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Biochemical biomarkers for multiple sclerosis

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ABSTRACT

Introduction: Multiple sclerosis (MS) is the most frequent demyelinating disease of the central nervous system. Although there is currently no definite cure for MS, new therapies have recently been developed based on a continuous search for new biomarkers.

Development: MS diagnosis relies on the integration of clinical, imaging and laboratory findings as there is still no single pathognomonic clinical feature or diagnostic laboratory biomarker. The most commonly laboratory test used is the presence of immunoglobulin G oligoclonal bands (OCB) in cerebrospinal fluid of MS patients. This test is now included in the 2017 McDonald criteria as a biomarker of dissemination in time. Nevertheless, there are other biomarkers currently in use such as kappa free light chain, which has shown higher sensitivity and specificity for MS diagnosis than OCB. In addition, other potential laboratory tests involved in neuronal damage, demyelination and/or inflammation could be used for detecting MS.

Conclusions: CSF and serum biomarkers have been reviewed for their use in MS diagnosis and prognosis to stablish an accurate and prompt MS diagnosis, crucial to implement an adequate treatment and to optimize clinical outcomes over time.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune inflammatory neurological disorder, classified as a demyelinating and degenerative disease that affects the central nervous system (CNS) [1]. Its prevalence has increased worldwide in recent years, with 2.5 million cases reported around the world and 700,000 in Europe [2,3]. Specifically, in Spain, the prevalence is considered medium–high, with 180 cases per 100,000 inhabitants [4].

MS is the most frequent demyelinating disease of the CNS and considered the leading cause of non-traumatic disability in young adults. Although it can occur throughout life, symptoms usually start at 25–30 years of age and mostly in women [5–7].

Most MS patients have initially presented a clinical isolated syndrome (CIS), defined as a single demyelinating event affecting the CNS of at least 24 h duration with no association to other organic disease

Abbreviations: APC, antigen-presenting cells; BBB, blood brain barrier; CIS, clinical isolated syndrome; CHI3L1, chitinase-3-like 1; CNS, central nervous system; CSF, cerebrospinal fluid; CXCL12, chemokine ligand 12 or stromal cell-derived factor; CXCL13, chemokine ligand 13 or B lymphocyte chemoattractant; DUSP, dual-specificity MAPK phosphatases; EBV, Epstein Barr virus; ECL, electrochemiluminescence; EDSS, expanded disability status scale; ELISA, enzyme-linked immunoassay; FLC, free light chain; GFAP, glial fibrillary acidic protein; HSP, heat shock protein; κFLC, Kappa free light chain; λFLC, Lambda free light chain; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MRI, magnetic resonance imaging; MS, multiple sclerosis; Nfs, neurofilaments; NfH, Nf composed of heavy chains; NfL, Nf composed of light chains; NfM, Nf composed of medium chains; OCB, oligoclonal bands; OPN, osteopontin; PPMS, primary progressive multiple sclerosis; PRMS, progressive recurrent multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; Treg cells, regulatory T cells; VCAM1, vascular cell adhesion molecule 1.

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(fever, infections, metabolic disorders, etc.) [8–10]. Although these patients can convert to Relapsing Remitting MS (RRMS), disease progression in highly variable with the majority characterized by recurrent onset of clinical symptoms with full or partial recovery after the flare.

Therefore, there have been described four MS types: RRMS, Secondary Progressive MS (SPMS) that leads to an irreversible progressive disability, Primary Progressive MS (PPMS) and Progressive Recurrent MS (PRMS) [11].

The disease may debut with flare-ups and different symptoms, depending on the location of the lesions in the CNS, commonly called plaques. A flare-up is the appearance of new neurological symptomatology within a period of at least 24 h or a significant deterioration of preexisting symptoms that had been stable or absent for at least 30 days [12]. These outbreaks can cause lesions at any CNS level, reflecting inflammatory activity and producing different symptoms such as fatigue, blurred vision or eye pain, weakness, coordination problems, sensory symptoms or several of them together. In that case, it is considered a multifocal outbreak [1,13].

Currently there is no definite cure for MS, although new therapies have recently been developed increasing the effectiveness of the treatment and improving the disability-free life expectancy [14]. More research and development of new biomarkers is essential to improve the diagnostic of the disease and therefore to initiate an early treatment to prevent disease progression. Thus, the main aim of this manuscript is to critically review the use of cerebrospinal fluid (CSF) and serum biomarkers to achieve the most accurate MS diagnosis and to evaluate its utility in follow-up and prognosis.

2. Etiology

The main cause of the disease is an unbalance in the autoimmune system [15], but its etiology and underlying pathogenic mechanisms are currently unknown. Although MS is not an inherited disease, a strong genetic component is involved in its etiology [2]. Also, environmental triggers have been observed [16].

2.1. Gene association

Different HLA II alleles could be involved in the development of MS. Populations with the HLA DRB1*1501-DQB1*0602 (HLA DRB2) haplotype are more susceptible to develop MS [17,18]. The full mechanism remains unknown, but it is believed that HLA DR2 has a specific binding gap for CNS self-antigens, which will be presented to T cells. Then the activated T cells may attack the CNS, thereby increasing the production of Th1 lymphocytes in the area, ultimately favoring CNS inflammation. This haplotype is found in 25–30 % of the Northern European and US population. Other genetic risk factors recently identified are interleukin-2 (IL-2) receptor alpha gene and interleukin-7 (IL-7) receptor alpha gene [18,19].

On the other hand, there is a HLA ALA genetic variant, HLA A02, which is the second most abundant one. However, HLA A02 protectively acts against the onset of the disease probably by eliminating viruses related to MS, such as Epstein Barr virus (EBV) [20].

Furthermore, the existence of different allelic variants increases the risk of MS over one allelic variant alone. An overactivity of mitogen-activated protein kinase (MAPK) pathways in microglia would be related to MS presentation and progression, classically related to inflammation [21,22]. MAPK ERK overactivation may cause down-regulation of the Wnt/β-catenin pathway, which leads to a microglial phenotype causing hypomyelination [23,24]. MAPK ERK also causes an overexpression of vascular cell adhesion molecule 1 (VCAM-1), a key adhesion molecule that induces the translocation of leukocytes to inflamed tissue [25]. A dual-specificity MAPK phosphatase (DUSP) is the negative feedback system that regulates MAPK pathways. An over activity of MAPK pathways could occur when DUSP are downregulated [26]. This downregulation is caused by low serum vitamin D, smoking habit [27] and prior EBV infection [15,28], all three known as risk factors for MS [29–32], despite the fact that their pathophysiologic mechanisms still remain unclear [33,34] (Fig. 1).

2.2. Immunopathology

Homeostasis defects in regulatory T cells (Treg cells), defined as CD4 + CD25 + FoxP3 + T cells, have been associated with some allelic variants in cytokines or co-stimulatory molecules genes [35]. Reduced suppressive effect of Treg cells is known in autoimmune diseases. Also, CD4 + CD25 + Treg cells functional loss has been described in the suppression of T cell immune response. That allows effector CD4 + T cells to migrate into the CNS crossing the blood brain barrier (BBB) and eventually to destroy myelin oligodendrocyte glycoprotein in MS patients [35–38]. In addition, Treg cells are capable of secreting proinflammatory cytokines [39] (Fig. 1).

2.3. Environmental triggers

Other hypothesis is that certain viral or bacterial autoantigens or super-antigens could produce cross-reactions by binding to antigen-presenting cells (APCs). These would travel through the bloodstream and reach the lymphoid organs, where they would activate T lymphocytes. The activated T lymphocytes would trigger CD8 + T lymphocytes and other B-lymphocytes, which could cross the BBB. Then, once inside the CNS, they would be able to generate a cytotoxic effect, producing pro-inflammatory cytokines such as IFN-γ, TNF-α and IL-17. These cytokines would trigger the activation of macrophages and microglia, which, in turn, secrete cytokines such as IL-12 and IL-23 and chemokines. These will then be responsible for inducing the recruitment of other lymphocytes, such as other CD4 + and CD8 + T-lymphocytes, B-lymphocytes and monocytes, to the CNS. CD4 + T cells turn into Th1 lymphocytes through exposure to cytokines, such as IL-12, and towards Th17 by exposure mainly to IL-17 and IL-23. Finally, these cells promote demyelination and damage to oligodendrocytes and neurons [40] (Fig. 1).

In addition, low sun exposure results in low serum vitamin D, which is also associated with increased predisposition to MS development [30,41]. Several studies have demonstrated a correlation between low serum vitamin D and higher disability [41–47]. Also, evidence shows that the risk of subsequent relapses is lower in CIS subjects with higher serum vitamin D [48,49]. For that reason, multiple studies have evaluated the use of vitamin D supplementation as treatment in MS. However, the *meta*-analysis conducted by James et al. [50], on the risk of MS relapses after vitamin D supplementation, showed no significant association between high-dose vitamin D treatment and risk of MS relapses. Similar results were found by Pozuelo-Moyano et al. [51] who reported no evidence of vitamin D being a beneficial treatment in MS.

3. Diagnostic criteria

Misdiagnosis of MS remains an issue in clinical practice. There are numerous CNS diseases to make a differential diagnosis with [52], such as migraine, fibromyalgia, nonspecific symptoms with abnormal magnetic resonance imaging (MRI), functional neurological disorder and neuromyelitis optica [53]. McDonald criteria revised in 2017 are the most widely employed for MS diagnosis [54].

To establish a diagnosis of MS according to that criteria, an individual must have evidence of CNS damage disseminated in space, plaques need to be present in multiple regions of the nervous system and/or there should be evidence of damage disseminated in time, or occurring at different points in time [54] (Table 1).

Moreover, it has been shown that early therapeutic intervention delays long-term disease progression and improves outcomes, which can be graded using the expanded disability status scale (EDSS). Therefore, an accurate diagnosis is needed especially in those patients with

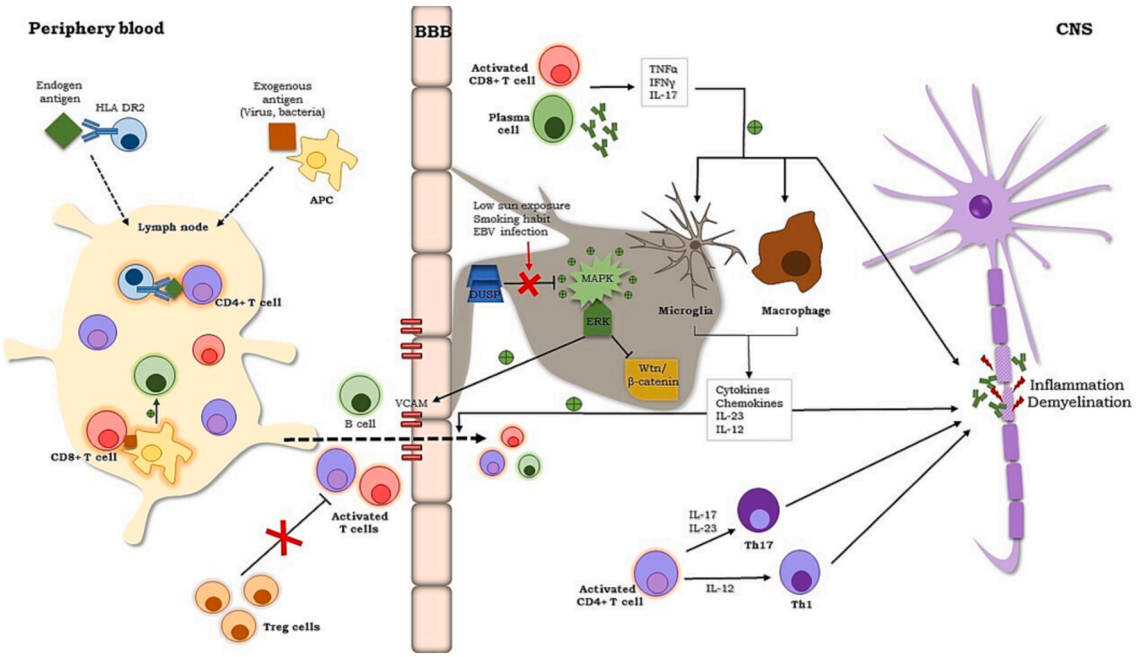

Fig. 1. Pathophysiological model of multiple sclerosis. APC, antigen-presenting cells; BBB, blood brain barrier; CNS, central nervous system; DUSP, dual-specificity MAPK phosphatases; MAPK, mitogen-activated protein kinase; Treg cells, regulatory T cells; VCAM1, vascular cell adhesion molecule 1.

neuromyelitis optica or CIS at high risk of developing RRMS, SPMS, or PPMS [55,56].

McDonald criteria include the use of MRI to establish the presence of disseminated lesions in space or time. In the latest update of these criteria, lesions in the cortical area of the brain were included, in addition to juxtacortical lesions, in order to consider dissemination in space. Furthermore, in addition to asymptomatic MRI lesions, symptomatic ones are currently taken into account when determining dissemination in space or time (except for lesions in the optic nerve in patients with neuromyelitis optica symptoms). According to the 2017 McDonald criteria the presence of IgG oligoclonal bands (OCB) in CSF can substitute the requirement of demonstrating dissemination in time. This improvement in the 2017 criteria compared to those of 2010, enables an early MS diagnosis in patients that meet the criteria for dissemination in space.

MS diagnosis relies on the integration of clinical, imaging, and laboratory findings, since there is still no single pathognomonic clinical feature or diagnostic laboratory biomarker that can effectively detect the disease. The most commonly laboratory test used is the presence of IgG OCB in CSF of MS patients, now included in 2017 McDonald criteria. Nevertheless, there are already in use biomarkers and proposed new serum and CFS biomarkers which could be helpful in diagnosis and prognosis of MS at different stages of the disease (Table 2).

4. Biomarkers used in clinical practice

4.1. IgG intrathecal synthesis. Oligoclonal bands and IgG index

The presence of higher IgG levels and IgG OCB in the CSF absent in serum are suggestive of intrathecal IgG synthesis by plasma cells and monoclonal B lymphocytes in the CNS [57]. Thereby, the detection of IgG OCB in CSF is considered the “gold standard” laboratory test to evidence elevated intrathecal synthesis. However, OCB detection is performed by isoelectric focusing technique followed by agarose gel electrophoresis. This is indeed a complex protocol with a high cost and methodological limitations such as the need of trained personnel and subjective observatory dependent interpretation. The importance of IgG OCB in MS diagnosis relies on its detection in 95 % of MS patients [49], although they could be found in other chronic inflammatory CNS diseases [58]. Moreover, their presence in CSF of CIS patients is a predictor to MS conversion [59].

Alternatively, IgG intrathecal synthesis can be determined also by the Reiber and Felgenhauer formula [60], Tourtellote formula [61], Schuller formula [62] and IgG or Tibbling Link index [63]. The most widely used formula is the IgG index. This evaluates the amount of IgG in the CSF compared to the levels in serum and is calculated as the ratio of IgG to albumin in CSF compared to the ratio of IgG to albumin in serum [64]. Albumin is included in the index because the albumin quotient (Qalb) CSF/serum, is used as a measurement of BBB dysfunction in MS [65]. An IgG index higher than 0.7 indicates increased intrathecal B cell response [66], and thus probably reveals a MS diagnostic, based on the fact that nearly 70 % of MS patients have increased IgG index [67,68]. However, there are other CNS diseases in which IgG index could increase [58].

4.2. IgM intrathecal synthesis

As IgG, IgM OCBs can be detected resulting from intrathecal production of IgM [69]. Its presence has been linked to increased risk of conversion from CIS to MS and to an aggressive evolution of the disease [64,70,71]. IgM index calculated using the formula CSF IgM × serum albumin/serum IgM × CSF albumin can be used to establish the intrathecal IgM synthesis. IgM index higher than 0.1 is considered increased and it predicts bad prognosis of the disease [72–74].

4.3. Kappa and Lambda free light chains

Kappa and Lambda immunoglobulins free light chain (κFLC, λFLC), are produced by B-lymphocytes during antibody synthesis. An increase in serum in both FLC production has been reported in inflammatory and autoimmune systemic diseases [75]. Whereas in CSF, intrathecal immunoglobulin synthesis is commonly observed in inflammatory disorders of the CNS (also when the origin is infectious) [76]. Different automated nephelometric assays are available for the detection of FLC [77]. The two most studied ones are Siemens® N-latex κFLC and λFLC [78] and Binding Site Freelite® FLC [79]. These two assays are

Table 1
McDonald Criteria 2017 summary for the diagnosis of MS.

McDonald Criteria 2017 for the diagnosis of MS	
CLINICAL PRESENTATION	**ADDITIONAL CRITERIA TO MAKE MS DIAGNOSIS**
…in a person who has experienced a typical attack/CIS at onset	
• 2 or more attacks and clinical evidence of 2 or more lesions OR 2 or more attacks and clinical evidence of 1 lesion with clear historical evidence of prior attack involving lesion in different location	None
• 2 or more attacks and clinical evidence of 1 lesion	**DIS** Additional clinical attack implicating different CNS site 1 or more MS typical T2 lesions in 2 or more areas of CNS
• 1 attack and clinical evidence of 2 or more lesions	**DIT** Additional clinical attack Simultaneous presence of both enhancing and non-enhancing MS typical MRI lesions, or new T2 or enhancing MRI lesion compared to baseline scan CSF oligoclonal bands
• 1 attack and clinical evidence of 1 lesion	**DIS** Additional attack implicating different CNS site 1 or more MS-typical T2 lesions in 2 or more areas of CNS. AND **DIT** Additional clinical attack Simultaneous presence of both enhancing and non-enhancing MStypical MRI lesions, or new T2 or enhancing MRI lesion compared to baseline scan (without regard to timing of baseline scan) CSF oligoclonal bands
…in a person who has steady progression of disease since onset	
• 1 year of disease progression (retrospective or prospective)	**DIS** 1 or more MS-typical T2 lesions 2 or more T2 spinal cord lesions CSF oligoclonal bands

comparable but not interchangeable for patient's follow-up. Thus, the same assay should be employed throughout the whole study period [80–82]. In comparison to OCB detection, FLC assays present important advantages, such as the simplicity of automated methods and the fact that the results are objective and quantifiable [83]. Furthermore, FLC methods are not influenced neither by hemolysis nor by long storage in CSF samples, indicating high stability [84]. Although κFLC and λFLC can be elevated in the CSF of MS patients, κFLC showed better correlation in MS diagnosis [85,86] and its index has higher sensitivity and specificity [87–91]. κ-index is the ratio between CSF and serum κFLC levels, taking into account the altered permeability of the BBB, through the Qalb, as described by Duranti et al. [92]. In addition, other κFLC-derived formulas are the ratio between κFLC and IgG in CSF, and the CSF to serum κFLC ratio (Q KFLC). All these κFLC parameters have been reported to have a diagnostic accuracy for MS diagnosis similar or superior to OCB [88–91,93,94]. Moreover, both κFLC formula and IgG OCB detection have been proposed as dual assays to improve the diagnostic accuracy for MS disease [93,95]. The prognostic role of κFLC absolute concentrations in CSF has been established in the conversion of CIS to MS [96–98]. In the same way, the prognostic role of the κ-index [99] and the CSF κFLC/IgG ratio [100] have been proven. Rosenstein et al.[101], have recently reported that high κ-index at baseline is predictive of progression independent of relapse activity. However, and in spite of the high sensitivity and specificity of κ-index, there is no consensus about its diagnostic cut-off values, being variable between studies [82,87,102–104].

5. Potential biomarkers

5.1. Neurofilaments

When axonal damage occurs, CNS neurofilaments (Nfs) are released [59]. Nfs are very stable cylindrical proteins composed of heavy (NfH), medium (NfM) and light (NfL) chains and α-internexin [105]. They are located in the neuronal cytoplasm, conferring stability to neurons and being extensively expressed in axons, especially NfL [106,107]. After damage, Nfs are released, reaching the interstitial fluid, and consequently, the CFS and the blood. CSF NfL increase under normal conditions with age, and it is associated with cognitive decline and motor impairment [108]. Both NfH and NfL chains have been studied in CSF as biomarkers in MS, being the NfL assay more sensitive [109,110]. Although CSF Nfs are increased not only in MS, but also in other neuronal pathologies, such as Alzheimer's disease, Creutzfeldt-Jakob disease, frontotemporal dementia, human immunodeficiency virus (HIV) associated dementia, amyotrophic lateral sclerosis, atypical parkinsonian disorders and traumatic brain injury [106].

Enzyme-linked immunosorbent assays (ELISA) were the first proposed for NfL quantification in CFS, nevertheless they could not be used for serum measurements due to its low sensitivity. NfL levels in serum are approximately 40 fold lower than in CSF. But it has been demonstrated that serum NfL could be measured in CIS and MS patients by using an electrochemiluminescence (ECL) based assay. Nevertheless its sensitivity was not the optimal [111–114]. A new single molecule array (Simoa) assay has shown 25 fold higher sensitivity than ECL assays, allowing the use of serum for the study of MS and avoiding the invasive procedure of lumbar puncture [115].

Higher NfL levels are detected in RRMS and progressive MS patients compared to healthy control subjects [110,116,117]. Patients with RRMS have shown higher NfL levels when clinical exacerbation occurs or when having contrast-enhancing lesions, while in progressive MS forms there is no correlation between NfL levels and disease activity [118,119]. As well as for CSF NfL, the main utility of serum NfL is the prognosis value. The association between NfL in serum and EDSS has been studied, finding a direct relation between serum NfL and MS severity score [118,120–122]. In addition, it has been shown that NfL decrease after natalizumab [55,110,123], rituximab [124] or fingolimod [125,126] treatment, showing promising utility for treatment follow-up in MS patients. However, there is no consensus in serum NfL thresholds, because of the lack of harmonization between different assays and the within-individual fluctuating levels during relapsing crisis happen [120,122,127–129].

5.2. Tau protein

Tau protein belongs to the microtubule-associated proteins family as a heat stable protein essential for microtubule assembly [130]. It is released upon neuronal damage and can be measured in CSF [131,132]. Its use in MS is controversial as some studies correlate higher CSF tau protein concentrations with disease progression [133] or with the time to next relapse [134]. Meanwhile others do not find differences in tau protein concentrations in MS patients compared to the control group nor a correlation between tau protein and EDSS scores [135,136].

5.3. Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is the major intermediate cytoskeletal protein in the astrocytes [137,138]. GFAP is released rapidly subsequent to axonal degeneration and it has been widely studied as CSF biomarker in traumatic brain injury [139,140]. Many studies have reported higher levels of GFAP in CFS and in blood in MS patients compared to healthy controls [141–150]. It has also been established that patients with relapsing MS have higher GFAP levels in CSF compared to those with MS in remission [143,145,146,150,151]. A

Table 2
Classification of MS biomarkers.

Biomarker	Biology	Sample	Pathology	Utility	Result in MS
IgG OCB	Intrathecal IgG synthesis	CSF	Inflammation	Diagnosis	↑
IgM index	Intrathecal IgM synthesis	CSF	Inflammation	Prognosis	↑
KFLC	Secreted by B-lymphocytes	CSF	Inflammation	Diagnosis/Prognosis	↑
Neurofilament	Axonal protein	CSF/ blood	Neuronal damage	Diagnosis/Prognosis	↑
Tau protein	Microtubule structural protein	CSF	Neuronal damage	Controversial	Controversial
GFAP	Cytoskeletal protein in the astrocytes	CSF/ blood	Neuronal damage	Diagnosis/Prognosis	↑
S100β	Ca^{2+} and Zn^{2+} binding protein secreted by astrocytes	CSF	Neuronal damage	Diagnosis/Prognosis/ Treatment efficacy	↑
MBP	Part of the myelin sheath, synthesized by oligodendroglia	CSF	Demyelination	Diagnosis	↑
CHI3L1	Extracellular monomeric single-chain glycoprotein expressed in astrocytes macrophages, chondrocytes, synovial cells, osteoblasts, and neutrophils	CSF	Neuronal damage/ Inflammation	Diagnosis/Treatment efficacy	↑
Osteopontin	Extracellular matrix protein secreted by activated macrophages, leukocytes and activated T lymphocytes	CSF/ blood	Inflammation	Prognosis	↑
CXCL12	Cytokine	CSF	Inflammation	Diagnosis	↑
CXCL13	Cytokine	CSF/ blood	Inflammation	Prognosis	↑
CD163	Monocyte/macrophage specific membrane marker	CSF/ blood	Inflammation	Prognosis	↑
CD5 + B cells	B cells	Blood	Inflammation	Prognosis	↑ RRMS ↓ SPMS
Tubulin β	Microtubules component	CSF	Neuronal damage	Potential in prognosis	↑
HSP70	Chaperones	CSF	Inflammation	MS inflammation biomarker	↑

Cerebrospinal fluid, CSF; Chemokine ligand, CXCL; Chitinase-3-like protein, CHI3L1; Glial fibrillary acidic protein, GFAP; Heat shock protein 70, HSP70; Immunoglobulin, Ig; Kappa free light chain, KFLC; Oligoclonal bands, OCB; Mielin basic protein, MBP.

correlation between CSF NfL [120,122] and GFAP [147,149] with EDSS has been described suggesting their role in disable progression prediction. However, Jiang et al.[152], have recently reported that GFAP at baseline correlate with lesion volume but not with disease progression measured though EDSS, Timed 25-Foot Walk, 9-Hole Peg Test, and composite confirmed disability progression tests. Serum GFAP has been reported to be higher in MS patients than in healthy controls and also higher in PPMS than in RRMS patients [142,143,145]. In addition, patients with RRMS showed no significant differences in serum GFAP compared to healthy controls. Serum GFAP could also be used to differentiate MS subtypes, being an adequate marker to assess disease progression [143].

5.4. S100 β

S100β is a small Ca^{2+} and Zn^{2+} binding protein, mostly secreted by astrocytes, but also by oligodendrocytes and certain neuronal subpopulations [153,154]. S100β promotes neuronal proliferation, oligodendrocyte differentiation, and astrocyte morphology maintenance [155]. It has been also reported to control the activation of GFAP, the polymerization of tubulin and DNA repair [156]. S100β can either act as a neurotrophic or as a neurotoxic molecule in vitro, depending on the concentration attained [157–160]. S100β is increased in CSF [161] and serum [162] of patients with MS on acute phase, but also during the course of the disease. It could also be increased in acute brain damage including strokes [163], rapid parenchymal destruction [164] or traumatic brain injuries [165]. Petzold et al. [150] demonstrated a significant increasing trend in S100β levels from PPMS to SPMS and then to RRMS. Moreover, S100β was higher in CSF and serum of MS patients at the time of diagnosis of RRMS compared to healthy control patients [162]. However, additional studies should be performed to evaluate whether S100β concentrations are associated with different MS stages and therefore it could potentially be used as a prognostic tool. Moreover, since S100β was recently reported to decrease upon MS treatment with immunosuppressive [166] or natalizumab [167], it can be also considered as a biomarker of treatment efficacy.

5.5. Myelin basic protein

Myelin basic protein (MBP) is part of the myelin sheath together with myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein [168]. MBP is synthesized by oligodendroglia cells in various isoforms that later undergo a relatively large number of posttranslational modifications, such as deamination, citrullination and phosphorylation [169]. Also MBP is generally considered to maintain the compaction of the myelin sheath [170]. Detectable concentrations of MBP in CSF have been found in acute demyelination [171], but not only in MS patients [172,173]. MPB also increases in acute disseminated encephalomyelitis [174,175], encephalitis [176], acute cerebral infarction [177] and neuro-Behcet's disease [178]. It should also be noted that higher CSF MBP concentrations are not related to MS severity or prognosis [179]. In addition, CNS inflammation correlates with increased MBP deamination leading to higher levels of citrullinated MBP [180,181] which could be used as a prognosis biomarker. Its correlation with MS severity has been proven to be strong, although once again, it increases in other neurology pathologies too [182,183].

Anti-myelin antibodies (anti-MOG and anti-MBP) have also been widely studied. It is considered that the MBP increase after a demyelinating event is followed by an immunoactivity phase [184]. The capacity of anti-myelin antibodies to estimate individual risk for progression of MS disease remains unclear. Concurrently, there are studies that suggest an association between serum anti-myelin antibody status and MS prognosis [185]. However, Kuhle et al. [186] did not find significant differences in MS diagnosis or risk progression between patients showing or not anti-MOG antibodies, anti-MBP antibodies, or both.

5.6. Chitinase-3-like 1

Chitinase-3-like 1 (CHI3L1), also known as YKL-40, is an extracellular monomeric single-chain glycoprotein expressed in astrocytes macrophages, chondrocytes, synovial cells, osteoblasts, and neutrophils [127,187,188] all of them involved in tissue remodeling and inflammation [189]. CHI3L1 increases in CSF in neuronal damage, such as in MS [190,191], but also in other CNS neuroinflammatory pathologies

[192]. It has been shown CIS patients exhibit lower CSF CHI3L1 than patients diagnosed with MS [193]. In addition, CSF CHI3L1 directly correlates with the risk of CIS conversion to MS [194] and with a rapid conversion at high concentrations. A meta-analysis recently conducted by Floro et al. [195], reported higher CHI3L1 in the remission stages of MS than during relapse. These findings suggest that CHI3L1 is possibly a reliable biomarker for clinical practice to monitor the course of MS even in the later stages. Schneider et al. [196] recently demonstrated that CHI3L1 correlates with spinal cord atrophy in progressive MS and also with EDSS [197]. Furthermore, CHI3L1 has utility in the evaluation of MS patient's response to IFN-gamma treatment, showing higher concentrations in the non-responder group [198]. However, serum CHI3L1 needs to be further studied.

5.7. Osteopontin

Osteopontin (OPN) is an extracellular matrix protein secreted by activated macrophages, leukocytes and activated T lymphocytes involved in inflammation and autoimmune disorders, including MS [199]. Higher levels of OPN have been reported in CSF and blood of MS patients [200]. Likewise, increased OPN concentrations in CSF and plasma have been associated with MS progression. Orsi et al. [201] described that higher CSF OPN is related with increased 10-year lesion size, suggesting the importance of inflammation in long-term disease progression. In addition, Marastoni et al. [202] performed a study were CFS OPN showed a relationship with disease progression in RRMS patients after being treated with dimethylfumarate.

5.8. Chemokine ligand

Chemokine (C-X-C motif) ligand 13 or B lymphocyte chemoattractant (CXCL13) is involved in MS pathogenesis [203]. Both serum and CSF CXCL13 expression are increased in MS patients and CIS [204]. Recently, DiSano et al. [205] have evaluated the use of a CXCL13 index, calculated as IgG index or KFLC index, to establish whether or not the production is intrathecal. CXCL13 index has proven to be a reliable biomarker for MS prognosis and follow-up when used alone or in combination with NfL. Moreover, CXCL13 has higher sensitivity than NfL in the prediction of future neuroinflammatory activity in MS patients. This is because it only increases in CSF when neuroinflammation occurs [206,207].

On the other hand, stromal cell-derived factor or C-X-C motif chemokine 12 (CXCL12) is a potent chemo-attractant molecule for different immune cells, including monocytes, T cells, B cells, and plasma cells [208]. Also it is primordial in neuronal development [209,210]. Whereas CSF CXCL12 is elevated in both active and inactive MS, CXCL13 CSF levels were increased in MS active disease only, suggesting it is more specific to evaluate disease progression, as described by Krumbholz et al. [211].

5.9. CD163

CD163 is a monocyte/macrophage specific membrane marker cleaved from the surface of activated macrophages as a soluble form (sCD1639) when inflammation of the CNS occurs. sCD163 can be detected in blood and CSF [59]. Fabriek et al. [212] found an up-regulation of plasma sCD163 and a down-regulation of membrane CD163 in MS patients compared to healthy controls. This conclusion suggests a higher release of this biomarker in MS patients. Furthermore, the sCD163 CSF/serum ratio was significantly increased in MS patients reflecting macrophage activation in MS inflammatory lesions [213].

5.10. CD5 + B cells

B cells have been increasingly recognized as major players in the pathogenesis of MS, as they are involved in other autoimmune diseases [214,215]. CD5 + B cells can be determined in blood samples by flow cytometry and its increased presence has been associated with the development of RRMS [216,217]. Also, it has been demonstrated that the percentage of CD5 + B cells is higher in patients with active disease [218]. Whereas CD5 + B cells have been reported to be decreased in SPMS, some authors suggest that patients with decreased CD5 + B cells and RRMS tend to develop SPMS [214]. In addition, this biomarker has been associated with further elevated risk of early CIS conversion to MS and a higher relapse rate in these patients, independent of IgG OCB presence and MRI findings [219].

5.11. Tubulin β

Tubulin is the major component of microtubules. An alpha (α) and a beta (β) subunits conform the heterodimeric tubulin which exists in six isotypes forms [220]. Tubulin β II is present in the brain and is increased in neuronal development and regeneration. Meanwhile, tubulin β III is also present in dorsal root ganglia and enhanced during axonal growing in the fetal and postnatal period [221,222]. In a preliminary study, Madeddu et al. [151] have suggested that tubulin β II could be a potential candidate for diagnosis. Also, that tubulin β III could be a possible prognostic biomarker of MS. Further studies should be performed to better establish the usefulness of these new biomarkers.

5.12. Heat shock protein 70

Heat shock proteins (HSP) are a group of chaperones that have homeostatic functions in CNS [223]. HSP70 is located in the cytosol protecting cells against lethal stress-induced damage, or in the cell membrane and the intracellular space playing an important role in the immune response [224,225]. Lechner et al. [226], conducted a study to determine whether HSP70 contributes to the neurodegenerative or inflammatory processes in MS. Their results conclude that HSP70 concentrations were significantly higher in patients with CIS or RRMS than in patients with PPMS or SPMS. This may be correlated with the inflammatory process in the first subgroup, which means HPS70 would be a useful biomarker to monitor inflammation in MS.

6. Conclusions

MS is a disabling disease, which needs to be diagnosed as early as possible, requiring a continuous search for new biomarkers and an attempt to achieve a solid consensus on their use. Thus, the latest McDonald's criteria for MS diagnosis included the presence of IgG OCB in CSF as a substitute for dissemination in time, enabling an early MS diagnosis in patients meeting the criteria of dissemination in space, crucial to implement an adequate treatment optimizing clinical outcomes over time. Furthermore, encouraging studies have been undertaken on new potential biomarkers in CSF and blood serum that could be useful in the diagnosis and prognosis of different forms and stages of MS, even if they are not specific for MS but used for other CNS diseases. Despite this, more extensive research is needed to determine the clinical usefulness of these biomarkers and to assess its applicability in everyday practice.

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Author contributions

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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