent its actual mechanism of action. These antibodies are found in serum. In interferon therapy (IFN)-β, neutralizing antibodies are synthesized in up to 40% of patients, based on the type of IFN. This commonly is found during the first 2 years of treatment (57). Neutralizing antibodies against IFN-β have been reported to reduce its positive effect on annual relapse rate, severity of the disease, and MRI activity (58). Therefore, changes of treatments are suggested within 3 to 6 months if two positive test results are received (59). Neutralizing antibodies against IFN-β therefore indicate a prognostic biomarker for poor response to treatment. An indirect biomarker for the biological function of IFN-β is the myxovirus resistance protein A (MxA), which is an antiviral protein selectively induced by IFN-β. In this condition, measurement is performed using expression of MxA mRNA in blood cells. If neutralizing antibodies against IFN-β with a low to medium titer were found in a patient, the MxA level can be measured as additional information. With a low MxA level meaning low IFN-β bioavailability, a change in treatment should be selected (59).

Neutralizing Antibodies Against Natalizumab

Neutralizing antibodies can also be produced during treatment with natalizumab, the monoclonal antibody against integrin α4β1 and α4β7 on leukocytes. Generally of six percent of patients treated with natalizumab, neutralizing antibodies are found at least once. In most of patients, these occur during the first three months of therapy (60). The neutralizing antibodies lower the serum level of natalizumab and, with continuous presence, are

correlated with a reduced usefulness of the therapy. For example, a study by Vennagoor et al. (61) revealed a correlation of high neutralizing antibody titers with the number of episodes and brain lesions in MRI. Although there are currently no consensus for the common use of neutralizing antibodies against natalizumab as prognostic biomarkers for response to treatment, it is suggested that a corresponding test should be carried out within 3 to 4 months after the initiation of treatment and when the patient shows relapses (45). Since neutralizing antibodies are also correlated with the occurrence of infusion-related complications, they also can be used as a biomarker for therapeutic complications.

Neurofilament Light Chain

Biomarkers that have an association with disease severity in RRMS patients can be used as important markers for response to treatment. Since the release of NFL is associated to the occurrence of axon damage and the NFL concentration associates with disease severity, the protein could be such a biomarker for response to treatment (62). Several investigations have already reported an average decrease in the levels of NFL in CSF of MS patients after treatment with fingolimod, natalizumab, rituximab or mitoxantrone, or alemtuzumab (63, 64). In this regard, Gunnarsson and colleagues showed a decrease in NFL levels in comparison of healthy controls 6 to 12 months following the initiation of treatment with natalizumab (65). Fingolimod therapy also provide a significant decrease in NFL levels in CSF following 12 months based on the results of an investigation by Kuhle and colleagues, whereas no considerable change occurred in the control group (66). A decrease in NFL levels was also reported in serum following progression-modifying treatments, including mitoxantrone, natalizumab, and fingolimod (67). In an investigation by Akgün et al. in patients who received alemtuzumab using monthly serum NFL (sNFL) measurement, clinical or MRI disease severity was in line by an increase of sNFL level (64). Even declared symptoms that have not been known as clinical relapse before were associated with sNFL increase suggesting sNFL measurement to approve a relapse. SNFL increased about 1 month before the initiation of clinical symptoms with more increase and then decrease over the following 1 to 3 months. Monthly sNFLs were observed at higher levels in patients with disease activity that needed alemtuzumab retreatment in comparison with responder patients.

C-X-C Motif Chemokine-13

The C-X-C motif chemokine-13 (CXCL13) is known as one of the most important B cell chemoattractants and has a pivotal role in the recruitment of B cells into the CNS in MS patients. Therefore, higher levels of CXCL13 in the CSF of MS patients could be assessed compared to healthy controls. Furthermore, an association of higher levels of CXCL13 with disease severity was reported (68). In a study by Novakova and colleagues, patients who received natalizumab had lower CXCL13 levels compared to patients under treatment with IFN-β (69). Another investigation also showed a reduction in CXCL13 levels following conversion from teriflunomide, IFN-β, or glatiramer acetate to fingolimod (70). With respect to these findings, CXCL13 could be a promising biomarker for the assessment of efficacy of MS treatments.

Molecular Biomarkers for Therapeutic Side Effects

In addition to response to treatments, side effects are a decisive factor for the assessment of success of a treatment. Molecular biomarkers can be used as an important indicator for assess and predict adverse events.

Anti-varicella Zoster Virus Antibodies

Antibodies against VZV are approved biomarkers for complications of different RRMS treatments. Recently, we have indicated that the antibody level is correlated with the more associated cellular VZV response which is difficult to assess (21). Due to the changed immune response, the risk of herpetic infections is higher in patients receiving some immunemodulating treatments (23). To avoid VZV reactivation during the treatment, the anti-VZV antibody titer should be measured in serum before initiation of treatment with alemtuzumab, fingolimod, and cladribine in patients without history of chickenpox disease or vaccination (23, 71). In the patients who are seronegative, vaccination should be performed and the treatment should be initiated after 4 to 6 weeks in order to fully achievement of vaccination protection. Prophylactic administration of antiherpetics is also suggested for all patients who receive alemtuzumab. Regarding treatment with cladribine, herpes prophylaxis should

be noted if the lymphocyte is lower than 200/µl for the duration of grade 4 lymphopenia (72).

Anti-John Cunningham Virus Antibodies

Antibodies against the John Cunningham virus (JCV) are found in serum or plasma and is known as a biomarker for the development of progressive multifocal leukoencephalopathy (PML) during natalizumab therapy. The risk of PML is also increased by history of treatment with immunosuppressive agents and the duration of natalizumab therapy (73). Seropositive patients without previous immunosuppressive treatment are additionally differentiated based on the result of Anti-JCV antibody index for PML risk evaluation (74). It has been shown that the risk of development of PML increases significantly in patients with an index value higher than 1.5. Exact monitoring and, in some conditions, a treatment switch may be suitable in this case. Thus, anti-JCV antibodies are approved and useful biomarkers in treatment with natalizumab.

L-selectin Expression

L-selectin (CD62L) is a biomarker on the cell surface of lymphocytes. The proportion of CD4+ T cells with CD62L in peripheral mononuclear blood cells is another possible candidate for the assessment of PML risk in patients receiving natalizumab (75). Schwab and colleagues showed an association between the levels of CD62L and the JCV status and the JCV index (76). Furthermore, in this case-control study, lower values of CD62L proportion was associated with increased risk of developing PML. However, another case-control study with 21 PML patients who received natalizumab and 104 control patients who received natalizumab led to no association between CD62L and PML risk (77). Taken together, more investigations are necessary in this case to assess the efficacy of CD62L as a biomarker for evaluation of complications.

Conclusion

Molecular biomarkers can be used as effective tools for clinical decisions and are pivotal stage on the process to a personalized treatment for MS patients [3, 142]. An appropriate biomarker is known by high sensitivity and specificity as well as a simple, cost-effective, reproducible, and non-invasive measurement technique. The accuracy of diagnosis and estimation of prognosis of MS as well as the assessment of response to treatment and the evaluation of the risk of complications can be improved with the application of some approved biomarkers. Taken together, these biomarkers include oligoclonal bands and the IgG index, anti-AQP-4 antibodies, neutralizing antibodies against IFN-β and natalizumab, as well as anti-JCV and anti-VZV antibodies. Furthermore, there are some possible biomarker candidates such as NFL and CHI3L that need to be evaluated in further investigations. However, long-term studies in large cohorts will be required to improve the efficacy of biomarker candidates in clinical practice. Despite these initial improvements, biomarkers that can be used to predict the response to treatment even before the initiation of treatment and thus specialized therapy are still lacking. Therefore, there is still a need to evaluate and improve new biomarkers for different aspects of MS.

Declarations

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Author Contribution

Niloofar alsadat Nourian: Study design, data collection, writing draft of study.

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Conflict of interest

There is no conflict of interest.

Data Availability

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

1. Houen G, Trier NH, Frederiksen JL. Epstein-Barr Virus and Multiple Sclerosis. Frontiers in immunology. 2020;11:3315.
2. Novo JC, Felgueiras H. Neuro-ophthalmologic manifestations of multiple sclerosis other than acute optic neuritis. Multiple sclerosis and related disorders. 2020:102730.
3. Hauer L, Perneczky J, Sellner J. A global view of comorbidity in multiple sclerosis: a systematic review with a focus on regional

differences, methodology, and clinical implications. Journal of Neurology. 2020:1-12.
4. Barros C, Fernandes A. Linking Cognitive Impairment to Neuroinflammation in Multiple Sclerosis using neuroimaging tools. Multiple sclerosis and related disorders. 2020:102622.
5. Sapko K, Jamroz-Wiśniewska A, Marciniec M, Kulczyński M, Szczepańska-Szerej A, Rejdak K. Biomarkers in Multiple Sclerosis: a review of diagnostic and prognostic factors. Neurologia i neurochirurgia polska. 2020;54(3):252-8.
6. Group BDW, Atkinson Jr AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical pharmacology & therapeutics. 2001;69(3):89-95.
7. Porter L, Shoushtarizadeh A, Jelinek GA, Brown CR, Lim CK, de Livera AM, et al. Metabolomic biomarkers of multiple sclerosis: A systematic review. Frontiers in Molecular Biosciences. 2020;7.
8. van Munster CE, Uitdehaag BM. Outcome measures in clinical trials for multiple sclerosis. CNS drugs. 2017;31(3):217-36.
9. Nociti V, Santoro M. What do we know about the role of lncRNAs in multiple sclerosis? Neural Regeneration Research. 2021;16(9):1715.
10. Simon R. Sensitivity, specificity, PPV, and NPV for predictive biomarkers. JNCI: Journal of the National Cancer Institute. 2015;107(8).
11. Azzopardi L, Thompson SA, Harding KE, Cossburn M, Robertson N, Compston A, et al. Predicting autoimmunity after alemtuzumab treatment of multiple sclerosis. Journal of Neurology, Neurosurgery & Psychiatry. 2014;85(7):795-8.
12. Martinez B, Peplow PV. MicroRNAs in blood and cerebrospinal fluid as diagnostic biomarkers of multiple sclerosis and to monitor disease progression. Neural regeneration research. 2020;15(4):606.
13. Pannewitz-Makaj K, Wurster U, Jendretzky KF, Gingele S, Sühs K-W, Stangel M, et al. Evidence of Oligoclonal Bands Does Not Exclude Non-Inflammatory Neurological Diseases. Diagnostics. 2021;11(1):37.
14. Pryce G, Baker D. Oligoclonal bands in multiple sclerosis; Functional significance and therapeutic implications. Does the specificity matter? Multiple sclerosis and related disorders. 2018;25:131-7.
15. Gastaldi M, Zardini E, Leante R, Ruggieri M, Costa G, Cocco E, et al. Cerebrospinal fluid analysis and the determination of oligoclonal bands. Neurological Sciences. 2017;38(2):217-24.
16. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology. 2018;17(2):162-73.
17. Dobson R, Ramagopalan S, Davis A, Giovannoni G. Cerebrospinal fluid oligoclonal bands in multiple sclerosis and clinically isolated syndromes: a meta-analysis of prevalence, prognosis and effect of latitude. Journal of Neurology, Neurosurgery & Psychiatry. 2013;84(8):909-14.
18. Owens GP, Burgoon MP, Anthony J, Kleinschmidt-DeMasters BK, Gilden DH. The immunoglobulin G heavy chain repertoire in multiple sclerosis plaques is distinct from the heavy chain repertoire in peripheral blood lymphocytes. Clinical Immunology. 2001;98(2):258-63.
19. Musaeus CS, Gleerup HS, Høgh P, Waldemar G, Hasselbalch SG, Simonsen AH. Cerebrospinal fluid/plasma albumin ratio as a biomarker for blood-brain barrier impairment across neurodegenerative dementias. Journal of Alzheimer's Disease. 2020(Preprint):1-8.
20. Link H, Huang YM. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. Journal of neuroimmunology. 2006;180(1-2):17-28.
21. Kattimani Y, Veerappa AM. Complex interaction between mutant HNRNPA1 and gE of varicella zoster virus in pathogenesis of multiple sclerosis. Autoimmunity. 2018;51(4):147-51.
22. Brettschneider J, Tumani H, Kiechle U, Muche R, Richards G, Lehmensiek V, et 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.
23. Sotelo J. On the viral hypothesis of multiple sclerosis: participation of varicella-zoster virus. J Neurol Sci. 2007;262(1-2):113-6.
24. Hubbard JA, Szu JI, Binder DK. The role of aquaporin-4 in synaptic plasticity, memory and disease. Brain research bulletin. 2018;136:118-29.
25. Ortiz Salas PA, Gaviria Carrillo M, Cortés Bernal GA, Moreno Medina K, Roa LF, Rodríguez Quintana JH. Neuromyelitis optica spectrum disorder: Do patients positive and negative for anti-aquaporin-4 antibodies present distinct entities? A

Colombian perspective. Neurologia (Barcelona, Spain). 2020.
26. Seay M, Rucker JC. Neuromyelitis Optica: Review and Utility of Testing Aquaporin-4 Antibody in Typical Optic Neuritis. Asia-Pacific journal of ophthalmology (Philadelphia, Pa). 2018;7(4):229-34.
27. Mangiatordi GF, Alberga D, Trisciuzzi D, Lattanzi G, Nicolotti O. Human Aquaporin-4 and Molecular Modeling: Historical Perspective and View to the Future. Int J Mol Sci. 2016;17(7).
28. Weber MS, Derfuss T, Metz I, Brück W. Defining distinct features of anti-MOG antibody associated central nervous system demyelination. Therapeutic advances in neurological disorders. 2018;11:1756286418762083.
29. Lana-Peixoto MA, Talim N. Neuromyelitis Optica Spectrum Disorder and Anti-MOG Syndromes. Biomedicines. 2019;7(2).
30. McLaughlin KA, Chitnis T, Newcombe J, Franz B, Kennedy J, McArdel S, et al. Age-dependent B cell autoimmunity to a myelin surface antigen in pediatric multiple sclerosis. Journal of immunology (Baltimore, Md : 1950). 2009;183(6):4067-76.
31. Ramanathan S, Dale RC, Brilot F. Anti-MOG antibody: The history, clinical phenotype, and pathogenicity of a serum biomarker for demyelination. Autoimmun Rev. 2016;15(4):307-24.
32. Sremec J, Tomasović S, Tomić Sremec N, Šućur A, Košćak Lukač J, Bačić Baronica K, et al. Elevated Concentrations of Soluble Fas and FasL in Multiple Sclerosis Patients with Antinuclear Antibodies. Journal of clinical medicine. 2020;9(12):3845.
33. Nosal RS, Superville SS, Varacallo M. Biochemistry, Antinuclear Antibodies (ANA). StatPearls. Treasure Island (FL): StatPearls Publishing

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34. Pisetsky DS, Lipsky PE. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. Nature reviews Rheumatology. 2020;16(10):565-79.
35. Becker J, Geffken M, Diehl RR, Berlit P, Krämer M. Choosing wisely? Multiple Sclerosis and Laboratory Screening for Autoimmune Differential Diagnoses. Neurology International Open. 2017;1(04):E256-E63.
36. Szmyrka-Kaczmarek M, Pokryszko-Dragan A, Pawlik B, Gruszka E, Korman L, Podemski R, et al. Antinuclear and antiphospholipid antibodies in patients with multiple sclerosis. Lupus. 2012;21(4):412-20.
37. Ziemssen T, Akgün K, Brück W. Molecular biomarkers in multiple sclerosis. Journal of neuroinflammation. 2019;16(1):1-11.
38. Gabelić T, Radmilović M, Posavec V, Škvorc A, Bošković M, Adamec I, et al. Differences in oligoclonal bands and visual evoked potentials in patients with radiologically and clinically isolated syndrome. Acta Neurologica Belgica. 2013;113(1):13-7.
39. Tintore M, Rovira À, Río J, Otero-Romero S, Arrambide G, Tur C, et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. Brain. 2015;138(7):1863-74.
40. Kuhle J, Disanto G, Dobson R, Adiutori R, Bianchi L, Topping J, et al. Conversion from clinically isolated syndrome to multiple sclerosis: a large multicentre study. Multiple Sclerosis Journal. 2015;21(8):1013-24.
41. Tintoré M, Rovira A, Rio J, Tur C, Pelayo R, Nos C, et al. Do oligoclonal bands add information to MRI in first attacks of multiple sclerosis? Neurology. 2008;70(13 Part 2):1079-83.
42. Boscá I, Magraner M, Coret F, Alvarez-Cermeno J, Simo-Castello M, Villar L, et al. The risk of relapse after a clinically isolated syndrome is related to the pattern of oligoclonal bands. Journal of neuroimmunology. 2010;226(1-2):143-6.
43. Ignacio RJ, Liliana P, Edgardo C. Oligoclonal bands and MRI in clinically isolated syndromes: predicting conversion time to multiple sclerosis. Journal of neurology. 2010;257(7):1188-91.
44. Frau J, Villar LM, Sardu C, Secci MA, Schirru L, Ferraro D, et al. Intrathecal oligoclonal bands synthesis in multiple sclerosis: is it always a prognostic factor? Journal of neurology. 2018;265(2):424-30.
45. Sapko K, Jamroz-Wiśniewska A, Marciniec M, Kulczyński M, Szczepańska-Szerej A, Rejdak K. Biomarkers in Multiple Sclerosis: a review of diagnostic and prognostic factors. Neurol Neurochir Pol. 2020;54(3):252-8.
46. Yeo IJ, Lee CK, Han SB, Yun J, Hong JT. Roles of chitinase 3-like 1 in the development of cancer, neurodegenerative diseases, and inflammatory diseases. Pharmacology & therapeutics. 2019;203:107394.

47. Canto E, Reverter F, Morcillo-Suárez C, Matesanz F, Fernandez O, Izquierdo G, et al. Chitinase 3-like 1 plasma levels are increased in patients with progressive forms of multiple sclerosis. Multiple Sclerosis Journal. 2012;18(7):983-90.
48. Lambertsen KL, Soares CB, Gaist D, Nielsen HH. Neurofilaments: The C-Reactive Protein of Neurology. Brain sciences. 2020;10(1).
49. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, et al. Neurofilaments as biomarkers in neurological disorders. Nature reviews Neurology. 2018;14(10):577-89.
50. Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, Sandelius Å, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clinical Chemistry and Laboratory Medicine (CCLM). 2016;54(10):1655-61.
51. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, et al. Serum neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Annals of neurology. 2017;81(6):857-70.
52. Williams T, Zetterberg H, Chataway J. Neurofilaments in progressive multiple sclerosis: a systematic review. J Neurol. 2020.
53. Disanto G, Adiutori R, Dobson R, Martinelli V, Dalla Costa G, Runia T, et al. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. Journal of Neurology, Neurosurgery & Psychiatry. 2016;87(2):126-9.
54. Håkansson I, Tisell A, Cassel P, Blennow K, Zetterberg H, Lundberg P, et al. Neurofilament light chain in cerebrospinal fluid and prediction of disease activity in clinically isolated syndrome and relapsing–remitting multiple sclerosis. European journal of neurology. 2017;24(5):703-12.
55. Matute-Blanch C, Villar LM, Álvarez-Cermeño JC, Rejdak K, Evdoshenko E, Makshakov G, et al. Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome. Brain. 2018;141(4):1085-93.
56. Högel H, Rissanen E, Barro C, Matilainen M, Nylund M, Kuhle J, et al. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. Multiple Sclerosis Journal. 2020;26(2):210-9.
57. Gilli F, Bertolotto A, Sala A, Hoffmann F, Capobianco M, Malucchi S, et al. Neutralizing antibodies against IFN-β in multiple sclerosis: antagonization of IFN-β mediated suppression of MMPs. Brain. 2004;127(2):259-68.
58. Hegen H, Auer M, Deisenhammer F. Predictors of response to multiple sclerosis therapeutics in individual patients. Drugs. 2016;76(15):1421-45.
59. Polman CH, Bertolotto A, Deisenhammer F, Giovannoni G, Hartung H-P, Hemmer B, et al. Recommendations for clinical use of data on neutralising antibodies to interferon-beta therapy in multiple sclerosis. The Lancet Neurology. 2010;9(7):740-50.
60. Khoy K, Mariotte D, Defer G, Petit G, Toutirais O, Le Mauff B. Natalizumab in Multiple Sclerosis Treatment: From Biological Effects to Immune Monitoring. Front Immunol. 2020;11:549842.
61. Vennegoor A, Rispens T, Strijbis EM, Seewann A, Uitdehaag BM, Balk LJ, et al. Clinical relevance of serum natalizumab concentration and anti-natalizumab antibodies in multiple sclerosis. Multiple Sclerosis Journal. 2013;19(5):593-600.
62. Thebault S, Booth RA, Freedman MS. Blood Neurofilament Light Chain: The Neurologist's Troponin? Biomedicines. 2020;8(11).
63. Kapoor R, Smith KE, Allegretta M, Arnold DL, Carroll W, Comabella M, et al. Serum neurofilament light as a biomarker in progressive multiple sclerosis. Neurology. 2020;95(10):436-44.
64. Akgün K, Kretschmann N, Haase R, Proschmann U, Kitzler HH, Reichmann H, et al. Profiling individual clinical responses by high-frequency serum neurofilament assessment in MS. Neurology-Neuroimmunology Neuroinflammation. 2019;6(3).
65. Gunnarsson M, Malmeström C, Axelsson M, Sundström P, Dahle C, Vrethem M, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Annals of neurology. 2011;69(1):83-9.
66. Kuhle J, Disanto G, Lorscheider J, Stites T, Chen Y, Dahlke F, et al. Fingolimod and CSF neurofilament light chain levels in relapsing-remitting multiple sclerosis. Neurology. 2015;84(16):1639-43.
67. Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, Gunnarsson M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. 2017;89(22):2230-7.

68. Londoño AC, Mora CA. Role of CXCL13 in the formation of the meningeal tertiary lymphoid organ in multiple sclerosis. F1000Research. 2018;7:514.
69. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, Malmeström C, et al. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. Journal of neurochemistry. 2017;141(2):296-304.
70. Novakova L, Axelsson M, Khademi M, Zetterberg H, Blennow K, Malmeström C, et al. Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis. Multiple Sclerosis Journal. 2017;23(1):62-71.
71. Marrie RA, Wolfson C. Multiple sclerosis and varicella zoster virus infection: a review. Epidemiology and infection. 2001;127(2):315-25.
72. Cook S, Leist T, Comi G, Montalban X, Giovannoni G, Nolting A, et al. Safety of cladribine tablets in the treatment of patients with multiple sclerosis: an integrated analysis. Multiple sclerosis and related disorders. 2019;29:157-67.
73. Paz SPC, Branco L, Pereira MAC, Spessotto C, Fragoso YD. Systematic review of the published data on the worldwide prevalence of John Cunningham virus in patients with multiple sclerosis and neuromyelitis optica. Epidemiology and health. 2018;40:e2018001.
74. Schwab N, Schneider-Hohendorf T, Hoyt T, Gross CC, Meuth SG, Klotz L, et al. Anti-JCV serology during natalizumab treatment: Review and meta-analysis of 17 independent patient cohorts analyzing anti-John Cunningham polyoma virus sero-conversion rates under natalizumab treatment and differences between technical and biological sero-converters. Multiple sclerosis (Houndmills, Basingstoke, England). 2018;24(5):563-73.
75. Voortman MM, Greiner P, Moser D, Stradner MH, Graninger W, Moser A, et al. The effect of disease modifying therapies on CD62L expression in multiple sclerosis. Multiple sclerosis journal - experimental, translational and clinical. 2018;4(3):2055217318800810.
76. Schwab N, Schneider-Hohendorf T, Pignolet B, Spadaro M, Görlich D, Meinl I, et al. PML risk stratification using anti-JCV antibody index and L-selectin. Multiple Sclerosis Journal. 2016;22(8):1048-60.
77. Schwab N, Cahir-McFarland E, Schneider-Hohendorf T, Wiendl H, Ransohoff RM, Lieberman L, et al. CD62L is not a reliable biomarker for predicting PML risk in natalizumab-treated R-MS patientsAuthor Response. Neurology. 2016;87(9):958-9.