Fluid phase biomarkers in multiple sclerosis

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Purpose of review
Multiple sclerosis (MS) is highly heterogenic disorder with respect to clinical course, diagnosis, and treatment response. There is an urgent need to search for simply and reliable fluid body biomarker which would assist the diagnosis and prediction of clinical and treatment prognosis.

Recent findings
'Traditional' MS biomarkers, with exception of cerebrospinal fluid oligoclonal bands, still are having limited clinical value. Therefore, there is growing interest in novel molecules and ingredients. The most robust results have been generated with regard to cerebrospinal fluid and serum levels of neurofilament light chains (NfL). However, there are still some limitations related to specificity of NfL which delays its use in everyday practice. We present a new approach to search for biomarkers involving extracellular RNA, particularly microRNA (miRNA), and small extracellular vesicles. MiRNA represents an important molecular mechanism influencing gene expression, including those involved in MS pathogenesis and extracellular vesicles transfer multiple cargo, including myelin molecules from parental cells of central nervous system to the long-distance targets.

Summary
MiRNAs which control gene expression in cells involved in autoimmune processes in MS as well as extracellular vesicles transferring myelin content might generate a new promising categories of biomarkers of MS.

Keywords
biomarkers, extracellular vesicles, multiple sclerosis, neurofilaments, RNA

INTRODUCTION

Multiple sclerosis (MS) is a common autoimmune disease of central nervous system (CNS). The clinical presentation, prognosis, and treatment response are highly heterogenic. MS diagnosis depends primarily on clinical symptoms and MRI findings. One of the major challenges in MS is development of simple and reliable fluid body biomarker for its diagnosis, prediction of clinical course, and treatment outcomes. Several studies have been performed for search of such biomarkers within cerebrospinal fluid (CSF) and serum over the last years. Some of them like oligoclonal bands (OCBs) entered clinical practice and others are still waiting for validation. In this review, we will present MS fluid body biomarkers with emphasis on the new findings related to extracellular vesicles and extracellular RNA (exRNA).

Oligoclonal bands in the cerebrospinal fluid

Despite the great efforts to find new fluid biomarkers, CSF OCBs still remain the most reliable diagnostic and prognostic biomarker of MS. IgG OCB represent more than 95% of CSF OCBs, and determined by immunofixation can be found in 90% of patients with MS (pwMS) [1]. CSF OCB should have different pattern from serum OCBs confirming their intrathecal origin. The finding of OCBs is highly sensitive for MS diagnosis but since OCBs can be present in CSF of patients with other inflammatory or autoimmune conditions specificity is limited [2]. Nevertheless, OCBs represent one of MS diagnostic criteria according to the 2017 McDonald criteria update. Antigen specificity of IgG OCBs is not known. OCB can be already detected in patients with radiologically isolated syndrome

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KEY POINTS

- Serum levels of NfL correlate with several clinical and MRI measures in patients with MS but still have limited specificity related to MS.
- miRNA serum levels correlate with gene expression program related to inflammatory and demyelinating processes in MS.
- Extracellular vesicles and their cargo secure the communication between CNS and peripheral immune system.
- miRNA and extracellular vesicles might represent new promising biomarkers in MS.

(RIS) and clinically isolated syndrome (CIS), although with lower frequency than in patients with confirmed MS and represent strong predictive factor for RIS and CIS conversion to MS with hazard ratio more than 10 [3]. The presence of OCBs correlate also with disability progression and brain atrophy [4]. Similar to IgG OCBs a predictive value of MS progression was attributed to IgM OCBs. Significantly, IgM OCBs showed immunoreactivity for myelin lipids [5]. Both categories of OCBs were shown to correlate with increase number of new and active MRI lesions [6]. OCBs correlate also with an increased retinal axonal loss measured with optical coherence tomography [7].

Immunoglobulin IgG and IgM indexes

IgG and IgM indexes represent biomarkers related to increased immunoglobulin synthesis within CNS of pwMS. The IgG index more than 0.7 and IgM more than 0.1 indicate intrathecal synthesis of both classes of Ig [8]. The IgG index serves as a biomarker of MS diagnosis as well as predictor of disease progression and MRI activity [9]. Although it has been established that the IgG index correlates with the presence of OCBs in the CSF, studies showed that the index has lower diagnostic sensitivity for MS [10]. Several studies indicated that the production of intrathecal IgM may be associated with a worse prognosis of MS [11]. This parameter was also shown to predict faster conversion from CIS to MS [12].

Kappa-free and lambda-free light chains

In MS, plasma cell secretion of free light chains occurs in excess relative to the total amount of intact immunoglobulins. Kappa-free light chains (KFLC) have been found to be increased in the CSF of MS patients and they correlated with higher level of disease disability [13]. The increased amounts of KFLC did also predict disability progression [14] and higher rate of conversion from CIS to confirmed MS [15]. It was calculated that the KFLC index greater than 5.9, had a 96% diagnostic sensitivity for MS [16]. The KFLC/lambda-free light chains CSF ratio also appears to have a prognostic value in CIS conversion similar to OCBs.

Chitinase-3-like precursor

Chitinase-3-like precursor (CHI3L1), also known as YKL-40, is microglia, macrophages, astrocytes, and epithelial cells secreted glycoprotein during CNS pathogenic processes related to inflammation, extracellular tissue remodeling, and fibrosis. CHI3L1 was found to correlate with diagnosis of MS [17] and faster conversion from CIS to confirmed MS [18]. High CSF levels of CHI3L1 have also correlated with secondary progressive MS [19] and predicted higher rate of development of active MRI lesions [20]. Recently it was shown that patients with high disability progression exhibited significantly higher CSF CHI3L1 levels compared with patients with low disability progression [21].

Chemokine ligand 13

Increased levels of several proinflammatory cytokines and chemokines were suggested to correlate with MS diagnosis and disease activity. The most reliable data were generated with chemokine ligand 13 (CXCL13) which interacts with the CXCR5 receptor and results in the activation of B and T cells. CXCL13 is also a B-cell chemoattractant that aids in the formation of B-cell follicles [22]. The CSF levels of CXCL13 are increased in pwMS patients and predicted conversion of CIS to confirmed MS [23].

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is a cytoskeletal protein found in astrocyte intermediate filaments. GFAP is elevated in the CSF of pwMS, reflecting ongoing astrocyte involvement in CNS injury. It was suggested that higher CSF levels of GFAP can predict greater disease severity [24].

Myelin proteins

Myelin and oligodendrocyte glycoprotein (MOG) was recently associated with a new demyelinating disorder MOGAD. MOGAD is classified as a member of NMOSD. Detection of anti-MOG antibodies in serum is a prerequisite for diagnosis of MOGAD and appeared to differentiate MOGAD from MS [25].

Higher levels of myelin basic protein (MBP) in the CSF of MS patients have been reported during

active demyelination and showed some correlation with MS clinical relapses. However, because of inconsistency of the results, MBP is not currently considered a reliable biomarker of MS activity [26].

Neurofilaments

Neurofilaments (Nfs) are the major cytoskeletal proteins of neurons in both the CNS and PNS comprising light (NfL), medium, and heavy (NfH) neurofilament chains [27]. Nfs are found in dendrites and the neuronal soma although they are most abundant in myelinated axons. Nfs promote the radial growth of axons and promoting a higher conduction velocity [28]. It has been long established that Nfs subunits are actively involved in the pathogenesis of axonal dysfunction and degeneration both as causative agents for disease and as markers for disease activity and progression [27]. They are released to CSF and blood after any structural damage both in neuronal soma and axons. Therefore, they represent attractive candidates for biomarkers for neurodegenerative, demyelinating, inflammatory, ischemic, metabolic, and posttraumatic neurological disorders [29]. In MS extensive studies have been conducted with that respect [30]. The initial approach was to detect its raised concentrations in CSF and to correlate it with acute and chronic disease course [31]. With time it appeared that NfL represents the most validated and reliable measure of Nfs in biofluids of pwMS [32]. The real revolution in Nfs field occurred after establishing ultrasensitive assays (single-molecule array assay, Simoa) enabling NfL measurement in serum. Several studies using Simoa technique validated NfL levels as biomarkers in MS prognosis, monitoring of disease activity, and treatment responses [33].

It has been repeatedly reported that high levels of serum NfL are present in the early or even prodromal stages of MS. In one recent study, serum NfL levels were increased in pwMS a median time of 6 years before the first clinical symptoms [34▪▪]. CSF NfL levels and OCBs were independent risk factors for the development of CIS and clinically definite MS in RIS syndrome [35]. NfL levels were also increased in patients with CIS and its levels correlated with shorter time of conversion to clinically definite MS [36].

In a large, prospective, multicenter study recruiting patients with CIS and early RRMS, the assessment of serum NfL increased diagnostic accuracy and facilitated prognosis of the disease course over the next 4 years [37]. It was found that longitudinal measurement of serum NfL rather than absolute cutoff values are recommended for clinical decision-making process.

Nfs showed also correlation with clinical activity and the rate of progression over time. Early studies showed the increase of NfH in CSF of pwMS during acute relapse. Accordingly, an inverse correlation with the clinical recovery was observed [38]. This was further confirmed over longer follow-up, showing increases of the CSF NfH in frequently relapsing patients [39]. Further studies showed close correlation between serum NfL increased levels and MRI activity measures [40,41▪,42]. Importantly, a strong correlation of high levels of serum NfL with long-term clinical progression as well as with brain and spinal cord atrophy was also reported [40]. Recently, numerous studies showed that registered and newly developed MS drugs decrease neuro-axonal damage quantified by changes in NfL concentrations in serum [43].

Extracellular vesicles

Extracellular vesicles are nanosized membrane particles released by every cell type, and they can be found circulating in biological fluids [44,45]. Their molecular composition is representative of that of parental cells, which makes them ideal biomarkers providing information on elusive cells or difficult to access tissues, as in neurological disorders [44]. Extracellular vesicles have been described in the CSF of pwMS, both of oligodendroglial [46] and myeloid origin [47]. The field has raised increased interest when extracellular vesicles relevant to MS pathogenesis, including neural-derived extracellular vesicles, have been described in the blood of pwMS [48,49,50,51]. Detection and characterization of extracellular vesicles might represent, however, a technical challenge [52]. Diagnostic, prognostic, disease, and treatment monitoring values of extracellular vesicles detection, both in CSF and blood, have been proposed for MS [53,54,55,56].

Concerning diagnosis, exosome loaded with myelin proteins have been detected at increased levels in the blood of pwMS in an active phase of the disease or having a secondary progressive form of the disease [49]. This may suggest that myelin-loaded extracellular vesicles may represent a cell-specific tissue-damage biomarker. Similarly, CSF myeloid cells-derived extracellular vesicles are clearly increased in pwMS, but also in NMOSD patients representing a putative marker of microglia activation, rather than a disease-specific biomarker [47]. When looking at the global transcriptomic profile of serum small extracellular vesicles some discriminative power has been described [45], suggesting that with technical advancements we might identify an MS-specific signature with a diagnostic value. It was shown that blood extracellular vesicles

concentration was altered in relapsing MS and, in particular, endothelium-derived extracellular vesicles were increased in stable MS patients [57]. Finally, it has been described that sulfatides on plasma-derived extracellular vesicles are able to distinguish pwMS from healthy donors, suggesting that also lipid composition of extracellular vesicles may be informative [58].

Several studies have addressed the prognostic value of extracellular vesicles. A very recent study has highlighted that CSF extracellular vesicles of myeloid origin display high sensitivity and specificity in distinguishing persons with RIS, with an extremely high risk of evolving toward MS [59▪]. Elevated myeloid CSF extracellular vesicles also identify persons with CIS that will have shorter time to convert to MS as well as predict high disease activity in relapsing MS and increased disability progression in progressive forms of MS [60▪].

Blood extracellular vesicles may represent a suitable biomarker to monitor MS disease activity, representing a practical and potentially high-frequency test, alternative to the gold standard MRI. Small extracellular vesicles carrying myelin proteins, for example, identify active disease in relapsing MS but are also increased in pwMS with a progressive disease, suggesting that this biomarker may capture also demyelination occurring in slowly expanding plaques typical of this disease form [49]. Extracellular vesicles transcriptome may provide more insight, with some extracellular vesicles-associated microRNAs (miRNAs) able to discriminate disease activity from remission with remarkable performances [48]. An interesting study reported lower levels of synaptopodin and synaptophysin in neural-enriched extracellular vesicles and higher levels of multiple complement cascade components in astrocyte-derived extracellular vesicles in the plasma from pwMS [61]. This may reflect the synaptic loss occurring in MS and may therefore be a relevant biomarker of disease activity also in progressive forms of the disease.

Some studies have explored the potential usefulness of extracellular vesicles as biomarkers of treatment effectiveness. In general circulating extracellular vesicles derived from platelets, total leukocytes, or monocytes, are decreased upon treatment [50]. Indeed, disease-modifying treatments decrease the ability of myeloid cells to release extracellular vesicles [62]. For one of these treatments, fingolimod, the inhibitory activity found *in vitro* [63] was not confirmed *in vivo* [64]. This apparent contradiction is probably easily explained by the effect of fingolimod on the migration of leukocytes rather than on extracellular vesicles release.

Extracellular microRNA

Encouraged by the results of the studies describing the presence of the exRNA in the various biofluids this form of RNA has been extensively tested as biomarker of various conditions including MS [65]. ExRNA can be encapsulated in the vesicles and secreted out of cells but also to be present in circulation in complex with RNA-binding proteins such as argonaute-2 as well as bound to HDL [66,67]. Some exRNA fragments can form self-protecting dimers to resist RNases [68].

The largest body of the studies so far were the analyses of the extracellular miRNA changes. miRNAs are small noncoding RNAs (approximately 20–22 nucleotides) that regulate gene expression by inhibiting translation and/or decreasing the stability of their mRNA targets [69]. They represent good biomarker candidates because of their high stability in various biological fluids [70]. Several studies have reported that changes in miRNA expressions might correlate with demyelination and inflammatory responses in MS [71]. In one of the largest studies 5 miRNAs (hsa-miR-484, hsa-miR-140–5p, hsa-miR-320a, hsa-miR-486-5p, and hsa-miR-320c) showed a significant difference between pwMS and healthy individuals [72]. In another large study, a group of pwMS ($n = 1088$) was stratified based on brain imaging and miRNA profiling was used to identify differences across the different MRI-based phenotypes [73]. miR-22-3p, miR-361-5p, and miR-345-5p were the most valid differentiators of the MRI phenotypes. A large body of a smaller studies has been published to identify serum/plasma miRNA biomarkers of MS. On the contrary, conflicting results and lack of replication are ongoing challenges for this type of studies [74]. Recently a first meta-analyses have been published generating an estimate of the relevance of circulating miRNA changes in pwMS [75,76]. A group of miR-145, miR-15b, miR-23a, miR-128-3p, and miR-191-5p have been highlighted as most concordantly reported markers dysregulated in serum of pwMS [76]. A potential of extracellular miRNA analysis in CSF, has also been demonstrated [77▪,78▪]. In a recent comprehensive exRNA analysis in blood and CSF from matching samples of pwMS and controls demonstrated widespread alterations and an opposing patterns of changes between these two compartments [78▪].

Extracellular noncoding RNA and viral RNA

ExRNA studies in MS have also revealed a potential role of other RNA species, beyond miRNA, as biomarkers of MS. In one study, three long noncoding RNAs (lncRNAs), nuclear paraspeckle assembly transcript 1, taurine upregulated 1 (TUG1), and 7SK

Table 1. Utility of multiple sclerosis biomarkers

Name	Source	Utility in		
		Diagnosis	Progression	Treatment outcomes
OCBs	CSF	+++	++	–
Ig indexes	CSF	++	++	–
FKLC	CSF	+	+	–
CHI3L1	CSF	++	++	+
CXCL13	CSF	+	+	+
GFAP	CSF	+	+	–
NfH	CSF	++	++	–
NfL	Serum	+++	+++	++
miRNA	Serum	++	++	ND
EVs	Serum	++	++	+

CHI3L1, chitinase-3-like precursor; CSF, cerebrospinal fluid; CXCL13, chemokine ligand 13; EV, extracellular vesicle; GFAP, glial fibrillary acidic protein; miRNA, microRNA; NfH, neurofilament heavy; NfL, neurofilament light chains; OCB, oligoclonal band.

small nuclear have been detected to be upregulated in relapsing-remitting MS patients respectively to controls sera [79]. Significantly, results of another study confirmed upregulation of TUG1 in secondary progressive MS versus controls. In addition, the significant upregulation of the long intergenic ncRNA 293 (LINC00293) and RP11-29G8.3 have been found parallel to downregulation of ncRNA 188 (LRRC75A-AS1) in PPMS [80]. Furthermore in a recent study two additional lncRNAs: MALAT1 and lnc-DC have been reported to be significantly increased in pwMS [81]. Finally, a nonhuman exRNA has also been detected in sera and CSF of pwMS, for example, human herpesvirus-6A and −6B-derived miRNAs [82].

Circular RNA

Another intriguing group of the ncRNAs with a potential regulatory properties in MS are circular RNA (circRNA) [83]. Several groups have recently published on the role of circRNA changes in immune cells in MS patients [84▪,85] as well as in MS animal model [86]. Intriguingly these reports have suggested an important role of circRNA for the function of the crucial MS-related cell population, like B and Th17 cells.

CONCLUSION

The fluid biomarker field in MS is developing rapidly reflecting unmet needs in this direction. Table 1. However, despite intensive investigations the only validated and clinically proven biomarker is CSF OCBs. The other 'traditional' MS biomarker like intrathecal Ig synthesis, kappa-free and lambda-free chains, chitinase-3-precursor require further investigations related to specificity and sensitivity in pwMS. Similarly, studies on markers of inflammatory component of MS pathogenesis, cytokines, and chemokines, provided variable and inconsistent results. A new hope for MS biomarkers is related to NfL measurements is serum. However, it should be remembered that Nfs do not discriminate between inflammatory or purely neurodegenerative processes in MS. Recently new categories of MS fluid biomarkers have appeared including exRNA, particularly miRNA, and small extracellular vesicles. MiRNA known to control wide range of cell genes expression are characterized by extraordinary stability in body fluids making them attractive biomarkers for complex disorders like MS. Extracellular vesicles cargo content, dependent on the cell of origin, makes them ideal biomarkers providing information on elusive cells or difficult to access tissues, as in the case of MS. With a wide array of cargo material including molecules related to MS pathogenesis, extracellular vesicles might represent a novel class of biomarkers in MS. Nevertheless, more studies are needed to firmly confirm the feasibility of extracellular vesicles and exRNA as MS biomarkers.

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Conflicts of interest

There are no conflicts of interest.

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