Biological reflections in body fluids of multiple sclerosis progression and multiple sclerosis-related fatigue

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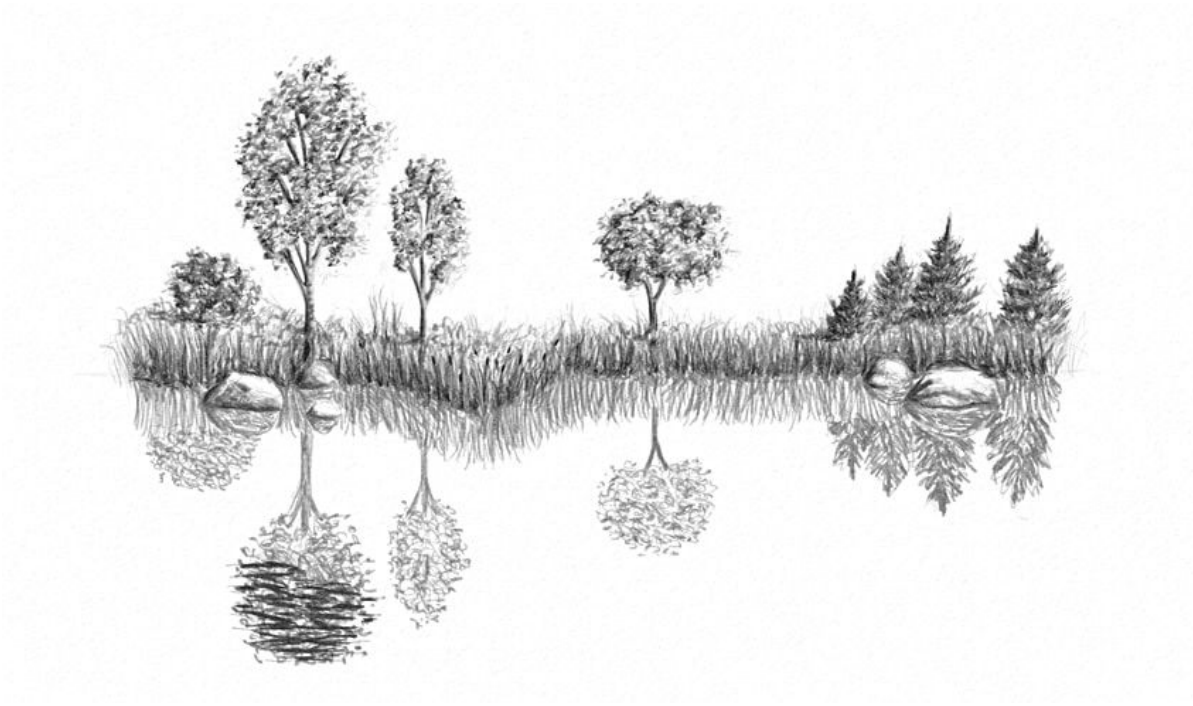
2020

Part 1. Chapter 2.

Body fluid biomarkers for multiple sclerosis —the long road to clinical application



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Nat. Rev. Neurol. 11, 585–596 (2015)

Abstract

There is a strong unmet clinical need for objective body fluid biomarkers to assist early diagnosis and estimate long-term prognosis, monitor treatment response and predict potential adverse effects in multiple sclerosis (MS). Here, we review recent studies (focusing on 2012 to early 2015) on body fluid markers in MS from the perspective of their clinical utility. Because the first step towards clinical implementation of a newly discovered biomarker is independent replication, we focus on biomarkers that have been validated in at least two independent cohorts. We also discuss recent data challenging earlier findings, and biomarkers for which new clinical uses are suggested. For early MS diagnosis and prediction of conversion from clinically isolated syndrome to MS, several new B-cell-associated candidate blood biomarkers have emerged. For prognosis, several novel axonal damage markers should be adopted to biomarker panels. The number of disease-modifying treatments for MS has increased sharply, but biomarkers for treatment response monitoring and adverse effect prediction are scarce, and markers for subtyping and staging of MS are still lacking. In view of the availability and implementation of several standardized protocols to optimize biomarker studies, we expect biomarker development for MS to be improved and accelerated, with clinical implementation in the near future

Introduction

To date, multiple sclerosis (MS) has remained a diagnosis of exclusion. Clinical evaluation and MRI have key roles in the diagnostic process, but MRI lacks specificity during the earliest stages of the disease. Cerebrospinal fluid (CSF) and blood have long been investigated as sources of accessible, dynamic and cost-effective biomarkers of MS that could, in addition to MRI, shed light on the ongoing pathological mechanisms. The discovery, several decades ago, of CSF-specific IgG oligoclonal bands (OCBs) [1] as sensitive diagnostic markers of MS, and their subsequent clinical implementation, fostered the search for additional and more-specific body fluid markers.

Since its discovery in 2004, serum anti-aquaporin 4 IgG (AQP4-IgG) has emerged as a highly specific marker for neuromyelitis optica (NMO), assisting clinicians in differentiating this pathophysiologically distinct entity from MS. As the clinical heterogeneity of MS and related demyelinating diseases of the CNS is increasingly under-stood, the need for objective markers for MS subtyping and prognosis becomes more pressing. Moreover, the expanding range of disease-modifying therapies (DMTs) for MS has generated a need for biomarkers to monitor treatment response and predict adverse effects. Promoted by these clinical demands, MS biomarker research is thriving, and data are being generated rapidly. The road to clinical application is long, however, and validation of the intended use in independent populations of distinct origin is challenging.

To help the clinician identify the most promising candidate biomarkers, we systematically reviewed recent MS body fluid biomarker literature from the perspective of clinical applications, focusing on protein and RNA bio-markers that dynamically change in relation to ongoing pathology, and evaluated the level of evidence on the basis of number confirmatory studies and the total number of patients included in these studies (Box 1) . Although of great interest, genetic biomarkers of MS are constitutive and beyond the scope of this Review (recently reviewed elsewhere [2]). Established and novel CSF biomarkers for MS (including biomarkers that have not yet been validated) have also been reviewed by other authors [1,3].

In this Review, we evaluate the recent proceedings in biomarker development from an applicability view-point. We discuss body fluid biomarkers that have been validated in at least two independent cohorts, recent data that challenge earlier findings, and known biomarkers for which new clinical uses are suggested. The Review is organized in sections

according to the intended clinical use of the candidate biomarkers. The tables summarize the evidence for positively validated biomarkers only, and details on patient numbers in each study are listed in supplementary tables. Finally, we illustrate the potential purpose of the various biomarkers within the clinical subgroups discussed in the Review [4,5].

Box 1 | Search strategy and definition of levels of evidence

We searched for "biomarkers" AND "multiple sclerosis" in the period from January 2012 to March 2015. If one of these papers referred to another relevant recent study, we also included that one. We included studies that had undergone at least initial validation for one of the clinical purposes in an independent cohort, either within the same publication or in different publications. We also included validation studies of research published before 2012. We did not apply selection criteria for number of patients included; numbers of patients in the studies are indicated in Supplementary Tables 1–4. For each subsection, we added search criteria to address the specific clinical question (clinically isolated syndrome, conversion from clinically isolated syndrome, neuromyelitis optica, progression, treatment). Taking into account all published biomarker studies, we divided the biomarkers into three categories reflecting the level of evidence. The classification was primarily based on the number of replication or validation cohorts, studies and number of patients included (irrespective of the division among subcategories within the study). Criteria for levels of evidence, as presented in the Tables, were defined as follows: weak (+): number of independent cohorts or studies = 2, total investigated population <100; intermediate (++): number of studies 2–3, total investigated population 100–200; strong (+++): number of studies ≥3, total investigated population >200. This classification should increase clarity, but the real number of patients needed to proof use of a biomarker is dependent on

Markers for CIS–MS conversion and diagnosis

Clinically isolated syndrome (CIS) refers to the initial clinical manifestation of a CNS demyelinating disease that can precede clinically definite MS (CDMS). Because of the high likelihood of conversion [6] from CIS to CDMS, diagnostic criteria have been revised over the years to enable earlier CDMS diagnosis and treatment. Most patients who would have been considered to have CIS a decade ago now fulfil McDonald 2010 CDMS criteria [7]. However, not all patients with CIS convert to CDMS, and an important area of body fluid biomarker research aims at discovering biomarkers for identification of those patients with CIS who are most likely to convert to CDMS (CIS converters) and benefit from early treatment. Here, we focus on clinically implemented and candidate biomarkers that predict CIS–CDMS conversion (Table 1 and Supplementary Table 1 online), as well as on biomarkers that can assist in the differentiation of early MS from related diseases. Indeed, as CIS converters and patients with MS represent a clinical continuum, and their conditions are likely to share underlying pathological mechanisms, biomarkers predicting CIS conversion could also be useful tools to assist early MS diagnosis, and vice versa. We do not discuss studies focusing on radiologically isolated syndrome, because no biomarker studies have been performed using this definition.

Table 1 | Promising novel biomarkers to identify CIS converters and support early MS diagnosis

Biomarker	Intended clinical purpose	Novel information (obtained 2012–2015)	Level of evidence	Pros and cons
Cerebrospinal fluid biomarkers				
IgG-OCBs	Support for diagnosis of MS	Confirmation[8,9] of numerous previous Studies[2]	+++	Clinically implemented; high sensitivity but low specificity when other inflammatory diseases of CNS are considered
	Identification of CIS converters	Confirmation[6] of previous studies[11]	+++	Clinically implemented; high predictive value for identification of CIS converters
IgM-OCBs	Identification of CIS converters	Confirmation[13] of a previous study[12]	++	Replication in additional cohorts needed
MRZ-specific IgG	Support for diagnosis of MS	Confirmation[19,20,23] of previous studies[18]	+++	Technically challenging Replication in additional cohorts needed
κ free light chains (κFLC)	Support for diagnosis of MS	Two confirmatory Studies[26,30] of previous[24–29] studies	++	Automatized analysis possible Replication in larger cohorts needed
C–X–C motif chemokine 13 (CXCL13)	Identification of CIS converters	One confirmatory study[41] of a previous study[43]	++	Replication in additional cohorts needed
Chitinase-3-like protein 1 (CHI3L1)	Identification of CIS converters	Two confirmatory Studies[48,51,104] of a previous study[50]	+++	Ready for preparations for clinical implementation
Neurofilament light chain (NfL)	Identification of CIS converters	Confirmation[63–65] of previous studies[58,61]	+	Replication in larger cohorts needed
Blood biomarkers				
miR-20a-5p	Support for diagnosis of MS	Confirmation[52] of a previous study[53]	+	Advantage of being blood-based Replication in larger cohorts needed
miR-22-5p	Support for diagnosis of MS	Confirmation[53,54] of a previous study[53]	+	Advantage of being blood-based Replication in larger cohorts needed

Levels of evidence: +, weak (number of independent cohorts or studies = 2, total investigated population <100); ++, intermediate (number of studies 2–3, total investigated population 100–200); +++, strong (number of studies ≥3, total investigated population >200). Abbreviations: CHI3L1, chitinase-3-like protein 1; CIS, clinically is isolated syndrome; CSF, cerebrospinal fluid; miR, microRNA; MRZ, measles–rubella–varicella zoster; MS, multiple sclerosis; OCBs, oligoclonal bands.

Humoral immunity biomarkers

IgG oligoclonal bands

CSF-restricted IgG-OCBs were first reported in patients with MS in the 1960's [1]. Although the presence of IgG-OCBs is not mandatory to diagnose MS according to the 2010 McDonald criteria,[7] their presence is still assessed as part of the MS diagnostic work-up in many MS centers. Two recent meta-analyses evaluated the sensitivity of IgG-OCBs, as measured by isoelectric focusing followed by immunofixation, and obtained values of 88% [8] and 94% [9] for a diagnosis of MS. The poor specificity of IgG-OCBs (61%) when considering MS versus other inflammatory diseases of the CNS, underlines the need for additional biomarkers to assist MS diagnosis. Interestingly, a recent study reported an association between the genetic marker HLA -DRB1*15 and the presence of IgG-OCBs [10], suggesting that genetic factors predispose to the development of IgG-OCBs.

The presence of CSF-restricted IgG-OCBs is a well-known independent prognostic factor for conversion from CIS to CDMS [11]. A recent large multicentre study further confirmed the strong prognostic value of IgG-OCBs in CIS conversion [6].

CSF-restricted IgG-OCBs are robust, clinically implemented biomarkers that need no further validation to confirm their clinical value. However, the lack of an auto-mated and quantitative method for their detection can limit their use in some clinical laboratories

IgM oligoclonal bands

In 2002, the presence of CSF-restricted IgM-OCBs was assessed in a small cohort of patients who had experienced the first clinical manifestations of MS [12]. Almost all patients who showed IgM-OCBs progressed to CDMS within 8 months, whereas only half of those without these bands progressed within 36 months [12]. Similarly, in a recent cohort study of patients with CIS, IgM-OCBs predicted conversion from CIS to CDMS: the mean time for a relapse was longer in IgM-OCB-negative patients than in IgM-OCB-positive patients [13]. At 12 months, 43% of IgM-OCB-positive patients, but only 23% of IgM-OCB-negative patients with CIS had converted to CDMS [13]. Interestingly, in most patients with MS, IgM-OCBs are directed against myelin lipids [14,15]. The principal method applied for assessment of immunoglobulin OCBs is isoelectric focusing followed by immunofixation. A multicentre validation study for this technique, published in 2015 [16], showed a good concordance (89.9%) and a kappa index of 0.71 between 13 centres. Together, these recent results implicate CSF IgM- OCBs as a promising prognostic marker for CIS–CDMS conversion, although the added value of CSF IgM-OCBs over IgG-OCBs remains to be established.

IgG directed against neurotropic viruses

IgG directed against the neurotropic viruses measles, rubella and varicella zoster—collectively referred to as MRZ—has repeatedly been reported in the CSF of patients with MS [17–20]. Though representing less than 2% of the total CSF-specific IgG, MRZ-specific IgG was reported in 80-90% of patients with MS, but was found in only 5% of patients with NMO, and is absent in patients with paraneoplastic neurological disorders, underlining the specificity of this analysis [19,21,22]. In a recent small study of IgG-OCB-negative patients with relapsing–remitting MS (RRMS), secondary progressive MS (SPMS) or primary progressive MS (PPMS), the prevalence of CSF-specific IgGs directed against two or more of the MRZ viruses was 24% in patients with MS and zero in the control groups (individuals with other inflammatory or noninflammatory neurological diseases) [23]. Thus, in addition to OCBs, determination of an intrathecal polyspecific antiviral immune response could be a useful marker to assist MS diagnosis. Anti-MRZ antibodies could also have prognostic value. Patients with CIS who showed two or more MRI T2-weighted lesions and were positive for MRZ- specific IgGs were recently reported to be at high risk of converting to RRMS [17]. To conclude, MRZ-specific IgGs are promising specific markers to support the diagnosis of MS, and could also have prognostic value for CIS conversion to CDMS.

However, the identification of MRZ-specific IgGs is technically challenging, which could limit their use.

κ free light chains

Besides immunoglobulins, plasmacytes also produce and secrete immunoglobulin free light chains (FLCs), which can be detected in both CSF and serum [24–28]. Since the late 1970s, multiple studies have reported an association between CSF FLCs and MS [24–28]. The analytical specificity of the earlier methods was questionable, but with the recent use of the highly sensitive nephelometric FLC assay [25], research in this field has been revived. CSF κ-FLC levels have been reported in over 50% of IgG OCB-negative patients with CIS [29], and two recent studies confirmed that both CSF κ-FLC levels and the κ-FLC index (κ-FLC–λ-FLC ratio) are increased in patients with CIS or RRMS compared with controls [26,30]. Moreover, a distinct pathological CSF FLC monomer–dimer pattern characteristic of RRMS was recently reported [27]. In summary, high CSF κ-FLC levels have potential as diagnostic markers of CIS and RRMS. The κ-FLC level also has the advantage of being assessed by an automated procedure.

Anti-KIR4.1 IgG

In a study published in 2012, antibodies directed against the glial inwardly rectifying potassium channel KIR4.1 (anti-KIR4.1) were detected in the serum of around half of a cohort of patients with MS [31]. Recently, the same research group reported a prevalence of over 50% for anti-KIR4.1 in children with MS [32]. These findings were interesting from a pathophysiological point of view, being the first to convincingly identify a potential brain tissue-specific antigenic target in MS. However, two recent studies using comparable ELISA methodology failed to reproduce these data, finding no evidence or low prevalence of anti-KIR4.1 autoantibodies in patients with MS [33,34]. Additional confirmation or invalidation in other cohorts of patients is needed in order to assess the pertinence of anti-KIR4.1 antibodies in MS.

Anti-myelin oligodendrocyte glycoprotein IgG

In the past, myelin oligodendrocyte glycoprotein (MOG) has been intensively investigated as a potential antigenic target in acquired CNS demyelinating diseases, and MOG autoantibodies (MOG-IgG) as diagnostic biomarkers of MS [35]. Though rarely detectable in the serum of adult patients with MS, MOG-IgG is frequently detected in children with MS or acute demyelinating encephalomyelitis (ADEM) [36]. A recent prospective study reported that serum MOG-IgG and other antibodies against myelin have no predictive value for CIS–CDMS conversion [37].

Inflammatory and immunological biomarkers C–X–C motif chemokine 13

In 2006, B-cell-attracting C–X–C motif chemokine 13 (CXCL13) mRNA was reported to be expressed in actively demyelinating MS lesions, but not in chronic inactive lesions or in the CNS of healthy controls [38]. In 2009, increased levels of CSF CXCL13 were detected in patients with CIS, RRMS, SPMS or PPMS compared with patients who had noninflammatory CNS disease [39].

The use of CXCL13 as diagnostic marker for MS is tempered by its apparent lack of specificity: elevated CSF levels of CXCL13 have recently been reported in patients with various other inflammatory or infectious diseases of the CNS [40–41]. Interestingly, a novel study on two large independent cohorts of patients with MS showed an inverse correlation between CXCL13 CSF levels and age, reflecting the predominantly neurodegenerative rather than inflammatory processes at later stages of MS [42]. In 2010, a 2-year prospective study reported higher CSF levels of CXCL13 in CIS converters than in non-converters [43].

These results were recently confirmed in an independent cohort [41], confirming the high potential of CXCL13 as a prognostic biomarker for CIS conversion; however, its lack of specificity impedes its use as diagnostic marker for MS.
Overall, biomarkers for B-cell activation and subsequent antibody secretion seem to predominate at early stages of MS. Whether the activation of B cells and the subsequent downstream effects are causative for MS remains inconclusive [1,44]. The importance of B cells in MS is further emphasized by the efficacy of anti-B-cell strategies [44].

Chitinase-3-like protein 1

The specific function of chitinase-3-like protein 1 (CHI3L1) is unknown, but its implication in various inflammatory and neoplastic diseases suggests both inflammatory and tissue remodeling roles [45,46]. In MS, immunohistochemistry and *in situ* hybridization revealed expression of CHI3L1 primarily in reactive astrocytes and microglia [47,48], and CHI3L1 protein levels were shown to increase on exposure of macrophages to IL-13 [49]. In a 2010 paper, CSF CHI3L1 levels were reported to be higher in CIS converters than in CIS non-converters [50]. These results were recently confirmed in two large and well-designed independent studies [48,51], supporting the robustness of CHI3L1 as a prognostic marker of CIS conversion. Although CSF CHI3L1 is a confirmed biomarker to identify CIS converters, its use as diagnostic biomarker for MS needs further replication.

Noncoding RNAs

Small noncoding microRNAs (miRNAs) regulate gene expression, and are being intensively investigated as a novel category of potential biomarkers. In 2010, miR-20a-5p was reported to be downregulated in whole-blood samples of a small cohort of treatment-naïve patients with RRMS, SPMS or PPMS compared with healthy controls [52,53]. Interestingly, most of the targets of miR-20a-5p are involved in T-cell regulation [52,53]. Another potential marker is miR-22-5p, which is upregulated in the blood [54,55] and brain lesions [56] in patients with MS. It should be emphasized that the sample sizes of these studies are very small, and that replication in larger cohorts is necessary.

Other biomarkers

Neurofilaments as axonal damage biomarkers

Neurofilaments are major components of the axonal cytoskeleton, consisting of light (NfL), intermediate, and heavy (NfH) chains that can be detected in the blood and CSF after axonal damage [57–60]. In 2009, we showed that CSF NfL levels were higher in CIS converters than in non-converters [61]. Increased levels in patients with CIS have been confirmed in independent studies [62–64]. Interestingly, increased levels of CSF-restricted NfL antibodies (NfL-IgG) [62] and CSF-restricted NfH anti-bodies (NfH-IgG) [65] were recently reported in CIS converters, suggesting that NfL-IgG and NfH-IgG are promising additional prognostic biomarkers of CIS conversion. However, these results, reported by the same group, require further validation in independent cohorts.

Cholesterol as a membrane homeostasis biomarker

There is currently great interest in 24-hydroxycholesterol (24-OHC) as a biomarker of MS. Slightly decreased levels of serum 24-OHC have been reported in patients with MS—particularly patients with PPMS and older patients with RRMS—compared with healthy controls [66–68] The small differences and overlap in values between groups prohibits clinical application as a single diagnostic marker, although the brain specificity and potential for monitoring biological response to statin therapy sustain interest in this molecule [69].

Markers for NMO differential diagnosis

NMO is a rare autoimmune disease of the CNS, with a typical clinical pattern including longitudinal extensive transverse myelitis and optic neuritis. Historically considered to be a variant of MS, NMO was defined as a pathophysiologically distinct entity in 2004 by the identification of pathogenic serum AQP4 autoantibodies (AQP4-IgG) [70,71]. The discovery of AQP4-IgG as an NMO biomarker resulted in the acknowledgement of an expanded spectrum of clinical disorders that previously would not have met the diagnostic criteria for NMO, now referred to as NMO spectrum disorders (NMOSD) [72]. Certain MS therapies, such as IFN-β and natalizumab, can exacerbate NMO, emphasizing the clinical importance of accurate differentiation of NMO and NMOSD from MS [73–75]. However, differentiating between NMO and CIS or early MS can be challenging, particularly during the early stages of NMO, and if AQP4-IgG is not detected.

Although the presence of AQP4-IgG is very specific for NMO, a critical review and comparison of 59 studies [76] found that its sensitivity ranges from 12.5% to 100%, with a median of 62.3%, depending on the different assays used. The identification of seronegative NMO patients fostered the search for alternative biomarkers of NMO [77].

Humoral immunity biomarkers

Anti-aquaporin 4 IgG

AQP4-IgG is a robust and clinically implemented biomarker for NMO. Recently, recombinant antigen-based assays such as ELISA, cell-based assays or fluorescence-activated cell sorting (FACS), were proved to be more sensitive than tissue-based indirect immunofluorescence assays in detecting serum AQP4-IgG in patients in NMO [78]. For example, 61% of patients who tested negative by tissue-based indirect immunofluorescence were

actually seropositive by the novel assays [79]. Hence, the use of these tests rather than classic indirect immunofluorescence staining for diagnostic purposes will certainly increase the number of AQP4-IgG-positive patients with NMO. These recent findings emphasize the importance of optimizing detection methods for established biomarkers.

Anti-myelin oligodendrocyte glycoprotein IgG

Recently, several groups reported the presence of MOG-IgG in the serum of patients with NMO and NMOSD [80–82]. Conflicting data regarding the coexistence of AQP4-IgG and MOG-IgG has led to a debate: some groups report mutual exclusivity of these autoantibodies [80,82,83], whereas others suggest the existence of double-seropositive patients harbouring both AQP4-IgG and MOG-IgG [81,84]. Regardless of these conflicting data, MOG-IgG is absent in MS, making MOG-IgG a promising biomarker to discriminate MS from AQP4-IgG-negative NMO.

Anti-KIR4.1 IgG

Given the recent interest in anti-KIR4.1 for the diagnosis of MS (see above), one research group has investigated whether these antibodies could discriminate between MS and NMO. Serum anti-KIR4.1 was elevated in 26% of patients with MS and in 22% of patients with NMO, but only in 6% of healthy controls [85]. Thus, anti-KIR4.1 does not reliably discriminate between different demyelinating diseases, but the authors noted that they detected elevated anti-KIR4.1 levels during MS relapses, indicating potential for disease activity monitoring [85]. However, this finding has not yet been replicated by other research groups.

Haptoglobin as a marker for inflammation

The levels of haptoglobin—an acute-phase protein synthesized by the liver—are elevated in several inflammatory diseases [86]. Proteomic studies have previously identified CSF haptoglobin as a candidate biomarker for NMO [87,88]. In a small verification study using a combination of ELISA and western blot [89], CSF haptoglobin concentrations were confirmed to be higher in patients with NMO than in patients with MS, Alzheimer disease or noninflammatory neurological disease. In this study, no differences in serum haptoglobin levels were detected between the NMO and control groups, in contrast with previous data from other groups [90]. The added value of haptoglobin over AQP4-IgG for diagnosis of NMO remains to established, especially given that it requires additional CSF analysis.

Summary

Overall, anti-AQP4-IgG remains the most robust biomarker for NMO diagnosis (Table 2 and Supplementary Table 2 online), particularly when detected with recombinant antigen-based assays. Additional biomarkers are still needed for accurate diagnosis of seronegative patients, which can be facilitated by more-sensitive methods, anti-MOG antibodies, or analysis of panels of biomarkers. The additional value of other markers, such as anti-KIR4.1 or haptoglobin, should be addressed in larger cohorts, taking into account the patients' anti-AQP4 status.

Table 2 | Promising novel biomarkers to aid NMO differential diagnosis

Biomarker	Intended clinical purpose	Novel information (obtained 2012–2015)	Level of evidence	Pros and cons
Blood biomarkers				
AQP4-IgG	Diagnostic support of NMO	Increased sensitivity for AQP4-IgG detection using recombinant antigen-based assays[79]	+++	Clinically implemented
MOG-IgG	Diagnostic support of NMO	Three recent studies[80–82]	++	Potentially useful in AQP4-IgG-negative patients Replication in additional cohorts needed
CSF biomarkers				
Haptoglobin	Discriminating NMO from MS	One confirmatory study[89] of previous studies [87,88]	+	Replication in larger cohorts needed Feasibility limited by being CSF-based

Levels of evidence: +, weak (number of independent cohorts or studies = 2, total investigated population <100); ++, intermediate (number of studies 2–3, total investigated population 100–200); +++, strong (number of studies ≥3, total investigated population >200). Abbreviations: AQP4, aquaporin 4; CSF, cerebrospinal fluid; MOG-IgG, IgG against myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NMO, neuromyelitis optica.

Markers for subtyping of MS

An increasing number of DMTs are available to stabilize RRMS but, to date, no efficient DMTs have been developed for PPMS and SPMS. A cornerstone in therapeutic clinical trials is early identification and inclusion of patients. However, diagnosis of PPMS, and detection of RRMS-to-SPMS transition, is often retrospective, based on clinical identification of progression defined as relentless accumulation of disability in the absence of relapse activity. Terms such as 'progressing', 'not progressing', 'active' and 'not active' are now added to the standard disease course description in the new definitions of the International Advisory Committee on Clinical Trials in MS [5].

Objective biomarkers for early subtyping of MS into relapse-onset versus PPMS, and for identification of active and non-active disease and progression, are critically needed. However, biomarker research addressing this particular clinical need lags behind other areas of MS biomarker research, as underlined by the smaller number of studies published in the past 3 years (Table 3 and Supplementary Table 3 online). Despite the clear need for biomarkers to subtype MS, only few recent studies have addressed this question; consequently, no biomarkers have been clinically implemented.

Humoral immunity biomarkers

A recent study assessed whether the IgG index or the number of IgG-OCBs could help discriminate CIS, RRMS, PPMS and SPMS, but no differences were detected between any of the subtypes [91]. A single study comprising three independent cohorts of patients with PPMS reported a correlation between CSF IgM-OCBs and gadolinium-positive lesions on MRI. The authors argued that the subset of CIS patients who are IgM-OCB- positive could have a more inflammatory disease type and, hence, might benefit from immunotherapy [92].

Noncoding RNAs as inflammatory markers

A recent study profiling miRNAs in the serum to differentiate RRMS from PPMS identified three differentially expressed miRNAs, which were further validated in two additional independent small cohorts within the same study. The authors found that miR-223 and miR-15b were most useful to discriminate PPMS from RRMS [93]. Interestingly, miR-223 was recently implicated in the regulation of CNS inflammasomes [94], and miR-15b as a promoter for neurogenesis [95].

N-acetylaspartate as axonal damage marker

N-acetylaspartate (NAA) levels are decreased on magnetic resonance spectroscopy96 and in the CSF of patients with SPMS in comparison with RRMS and CIS. In addition, decreased NAA levels correlate with increased Expanded Disability Status Scale (EDSS) scores, increased MRI lesion loads and decreased brain volume, but NAA levels are not altered in patients with early MS [64,97–99]. Reduced NAA levels in the later stages of disease suggest that NAA could be a marker for axonal damage in progressive forms of MS, but the variation within patient groups is too high for NAA to be used as a single marker.

Markers for MS prognosis

Clinically, and probably also pathophysiologically, MS is a heterogeneous disease both across disease subtypes and within a particular subtype. Within a subtype of MS, some patients experience early disability progression, while others remain disability-free many years after diagnosis. Here, we focus on recent studies that have addressed the predictive value of biomarkers within all subtypes of MS, as long-term prognosis is relevant for all subtypes (Table 4 and Supplementary Table 3 online).

IgM oligoclonal bands

A recent study reported that in CIS, IgM-OCB-positive patients showed an early increase in lesion load and brain atrophy, indicating that IgM-OCBs have prognostic value [100]. However, the cohort examined in this study was small, and replication in a larger cohort is needed. CSF-restricted IgM-OCBs have been reported for over a decade as prognostic biomarkers of early RRMS [101],
with patients harbouring IgM-OCBs having more-active disease in terms of relapse rate [102]. In addition, patients with IgM-OCBs were shown to convert earlier to SPMS [101].

Chitinase-3-like protein 1

As discussed above, CHI3L1 is a very promising candidate biomarker to identify CIS converters, and CSF CHI3L1 was recently analysed in relation to disability progression. In an RRMS cohort, elevated CSF CHI3L1 levels were associated with earlier progression to high EDSS scores [103].
In a retrospective study of patients with optic neuritis as a first demyelinating event, high CSF levels of CHI3L1 predicted long-term cognitive impairment [104].

Noncoding RNAs

As described above, miRNAs have potential as biomarkers in MS. One recent study showed that elevated levels of miR-92a-1 and miR-454 were associated with more-severe disease and increased disability [105].

Table 3 | Promising emerging blood-based biomarkers for subtyping of MS

	purpose	(obtained 2012–2015)	evidence	
IgM oligoclonal bands	Subgrouping of PPMS	Initial study including three independent cohorts[92]	++	Replication in independent studies needed
miR-223	Discriminating RRMS from PPMS	Initial study including two independent cohorts[93]	+	Replication in independent studies with larger cohorts needed
miR-15b	Discriminating RRMS from PPMS	Initial study including two independent cohorts[93]	+	Replication in independent studies with larger cohorts needed

Levels of evidence: +, weak (number of independent cohorts or studies = 2, total investigated population <100); ++, intermediate (number of studies 2–3, total investigated population 100–200). Abbreviations: miR, microRNA; MS, multiple sclerosis; PPMS, primary progressive MS; RRMS, relapsing–remitting MS.

Table 4 | Promising novel prognostic biomarkers

Biomarker	Body fluid	Intended clinical purpose	Novel information (obtained 2012–2015)	Level of evidence	Pros and cons
Neurofilament light chain	Blood	Clinically isolated syndrome prognosis	Confirmation[99] of previous Studies[59,61]	+	Advantage of being blood-based Replication in larger cohorts needed
IgM oligoclonal bands	Cerebrospinal fluid	Prognosis of relapsing–remitting multiple sclerosis	Confirmation[100] of previous Studies[12,13,14,101,102,128]	+++	Ready for preparations for clinical implementation

Levels of evidence: +, weak (number of independent cohorts or studies = 2, total investigated population <100); +++, strong (number of studies ≥3, total investigated population >200).

Axonal damage biomarkers

Neurofilaments

Above, we mentioned CSF NfL as a promising prognostic biomarker for CIS conversion, and neurofilaments have also been investigated as early markers of long-term prognosis in CIS, RRMS and PPMS. In a cohort of patients with CIS, we found that CSF NfH levels correlated with physical disability and brain volume loss over 1 year [64]. In a cohort of patients with RRMS who were followed up for a median of 14 years, CSF NfL levels at diagnosis correlated with long-term MS Severity Score (MSSS) [106]. Moreover, conversion to SPMS was more likely in cases with high NfL levels (>386 ng/l) than in those with low levels (<60 ng/l) [106]. In a cohort of patients with SPMS or PPMS, we recently identified NfH as a predictor of ongoing disability (as measured by the MSSS), and NfL as a predictor of EDSS annual increase [99]. A promising sensitive immunoassay for quantification of NfL in serum has recently been developed [105], and serum NfL levels were reported to predict recovery after a spinal cord lesion in RRMS [60,107]. In addition, serum NfL was recently shown to be elevated in patients with CIS, and this elevation was associated with the number of T2-hyperintense and gadolinium-enhancing lesions, and with increased disability status [108].Overall, neurofilament proteins can be used for prognostic evaluation at a group level, but their prognostic value in individual patients seems limited, and more extensive validation (including longitudinal cohort studies) is required.

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is an astrocytic cytoskeletal component, and elevated levels of this protein have been reported in progressive subtypes of MS [59,109]. In a 2011 paper, CSF GFAP levels were reported to have predictive value for neurological disability 8-10 years later [110]. Recently, in a cohort of patients with CIS or RRMS, high levels of CSF GFAP were associated with earlier progression on the EDSS [103].

Summary

Prediction of prognosis in any given subtype of MS remains difficult, and should be assessed in longitudinal biomarker studies. This area of research is of paramount importance both for treatment selection and patient counselling.

Markers for treatment response in RRMS

The number of DMTs that are available to treat RRMS is growing, but body fluid biomarkers for treatment response evaluation and adverse effect prediction remain scarce (Table 5 and Supplementary Table 4 online). It would be extremely helpful to be able to predict which individual will benefit from a particular type of DMT, for example, to foresee who will respond to anti-B-cell therapy (such as rituximab or ocrelizumab), or who will benefit more from S1P receptor subtype modulators (such as fingolimod or siponimod).

Biomarkers predicting treatment non-response

Anti-interferon antibodies

Parenteral administration of a protein drug often induces an antibody response. The use of anti-IFN-β NAb assays and/or bioactivity measurements, such as quantification of myxovirus-resistance protein A (MxA), is well established in clinical practice [111]. MxA is usually upregulated on IFN-β injection; however, if IFN-β NAbs are present, this response is attenuated. 3–20% of patients with MS have high NAb titres and/or lack ofMxA induction after sustained (12–24 months) IFN-β therapy; in these cases, switching to alternative drugs should be considered [111]. Several HLA class II alleles and short nucleotide polymorphisms have been suggested to be associated with anti-IFN-β NAb titres [112,113], but no validated biomarkers are currently available to reliably predict the development of anti-IFN-β NAbs in individual patients. Recent work comprising a prospective European MS cohort suggested a role for an early increase in binding antibody titers, which reliably predicted anti-IFN-β NAbs; moreover, the investigators identified CXCL10 as a promising predictor of NAb-associated IFN-β response attenuation [114]. Retrospective analysis of the BENEFIT trial data suggested that in early MS, high levels of anti-IFN-β1b NAb predict slow anti-IFN-β1b–NAb dissociation rate, raising the possibility of early differentiation of persistently NAb-positive patients from transiently positive patients [115]. Another recent study on IFN-β-treated patients suggested that higher levels of serum MxA mRNA predicted a lower risk of EDSS progression [116].

Anti-natalizumab antibodies

Several studies have suggested that persisting antibodies against natalizumab have clinical relevance [117,118]. These antibodies develop early during treatment, persist in about 6% of patients, and are associated with a decrease in treatment efficacy and adverse reactions to natalizumab infusions. However, lack of efficacy and infusion reactions will generally not be missed clinically and radiologically in natalizumab-treated patients, as relapses and new, active and/or enhancing lesions rarely occur during such treatment in daily practice, limiting the clinical relevance of anti-natalizumab antibodies.

Inflammatory and immunological biomarkers

In 2010, high concentrations of IL- 17F before initiation of therapy were reported to be associated with lack of response to IFN- β [119]. However, in a recent large study, levels of IL- 17F measured at baseline and at 6 months after treatment initiation did not correlate with lack of response to treatment after 2 years, as measured by clinical and MRI outcomes [120]. Only very high levels of IL- 17F (>200 pg/ml) found in eight patients (4.4%) were associated with non-responsiveness to IL- 17F.

Levels of miRNAs in the blood also could have a role as biomarkers of the biological response to IFN- β [121–123]. Data from a recent longitudinal study suggest that dynamic changes in miRNA levels could serve as biomarkers of the biological effects of IFN- β therapy, and can have predictive value for clinical response in individual patients [123].

Biomarkers predicting adverse effects

There is an evident need to better predict the risk of PML in patients with MS who are on DMTs, especially natalizumab and—probably to a lesser extent—dimethylfumarate (DMF).

Anti-JCV antibodies

The development of PML during natalizumab treatment is clearly associated with the presence of anti-JCV antibodies in the blood [124,125]. Recent evidence suggests that JCV antibody levels, measured as a serum or plasma index, enables further stratification of the risk of PML in JCV-seropositive patients [126]. In patients without immunosuppressant use before natalizumab, a low anti-JCV antibody index indicates a low PML risk, even in anti-JCV antibody-positive patients. In patients with prior immunosuppressant use, this association was not shown [126]. Thus, anti-JCV antibodies can aid identification of patients who can continue natalizumab with only a minor risk of PML, and those who should be switched on other medications.

l-selectin-expressing CD4+ T cells

To further identify biomarkers that predict PML risk during natalizumab treatment, flow cytometry in peripheral mononuclear blood cells suggested that numbers of l-selectin-expressing CD4+ T cells were lower in patients who had received long-term treatment with natalizumab than in patients not using natalizumab, and in healthy controls [127]. Moreover, in patients who developed PML, a ninefold decrease in the number of l-selectin-expressing CD4+ T cells was found in the pre-PML samples. The applicability and the additive value of this cell-based assessment in comparison with the JCV index should be established in further work.

IgM oligoclonal bands

In 2015, lipid-specific IgM oligoclonal bands in the CSF were demonstrated to reflect a decreased risk of natalizumab-associated PML [128]. The authors of the study speculated that IgM might neutralize the immuno-suppressive properties of natalizumab; this possibility needs to be confirmed in further studies.

Summary

The small number of validated biomarkers for treatment response (Supplementary Table 4 online) indicates that personalized medicine for MS is still in its infancy. Nevertheless, clear progress has been made, especially in the immunogenicity field (applicability of neutralizing antibodies [NAbs]) and in pharmacovigilance (JC virus [JCV] index, and risk of progressive multifocal leuko-encephalopathy [PML]). Companies have the opportunity to investigate treatment response biomarkers in phase III trial data sets, and large MS centres, and MS research networks such as the BioMS, are in a position to test biomarkers in individual patients in relation to different drugs.

Table 5 | Promising novel blood biomarkers for treatment response and adverse effects

Biomarker	Intended clinical purpose	Novel information (obtained 2012-2015)	Level of evidence	Pros and cons
Anti-IFN-β NAbs	No response to IFN	Confirmation of numerous studies[110]	+++	Clinically implemented Identifies only a fraction of non-responders
Anti-natalizumab NAbs	No response to IFN	Confirmation[124] of initial clinical purpose[116,117]	+++	Little add-on value on clinical observation
miRNAs (miR-29 family; miR-26a-5p)	No response to IFN	Two studies[120,122]	+	Small study size
Anti-JCV antibodies and JCV index	Predicting risk of PML in natalizumab-treated Patientsp[23]	Confirmation[123] of an initial study[123]	+++	Clinically implemented biomarker

Levels of evidence: +, weak (number of independent cohorts or studies = 2, total investigated population <100); +++, strong (number of studies ≥3, total investigated population >200). Abbreviations: IFN, interferon; miR, microRNA; NAbs, neutralizing antibodies.

Discussion

Good quality validation studies have been reported for several biomarkers for different clinical purposes (Tables 1–5). Validation for the intended purpose, at both technical and clinical levels, is an important aspect in biomarker studies. Here, we focused on clinical validation in independent cohorts or studies, and defined the level of confidence on the basis of the number of clinical studies and patients in cohorts (Figure 1). However, technical validation of the assays used is equally important, and validation by independent methods would further strengthen the evidence for the various biomarkers. In addition, before they can be implemented in clinical practice and guidelines, the biomarkers must be validated in larger cohorts than were used in the majority of studies reported to date.

Increased understanding of MS pathophysiology can facilitate identification of novel biomarkers; for example, the discovery of the role of axonal damage in MS implicated neurofilaments as prognostic markers. That said, with the advances in 'omics' technologies, we now have the tools to systematically screen body fluids for novel biomarkers for different clinical purposes. One of the consequences of adopting the 'omics' approach is a shift from the old, hypothesis-based single-marker paradigm towards compilation of panels of multiple biomarkers that reflect multiple disease mechanisms. Thus, endo-phenotyping and molecular profiling (defining a molecular signature without a priori definition of the pathway to which the molecule belongs) represent new avenues for biomarker research. There are several arguments for adopting such an approach. First, until we fully understand the mechanisms of MS, we must be open to novel mechanisms.

Second, hypotheses based on post-mortem evaluation cannot be directly extrapolated to the in vivo situation. Third, the pathophysiology of MS is heterogeneous, with multiple mechanisms involved at every clinical or pathological stage. Last, the assignment of biomarkers to one specific pathway is too restricted, because molecules can be involved in multiple mechanisms; for example, NfL is an axonal protein, but axonal damage can be the direct consequence of inflammation, so NfL also participates in inflammatory pathways. It should be noted that our knowledge of the function and cellular location of proteins is still very limited and largely influenced by specialities with a large capacity for biomarker research, such as cancer and other non-neurological diseases, and the translatability of these findings to MS is unknown

Conclusions

Development of novel biomarkers is always a long process (Figure 2), and optimal use of available resources is required. International collaboration is essential for standardization of all aspects of biomarker studies, including biobanking procedures and study design, and to enable construction of sufficiently powered cohorts and optimally replicate findings.

The BioMS network has taken several steps to standardize all aspects of biomarker research and validation (Box 2). These procedures have also been useful for researchers focusing on other biomarkers. For example, dementia researchers have adopted the standardized bio-banking protocol; moreover, they have developed additional standards, such as for biomarker assay evaluation, driven by experience and problems during the clinical validation studies for Alzheimer disease biomarkers. The experiences gained from MS and Alzheimer disease biomarker research, and the resulting guidelines, are of great benefit for biomarker studies for any neurological disease; indeed, these protocols are now widely adopted and employed as useful tools for performing high-quality biomarker studies in other fields of neurology. Guidelines and collaboration within research networks, such as the BioMS network, will further pave the way for optimization of biomarker development. Such harmonized collaboration will also accelerate implementation in clinical practice for the currently prioritized clinical needs, most importantly progressive MS and prediction of treatment response.

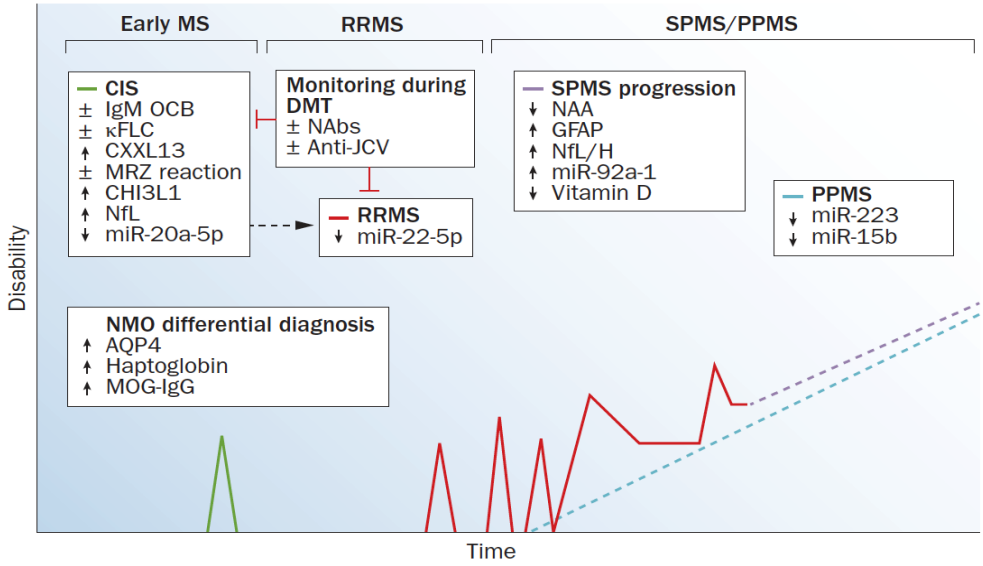

Figure 1 | Biomarkers associated with different clinical phases in MS. This overview shows how biomarkers discussed in this Review are linked to specific clinical stages of MS. The arrows indicate expression changes in MS. ± indicates that the antibody can be either present or absent. Abbreviations: κFLC, κ free lightchain; AQP4, aquaporin 4; CHI3L1, chitinase-3-like protein 1; CIS, clinically isolated syndrome; CSF, cerebrospinal fluid; CXCL13, C–X–C motif chemokine 13; DMT, disease-modifying therapy; GFAP, glial fibrillary acidic protein; KIR4.1, potassium channel, inwardly rectifying subfamily J member 10; miR, microRNA; MOG-IgG, IgG against myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; MRZ, measles–rubella–varicella zoster; NAA, N-acetylaspartate; NAbs, neutralizing antibodies (against IFN-β or natalizumab); NfL/H, neurofilament light/heavy chain; NMO, neuromyelitis optica; O`CBs, oligoclonal bands; PPMS, primary progressive MS; RRMS, relapsing–remitting MS; SPMS, secondary progressive MS.

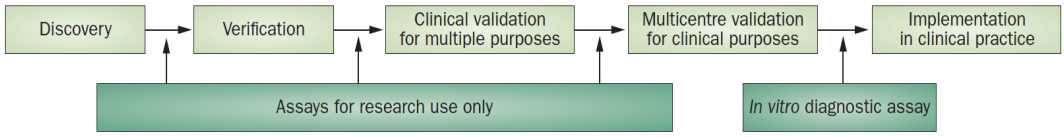

Figure 2 | Schematic representation of the process of biomarker development. The light green boxes indicate the steps to be followed, dark green boxes indicate the type (and quality) of assays that are typically used. The first three steps can be performed rapidly (in a couple of years), but the complete process to clinical implementation usually takes >20 years

Box 2 | Standardized tools to optimize biomarker studies for neurological diseases

- CSF collection and biobanking protocols [129–134]
- CSF collection quality auditing too(http://www.isber.org/surveys/?id=EQASurvey [135])
- Guidelines for exchange of biobanked samples
- Tools developed by the Joint Programming for Neurodegenerative Diseases, such as The Biospecimen Stability Testing SOP and Calculator Tool (http://www.isber.org/?page=STABCALC [135])
- Reference material for evaluation of quality of biobanked CSF and blood samples (available via authors of this paper at the department of Clinical Chemistry VU University Medical Centers; http://bit.ly/CSF-stability [136])
- Guidelines for selection of control groups [129,130]
- Guidelines for study design [129]
- Reporting framework [130]
- Standardized protocol for assay evaluation and reporting templates [137]

Abbreviation: CSF, cerebrospinal fluid.

Acknowledgements
We acknowledge support from the Dutch MS Research Foundation (grant no 14-358e "Facing MS progression"), Fonds NutsOhra (grant no. ZonMw 89000005) and the International progressive MS Alliance.

Author contributions
All authors provided substantial contribution to discussion of content of the article, and contributed to researching data for the article, and writing, reviewing and editing the article.

Supplementary information is linked to the online version of the paper at www.nature.com/nrneurol.

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