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Emerging imaging and liquid biomarkers in multiple sclerosis

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Abstract

The advent of highly-effective disease modifying therapy has transformed the landscape of multiple sclerosis (MS) care over the last two decades. However, there remains a critical, unmet need for sensitive and specific biomarkers to aid in diagnosis, prognosis, treatment monitoring, and the development of new interventions, particularly for people with progressive disease. This review evaluates the current data for several emerging imaging and liquid biomarkers in people with MS. MRI findings such as the central vein sign and paramagnetic rim lesions may improve MS diagnostic accuracy and evaluation of therapy efficacy in progressive disease. Serum and cerebrospinal fluid levels of several neuroglial proteins such as neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) show potential to be sensitive biomarkers of pathologic processes such as neuro-axonal injury or glial-inflammation. Additional promising biomarkers including optical coherence tomography, cytokines and chemokines, microRNAs, and extracellular vesicles/exosomes are also reviewed, among others. Beyond their potential integration into MS clinical care and interventional trials, several of these biomarkers may be informative of MS pathogenesis and help elucidate novel targets for treatment strategies.

Graphical Abstract

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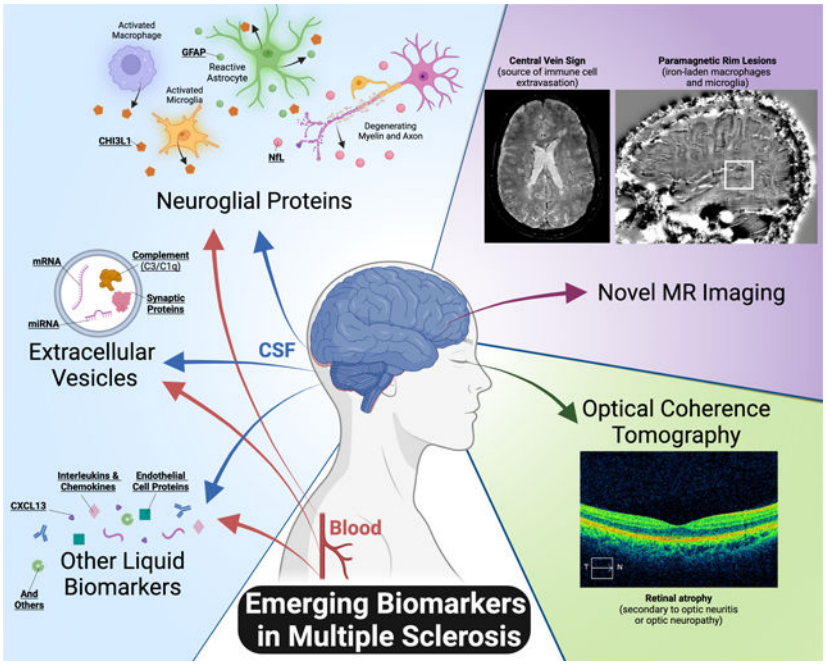

Several emerging imaging and liquid biomarkers reflecting underlying immunopathology have potential to aid in the diagnosis, prognosis, and treatment monitoring in people living with multiple sclerosis. Of particular note are novel MRI techniques and quantification of relevant neuroglial proteins in the blood.

Keywords

central vein sign; paramagnetic rim lesion; neurofilament light chain; glial fibrillary acidic protein; optical coherence tomography

Introduction

Multiple sclerosis (MS) is a complex neurologic disease with neuroinflammatory and neurodegenerative components that affects over 2 million people worldwide[1]. Current MS diagnostic criteria rely largely on clinical presentation and non-specific imaging and laboratory findings and thus misdiagnosis remains a significant issue[2–4]. Even after an accurate diagnosis of MS is made, the disease course and response to disease modifying therapy (DMT) are highly variable and are poorly predicted by currently clinically available biomarkers. With a wide array of medications of varying efficacy and safety available, treatments for prevention of the inflammatory, relapsing component of the disease have expanded significantly in recent years, but there has been far less forward movement in develop of therapy for insidious progressive decline or remyelination. This lack of reliable, accurate, non-invasive, and easily applied biomarkers significantly hinders MS research, prognostication, and DMT management decisions, particularly in progressive disease. This review highlights some of the emerging imaging and liquid biomarkers in people living with MS (PwMS) that have potential for improving MS diagnosis, quantifying current

disease activity, assessing response to therapy, and prognosticating future disease activity and disability.

MS Pathogenesis, Diagnosis, and Clinically Established Biomarkers

MS pathogenesis—In the setting of genetic predisposition and a range of possible environmental triggers such as viral[5] or toxin exposure[6], the CNS undergoes autoimmune inflammatory injury resulting in demyelination and axonal transection followed by varying degrees of remyelination, neurodegeneration, and gliosis. In PwMS, these processes occur in focal, characteristic lesions as well as more diffusely throughout the CNS, the extent of which varies by the individual and by phase of the disease. While classically MS was thought of as predominantly affecting the white matter of the CNS, MS is known to significantly involve the gray matter as well[7–10]. Potential antigenic triggers remain uncertain, but are likely CNS-derived; examples of candidates include myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipoprotein (PLP)[11]. New inflammatory demyelinating lesions in PwMS are largely driven by the adaptive immune system, with bouts of peripheral lymphocyte activation and infiltration into the CNS (initially, predominantly CD8+ T cells, but also B-cells and macrophages) and subsequent activation of local glia[12,13]. MS-related neurodegeneration and progressive decline in PwMS independent of new inflammatory lesions is not well understood, but is thought to be driven more by compartmentalized processes within the CNS and leptomeninges, such as reactive glia, ectopic meningeal lymphoid follicles, oxidative stress, and age-related iron deposition[14–16]. This smoldering, CNS-compartmentalized pathological activity is more difficult to monitor and current DMTs largely target the peripheral immune system and thus may be of limited benefit.

Diagnostic and monitoring challenges—Diagnosis of MS is frequently challenging due to complex, variable clinical presentations, relative non-specificity of biomarkers and imaging findings, and many potential MS mimics. Two important mimics, among many, are neuromyelitis optica (NMO) and myelin oligodendrocyte antibody disease (MOGAD). Both of these diseases cause inflammatory, demyelinating lesions in the CNS with significant clinical overlap with MS. MOG has more cortical features and better recovery, and is more likely to be monophasic than MS[17]. NMO is an astrocytopathy with severe attacks (such as simultaneous bilateral optic neuritis, or longitudinally-extensive cord lesions) with poor recovery[18]. Unlike MS, NMO does not seem to have a prominent neurodegenerative component. Over the last two decades disease-specific autoantibodies (anti-aquaporin-4 for NMO and anti-MOG for MOGAD) have been identified. Clinically available testing for these antibodies has made assessing for these conditions in an MS workup more routine, but seronegative MOGAD or NMOSD remains a challenge. Additionally, many other MS mimics remain resulting in both over and under diagnosis. Studies show that about 25% of those referred to an academic center with a diagnosis of MS are misdiagnosed and many people with an erroneous MS diagnosis are started on disease modifying therapy (DMT)[2–4]. There is urgent need for more sensitive and specific biomarkers in MS, as misdiagnosis, often lasting years, may result in preventable morbidity, unnecessary expenditures, and psychologic burden[2].

The current consensus diagnostic criteria for MS are the 2017 McDonald criteria[19]. The core of MS diagnosis requires clinical symptoms and radiologic CNS lesions disseminated in both space and time. Clinically definite MS is defined as two typical symptomatic clinical attacks along with certain paraclinical (imaging/cerebrospinal fluid (CSF)) findings; the 2017 McDonald criteria[19] also allows for MS diagnosis with only one clinical event with certain imaging/CSF findings that satisfy dissemination in time and space, and this has been shown to offer at least moderate specifity[20].

MS is currently clinically categorized as relapsing-remitting (RRMS), relapsing with secondary progression (SPMS), and primary progressive MS (PPMS). While lines between these categories are often blurred, RRMS consists of discrete clinical and radiologic attacks or relapses without disability progression between; SPMS begins as RRMS but in a later phase disability progression occurs between and distinct from acute relapses; and PPMS consists of insidious disease progression without any acute clinical attacks initially. SPMS and PPMS are often referred to collectively as progressive forms of MS (PMS). The vast majority of PwMS present as RRMS. Other entities on the demyelinating disease spectrum include clinically isolated syndrome (CIS) which defines one suspicious clinical event, but without adequately specific clinical or paraclinical data to satisfy current MS diagnostic criteria[21]. Radiologically isolated syndrome (RIS) involves imaging findings suspicious for MS but without any clinical symptoms attributable to MS[22]. Some people with CIS and RIS will go on to develop MS, but sensitive and specific biomarkers to predict who will convert to MS are needed and would allow for earlier initiation of DMT.

Current clinically established biomarkers—Key MRI features including location, morphology, number of lesions, and enhancement of lesions aid in MS diagnosis and prognosis. Gadolinium enhancement on MRI demonstrates an actively disturbed blood-brain barrier associated with peripheral-immune cell infiltration in MS. MS lesions tend to be ovoid and tend to occur in specific regions: periventricular, juxtacortical or cortical, infratentorial, or spinal cord. Despite some typical characteristics, MS lesions can sometimes be difficult to distinguish from other causes of white matter lesions such as microvascular disease, vasculopathies, systemic inflammatory conditions (e.g. Sjogren's), and leukodystrophies. Thus, routine MRI imaging techniques may have low specificity for a diagnosis of MS, particularly in people with CIS and RIS, though newer imaging techniques discussed below significantly improve imaging-based diagnosis of MS. In PwMS, neurologists monitor for disease activity and response to current therapies by assessing for new, demyelinating-appearing lesions, and by enhancement which suggests recent/current inflammatory activity. Newer imaging markers of disease activity, particularly progressive disease, under investigation are discussed below.

In addition to MRI imaging, CSF can help support or dissuade an MS diagnosis, such as in CIS or in suspected MS with atypical features. Oligoclonal bands (OCBs) unique to the CSF, in the setting of a clinically suspicious event or suspicious imaging finds, are an established risk factor for future MS disease activity, and greater than 90% of those with MS have CSF-restricted OCBs[23–25]. OCBs unique to the CSF are nearly always abnormal, and reflect intrathecal IgG production from B cell clones that reside within the CNS compartment. Importantly, OCBs are *not* specific for MS. The long list of other CNS

inflammatory conditions that may have OCBs unique to the CSF include neurosarcoidosis, paraneoplastic disorders, many neuro-infections, and Sjogren's. One systematic review found that, when considering only neuroinflammatory differentials, CSF-unique OCBs were only 61% specific for MS[26]. CSF IgG, in normal conditions, is present via diffusion from serum, and therefore, polyclonal OCBs that mirror those in the serum (which must be simultaneously assessed) do not suggest CNS-specific inflammation. A potential alternative to OCBs that is gaining interest is CSF kappa free light chain index[27], with some studies suggest it may be a more sensitive indicator for intrathecal IgG production[28].

The current gold standard to quantify disability is the Expanded Disability Status Scale (EDSS), which is vulnerable to intra- and inter-rater variability[16] and in many cases over-emphasizes ambulation and may not capture meaningful but subtler symptoms. Other clinical markers include patient-reported outcomes like the Patient Determined Disease Steps (PDDS). Potentially more nuanced clinical measures, such as actigraphy measuring daily steps and activity patterns, are undergoing investigation as a clinical tool as well.

Despite the current imaging and CSF biomarkers used in the clinic, there is a critical, unmet need for accurate, reliable, objective, and trackable biomarkers. This review highlights some of the emerging imaging and liquid biomarkers in field of MS that have potential for improving MS diagnosis, quantifying current disease activity, assessing response to therapy, and prognosticating future disease activity and disability.

Emerging Imaging Biomarkers

Central vein sign—For decades pathology studies have demonstrated that acute white matter lesions in MS are characterized by infiltration of monocytes and lymphocytes from a small central vein[29]. Recent studies have demonstrated that the presence of a central vein, or "central vein sign" (CVS), within white matter lesions can be reliably imaged by MRI (Figure 1, A and B). The CVS is relatively specific for MS pathologic processes and has the potential differentiate MS from mimicking diseases including migraine[30], cerebral small vessel ischemic disease[31,32], NMO spectrum disorder[33], and inflammatory vasculopathies[34], among others[35,36]. Some rare diseases like Behcet's disease may have a perivenular lesion burden similar to MS[34]. While different optimized MRI sequences have been developed to detect the CVS and several different CVS-based criteria (e.g. percentage of CVS lesions vs. CVS lesion count) have been proposed to date to distinguish MS from other mimicking neurological conditions, retrospective studies have shown excellent diagnostic discrimination in a meta-analysis (sensitivity 91%, specificity 96%)[37]. CVS is also detectable in patients diagnosed with RIS and CIS and may be able to help prognosticate those who will convert to MS[36,38,39]. While CVS seems poised to be a clinically useful biomarker for MS diagnosis, it does not appear to differentiate between MS subtypes (ie. RRMS vs. PMS) in the above studies. To date, the utility of CVS in MS diagnosis has mainly been studied in cross-sectional and retrospective studies. The "Central Vein Sign" A Diagnostic Biomarker in Multiple Sclerosis" (CAVS-MS) is a prospective, international, multicenter study that recently completed its recruitment of >400 subjects with and without typical MS presentations to evaluate CVS as an MS diagnostic biomarker, with the goal to rapidly translate CVS into clinical care[40].

Paramagnetic rim lesions—Many MS lesions after the resolution of the acute inflammatory phase remain demyelinated and a small subset can become chronic active lesions (CALs). CAL have a hypocellular, demyelinated center with peripheral iron-laded activated CD68+ microglia/macrophages and reactive astrocytes[41–43]. These lesions can slowly expand over time likely reflecting at least one potential mechanism of progressive disease[44,45]. Pathology studies have shown CALs are present in the majority of PwMS with a higher prevalence in more severe disease and progressive disease courses[8,46]. Susceptibility-based MRI sequences (ie. Gd-T_2*-EPI) identify paramagnetic rims around some non-enhancing chronic lesions (paramagnetic rim lesions, PRLs) that MRI-pathological correlation studies have been shown to identify with high-accuracy iron-enriched microglia at the edge of CALs[45,47,48]. PRLs are thus a reliable imaging surrogate of a at least a subset of CALs (Figure 1, C and D).

The presence of at least one PRL in the supratentorial brain is common in all MS phenotypes (~50% in RRMS and CIS) and a higher prevalence of PRLs are found in patients with progressive disease, highlighting potential prognostic implications[44]. PRLs are relatively specific for MS compared to other neurologic inflammatory disorders such as NMOSD, systemic lupus erythematosus, Behcet's disease, Sjogren's syndrome, CNS vasculitis, and neurosarcoidosis, with the exception of Susac Syndrome which features lesions resembling PRL in the corpus collosum (notably without CVS)[49]. This specificity is affirmed in a study that demonstrated that all CIS patients with at least one PRL developed RRMS over mean follow-up period of 4.6 years, outperforming oligoclonal bands[39]. Despite the high specificity, as only about half of MS cases have at least one PRL, the diagnostic sensitivity is poor. In the largest published study to date, the presence of at least one PRL had sensitivity of 52% and specificity of 93% for MS diagnosis[49]. In this same cohort CVS (present in >40% of an individual's visualized lesions) could significantly better discriminate MS from non-MS cases with high specificity (96%) and sensitivity (99%), though combining PRL and CVS criteria did improve specificity to 99%. This and other studies suggest that PRL might improve diagnostic specificity when combined with CVS, particularly in setting of high-suspicion of MS despite low frequency of CVS such as in the context of small vessel disease comorbidity. While the diagnostic utility of PRLs may be limited, a higher PRL burden associates with higher disability and MS severity[49], suggesting a role in prognostication particularly in progressive disease. This is underscored by the finding that more than half of MS cases on DMT continue to have PRLs, a marker of chronically inflamed lesions, even in PwMS receiving highly-effective antibody-based therapy. PRLs may therefore serve as a marker of persistent, low-level inflammation that may require adjunctive therapies to target and prevent lesion expansion and insidious clinical decline.

Other MRI biomarkers—Several other MRI imaging findings have evidence as biomarkers in MS prognosis, including brain atrophy[7,50,51], spinal cord atrophy[52–54], cortical lesions[9,55,56], enlarged perivascular spaces[57], and leptomeningeal enhancement[58]. CNS atrophy, both global and regional, serves as a surrogate of neurodegeneration. Cortical lesions also associate with progressive disease as well as cognitive symptoms[59–61]. Cortical lesions, particularly intracortical and subpial lesions, are difficult to detect on clinically

available MRI, currently limiting their potential utility[62,63]. A number of other challenges remain to be overcome before reliable detection of cortical lesions or quantitative MRI can be translated from the research setting to the clinic[64,65].

Optical coherence tomography—Retinal optical coherence tomography (OCT) is an emerging imaging biomarker that is rapid, practical, and non-invasive. OCT is increasingly utilized in trials and clinical centers in MS and in optic neuropathy patients, as adjunct information that in some situations can aid in differential diagnosis, prognosis, and monitoring response to therapy in MS and related conditions[66,67]. OCT allows for measurement of retinal layers on the scale of microns via an interference pattern generated by infrared light beam reflection. The retina is the most easily accessible part of the CNS and is a common site of blood-retina barrier breakdown, local inflammation, and degeneration. Specific measures of interest include the retinal nerve fiber layer (RNFL) and the ganglion cell-inner plexiform layer (GCIPL). Both these layers thin with loss or damage to the retinal ganglion cells whose axons make up the optic nerve. These measures correlate with and predict cerebral atrophy, MS course and disability, response to DMT, as well as low-contrast visual acuity[68–70]. Symptomatic optic neuritis (ON) occurs in at least half of MS cases, but regardless of known ON history, some immune mediated demyelination of the optic nerve and subsequent retinal ganglion cell pathology occurs in nearly all people with MS. RNFL data may be more limited by expected transient thickening during acute ON, gliosis-obscured atrophy, and poorer RNFL inter-scan reliability, versus the GCIPL[71]. Growing data is emerging about other retinal measures of interest. For example, about 5% of those with MS have microcystic macular pathology, which may be associated with a more severe MS course[72]. How differing pathological patterns in OCT may serve as a proxy for disease processes in the cerebrum remains an area of active investigation.

Emerging Liquid Biomarkers—Clinical history, physical exam, and MRI are currently the gold standard to diagnose and monitor clinical activity over time in MS. While current imaging biomarkers are excellent at identifying new active inflammatory lesions and novel biomarkers such as CVS seem most likely to contribute to increasing MS diagnostic accuracy, current clinically available imaging is limited in its ability to differentiate MS subtypes or identify and quantify subclinical disease and progressive disease where acute inflammation and new lesions may be absent. Additionally, imaging is expensive, time consuming (particularly for more advanced analyses), and difficult to standardize. Liquid biomarkers, particularly in the blood or other non-invasive body fluid, have great potential to meet the unmet need for pragmatic, cost-efficient, and repeatable markers.

Neuroglial proteins

Neurofilament Light Chain: Neurofilament light chain (NfL) is an emerging biomarker of neuro-axonal injury in several neurological conditions including MS. Neurofilaments are neuron-specific intermediate filaments that are components of the cytoskeleton. With loss of neuronal membrane integrity in the CNS, neurofilaments are released into the extracellular space and ultimately into the cerebrospinal fluid and the blood. NfL and other neurofilaments are not specific to MS and can be elevated from any cause of neuronal injury, including other CNS neuroinflammatory disorders such as NMO or MOGAD[73,74]. While

initial studies focused on detection of NfL in the CSF, newer more sensitive assays have allowed for detection of plasma and serum NfL (sNfL). Several studies have demonstrated that sNfL correlates tightly with CSF NfL in CNS disease, including in PwMS[75–77], making sNfL a potential non-invasive biomarker of neuronal damage within the CNS.

CSF and sNfL are higher in MS compared to healthy controls or individuals with non-inflammatory neurologic disorders[75–78], suggesting sNfL may improve MS diagnosis. Additionally, sNfL may be increased up to six years prior to first clinical symptoms suggesting neuroaxonal damage occurs during a prolonged MS prodromal phase[79]. Consistent with the above, several studies demonstrate that sNfL increases the sensitivity and specificity of differentiating patients with MS from both CIS and RIS[80–83], which could enable early DMT initiation in CIS and RIS patients with high-risk for conversion to MS.

In addition to aiding in diagnosis, sNfL may also be a biomarker for both concomitant and future disease activity. Several studies have shown that sNfL correlates with disease activity and baseline MRI lesion burden and can predict future acute clinical activity and new gadolinium-enhancing and T2 MRI lesions[75,76,80,84–87], as well as future brain and spinal cord atrophy and disability worsening[75,84–88]. Consistent with sNfL's role as a disease activity indicator, DMT use correlates with lower sNfL levels. More specifically, higher-efficacy monoclonal antibody therapies (i.e. ocrelizumab, natalizumab, alemtuzumab, rituximab) seem to lower sNfL levels with greater efficacy than oral therapies (i.e. dimethyl fumarate, fingolimod, teriflunomide), all of which were more efficacious in lowering sNfL than platform therapies (interferons and glatiramer acetate)[80,84]. This data suggests that sNfL may also be able to assess DMT-efficacy, with stable or low sNfL levels able to help exclude clinical or subclinical inflammatory disease activity.

While the growing evidence for sNfL's utility in inflammatory MS activity is strong, its role in PMS, where biomarkers are particularly needed, is less clear. Current data highlights that active inflammation is a major contributor to sNfL even in patients with progressive disease. Separating this acute inflammatory disease activity from insidious disease and gradual disability worsening that defines progressive MS presents a difficult challenge. There is significant disagreement in reported data comparing NfL (CSF and serum) between patients with RRMS and PMS, with several studies reporting higher NfL in PMS compared to RRMS, several lower in PMS compared to RRMS, and the majority finding no difference (reviewed in[89]). These discrepancies are likely explained by associations with other factors that differ between these groups and associate with NfL levels, especially age. Supporting this, analyses including age, disability status, recent relapses, and DMT-treatment status as covariates often results in loss of significance between these groups[75,85,90]. Similarly, some studies found a significant, albeit marginal, relationship between baseline sNfL and conversion from RRMS to SPMS, whereas other studies did not[91–93].

Overall, current data support that sNfL levels provide a good reflection of ongoing and future neuroaxonal damage in the setting of inflammatory disease activity in MS. While sNfL may be a useful biomarker across several aspects of MS care, to date sNfL has largely been investigated on a group level and only a handful of studies have looked at predictions on an individual level. Interpretation of individual sNfL levels are also complicated by

several common factors that associate with higher sNfL levels including age, body mass index, impaired renal function, diabetes mellitus, and active smoking, underscoring the need for normative sNfL data to correct for these factors[84,87,94]. Prospective clinical use of sNfL on an individual MS patient level has not yet been established. Its recent commercial approval will likely shed more light on its role in this context.

Chitinase-3-like 1: Chitinase-3-like 1 (CHI3L1/YKL-40) is a pro-inflammatory secreted glycoprotein of unclear function that has been purported as a potential marker of reactive astrocytes and microglia/macrophages, though it is also expressed on peripheral cells including monocytes, chondrocytes, neutrophils, and vascular smooth muscle cells, among other cell types[95,96]. Initial studies suggested that CSF CHI3L1 is primarily intrathecally produced and that CSF levels do not correlate with serum levels [95,97,98]. Elevated CSF CHI3L1 has also been shown to associate with higher-risk and faster time for conversion from CIS to MS, faster development of disability, brain MRI lesions, and brain atrophy and may decrease with DMT initiation[83,95–100]. A recent meta-analysis found that CSF CHI3L1 levels were higher in patients with MS compared to healthy controls, higher in people with PPMS compared to both RRMS and SPMS, and higher in those with CIS who converted to MS compared to those that did not convert[101]. Interestingly, pathologic studies have shown that in CALs with high inflammatory activity, CHI3L1 is expressed highly at the lesion edge in reactive astrocytes and CD68+ macrophages/microglia[95], emphasizing the possibility that CHI3L1 may in part associate with CALs with iron rims. Supporting this potential relationship, a recent study found that CSF CHI3L1 associates with PRLs in PwMS after their first demyelinating event [102]. In summary, CSF CHI3L1 is a potentially useful CSF biomarker in MS, perhaps particularly in CIS, but less invasive biomarkers (serum and imaging) may be more beneficial for tracking disease activity and response to therapy over time.

Glial Fibrillary Acidic Protein: Glial fibrillary acidic protein (GFAP) is the primary cytoskeletal protein in astrocytes and an established marker of astroglial reactivity (astrogliosis) and astrocyte damage. GFAP is also found in non-myelinating Schwann cells in the peripheral nervous system, Mueller cells in the retina, and in glia of the enteric nervous system, and to a lesser extent among other neurological and non-neurological cells. GFAP plays a role in the extension of astrocyte processes formed in response to injury, as part of the dynamic intermediate filament network, and supports interactions with neighboring neurons and the blood-brain barrier.

Similar to NfL, cell membrane permeability changes may facilitate leakage of GFAP. There may also be an increase in intracellular GFAP levels from increased expression as part of physiological or pathological injury response. GFAP may be released into the CSF and then through the CSF-blood barrier, and likely also released into the glymphatic system/direct venous drainage[103]. Correlation is high between CSF and blood GFAP in MS patients and controls[104], suggesting serum levels reflect CNS pathology. Though some degree of astrocyte response is likely beneficial, pathologically reactive astrocytes are proposed to be key drivers of neurologic damage in MS[105]. Much of the clinical data regarding GFAP is from post-traumatic brain injury outcomes[106], though recent studies highlight its promise

for use in MS. As with many liquid biomarkers in MS, differences between relapsing and progressive disease have been variable, largely complicated by variability of RRMS data gathered around time of active lesions versus in stable periods, expected and typical age differences between PMS vs RRMS, with PMS patients tending to be older and GFAP, as with NfL, higher with advancing age[104,107–109].

Some studies reported GFAP is mildly elevated in RRMS versus healthy controls and patients with non-inflammatory neurological disorders, though these findings may be driven by RRMS patients with recent relapses[74,104,110]; those with stable, inactive RRMS may have similar blood GFAP levels to healthy controls[74]. Interestingly, GFAP elevation or lack thereof around time of acute relapse has been inconsistent across studies[74]. Promisingly, some studies suggest that serum GFAP is elevated in progressive MS versus RRMS[78,108,111], though some of these studies found no difference between progressive versus RRMS after adjusting for age[104]. Blood GFAP has been shown to correlate with clinical disability in MS as measured by EDSS[107,109,111] and with lesion burden[104,107,111]. Though GFAP and NfL often correlate[104,107,109,111], instances where they diverge may provide particularly useful information. One large recent study found that elevated blood GFAP predicted poorer disability status in six months in PwMS, particularly in those with low sNfL and who did not have recent relapses[108]. Another group suggested a “glia score” that integrates multiple biomarkers (GFAP x CHI3L1 / sNfL), which they found was higher in SPMS than RRMS patients, and correlated with EDSS in SPMS patients[112]. Blood GFAP has also proven to be increased in NMO versus healthy controls or MS, with early data supporting exploration of use of GFAP:NfL ratio during acute relapse[74]. Assessing both NfL, GFAP, and perhaps other biomarkers simultaneously may be useful for differentiating MS across different stages of the disease and may assist with prognosis and response to therapy. Overall, data suggests it is possible that GFAP may better reflect progressive disease in PMS without relapse whereas sNfL may better reflect acute relapsing disease activity.

Parvalbumin: The budding success of NFL, GFAP, CHI3L1 and other neuronal and glial proteins as biomarkers of neurologic disease has led to the search of even more nuanced, cell-specific based markers of distinct CNS pathology. For example, parvalbumin is a protein specifically expressed in GABAergic interneurons and CSF levels could be a specific marker of grey-matter neurodegeneration in MS. Cortical neurodegeneration is associated with meningeal B-cell follicles and progressive disease. A recent study by Magliozzi et al. showed parvalbumin gene expression and parvalbumin-positive cell density in the motor cortex are decreased in PwMS versus controls. CSF parvalbumin levels negatively correlated with parvalbumin-positive cell density and were increased in MS compared to controls. CSF parvalbumin levels also associated positively with cortical lesion number and global cortical thickness on MRI, microglia density in the motor cortex, earlier age of MS onset, faster disability progression, and severity of cognitive impairment[113]. These initial results suggest CSF parvalbumin may reflect loss of cortical interneurons in MS and associated cortical neurodegeneration, atrophy, and cognitive decline. However, parvalbumin is also highly expressed in fast-contracting muscle fibers and thus serum parvalbumin is thought to

be an indicator of muscle pathology, limiting the ability of serum parvalbumin to be a useful biomarker for CNS disease.

Extracellular vesicles—Extracellular vesicles (EVs) are small lipid-bound particles that facilitate cellular communication through their contents of bioactive proteins, nucleic acids, and lipids. EVs released from CNS cells such as neurons, astrocytes, microglia, and oligodendrocytes can cross into the blood, urine, and tears. Cell surface receptors on EVs as well as their contents can be used to identify their parental cell of origin. This makes CNS-derived EVs in blood a non-invasive source of potential cell-type specific biomarkers. CNS-derived EVs from several cell types have been shown to play important roles in MS and animal models of demyelination including myelin damage, inflammatory signaling, blood-brain-barrier breakdown, and neuroplasticity. CNS-derived EVs, including myeloid and endothelial-derived EVs have been shown to be elevated in the CSF of PwMS particularly in association with acute active disease[114,115], suggesting they may serve as measures of neuroinflammation. Myeloid EVs are elevated in CIS and higher levels associate with a shorter time to further disease activity[81]. Recently several studies have started examining the EVs cargo including microRNAs, proteins, and lipids as potential biomarkers in MS (reviewed in[116]). One study by Galazka et al., showed that MOG was elevated in serum-derived EVs in RRMS during relapse and also in SPMS, potentially reflecting MOG within CSF-derived EV[117]. MOG, an immunogenic myelin protein expressed only on the surface of myelin sheaths and oligodendrocyte membrane, likely directly reflects oligodendrocyte pathology in this context.

The research of EVs as biomarkers in MS is in its infancy and much remains unexplored particularly regarding cell-type specific EVs and their relation to disease activity and prognosis. A recent study found that neuronal-enriched EVs had lower levels of synaptopodin and synaptophysin in MS compared to controls potentially reflecting synaptic loss in MS[118]. Versus controls, PwMS were found to have higher levels of multiple early classical complement cascade components in astrocyte-derived EVs. This suggests a potential link to astrocyte complement production, which is thought to opsonize synapses and has been implicated in several neurodegenerative disorders including MS. Importantly, these differences in synaptic and complement proteins were not found in total EVs or neat plasma, demonstrating that CNS-enriched EVs may prove to be unique reservoirs of biomarkers in neuroinflammatory diseases. In a follow-up study[119], altered mitochondrial complex activity in neuronally-enriched EVs was significantly associated with faster brain and retinal atrophy in MS, exemplifying that neuronal-derived EVs also have potential to provide a unique assessment of neuronal health and pathology in MS. Expanding the repertoire of cell-specific EVs, a recent study by Mazzucco et al., presented a method to isolate CNS-endothelial derived EVs from plasma with results from their pilot study suggesting increased levels of these EVs in PwMS with active disease compared to healthy controls and PwMS with stable disease or on high-efficacy therapy[120]. Thus CNS-derived EVs may be a biomarker of blood-brain barrier permeability and active disease in MS. Overall, preliminary findings support CNS-derived EVs and their contents as promising candidates to serve as novel biomarkers of disease activity and progression in MS and other neurological conditions.

CSF and serum inflammatory mediators and other potential liquid biomarkers

CSF and Serum Inflammatory Mediators: Several cytokines, chemokines, and other inflammatory mediators are altered in the CSF and sometimes blood in MS and some have correlated with disease activity and future disability (Table 1). One example is CXCL13, a major chemoattractant involved in recruiting B-cell and some T-cell subsets, including follicular T-helper cells, into the CNS. CXCL13 has been implicated in the formation of ectopic lymphoid follicles in the CNS in MS, particularly progressive MS[121]. CXCL13 has repeatedly been shown to be elevated in the CSF in MS, especially RRMS[122–127]. Some studies report a correlation between CSF and serum CXCL13 levels, including in PwMS[128]. CSF CXCL13 can be used to predict conversion to MS from CIS[129–131]. CXCL13 CSF levels also correlated with current and future disease activity, particularly in RRMS, including relapse rate, disability, and MRI lesions[124,127,128]. CSF and serum CXCL3 levels decreased with steroids, with DMT including B-cell depleting therapy, and in some cases may predict response to therapy[127,132–134].

However, several common issues exist with many of these inflammatory mediators as biomarkers in MS. Many of these molecules are elevated in other inflammatory neurologic conditions, limiting their diagnostic specificity. For example, CXCL13 has been shown to be elevated in NMO, neurosarcoidosis, primary CNS lymphoma, idiopathic transverse myelitis, Lyme neuroborreliosis, and viral and bacterial meningitis[123,124,126,135,136]. Moreover, many are relatively small molecules that can cross the blood-CSF and/or blood-brain barrier, so serum levels may contribute greatly to CSF concentrations, requiring correction[137]. While CXCL13 levels correlate between CSF and serum in PwMS in some studies, this correlation is markedly stronger in people with non-inflammatory neurologic conditions[128] Thus, serum levels are not a good predictor of CSF levels, particularly when CSF levels are high. Many of these potential biomarkers that are produced in the periphery also do not correlate, or do not correlate well, with their CSF levels, which may more accurately reflect intrathecal pathology. Additionally, some of these potential biomarkers that are only produced significantly in the CNS are not detectable reliably in the blood using standard commercially available assays, with more sensitive assays not readily available in clinical laboratories. The inability to assay these biomarkers in the blood limits their promise as applicable biomarkers.

Micro RNAs: MicroRNAs (miRNA/miR) play a vital role in gene-regulation, through targeting messenger RNAs for cleavage or translational repression. Several miRNAs have been shown to regulate processes critical to MS including oligodendrocyte development, myelination, and inflammatory responses[138,139]. Several studies have profiled miRNAs in blood, other biological fluids, or cells in MS, with some miRNAs differing between MS and healthy controls, MS subtypes, or with outcomes such as MRI lesions, disability, or response to therapy[138,140–143]. Though there are conflicting results and lack of replication in the miRNA biomarker literature, several miRNAs with known roles in inflammatory signaling including regulation of lymphocyte subsets have been identified in multiple MS studies including miR-145, miR-155, and miR-92a [140,143–148]. The role of miRNAs as biomarkers and as potential therapies in MS are reviewed in detail elsewhere[149,150].

Vitamin D: Decades ago epidemiological studies first noted that MS prevalence is lowest along the equator and increases with increasing latitude [151]. Scientists hypothesized that perhaps sunlight dependent biology, such as Vitamin D synthesis, was involved in this phenomenon. Observational studies supported this notion in finding that individuals with low serum 25-hydroxyvitamin D levels or lower vitamin D intake have higher risk of developing MS and having more severe MS disease[152–155]. Vitamin D has known immunologic effects on both the innate and adaptive immune system[156]. Further supporting a role for Vitamin D in CNS autoimmunity, Vitamin D supplementation suppressed experimental autoimmune encephalomyelitis[157,158], the CD4+ T-cell dependent demyelinating mouse model of MS, and moreover the therapeutic effects required Vitamin D receptor function in T cells[159]. However, numerous trials indicate that Vitamin D supplementation provides little, if any, benefit in PwMS[160]. Furthermore, risk for lower serum 25-hydroxyvitamin D as determined by polygenic risk scores was not associated with worse disease outcomes in PwMS[161]. Several ongoing Vitamin D supplementation studies in MS may provide additional insight into the potential role of Vitamin D in PwMS. At the current time, Vitamin D levels are not an adequately sensitive or specific diagnostic or prognostic tool.

Epstein Barr virus related-biomarkers—Epidemiologic research to identify a viral trigger of MS over the last few decades has suggested that Epstein Barr Virus (EBV) infection may be necessary but not sufficient for development of MS in most, if not all, cases[162–164]. A recent study by Bjornevik et al. demonstrated a 32-fold increased risk of MS after EBV infection, but not other viruses, in a large U.S. military cohort[165], reigniting the field's interest in EBV as a putative causal agent of MS. Several studies suggest that symptomatic EBV infection (i.e. infectious mononucleosis) confers a higher risk of MS than asymptomatic EBV infection[163,166–168]. Additionally, higher titers of anti-EBV-nuclear antigen (EBNA) antibodies associate with increased MS risk[162,166]. These findings may suggest that individuals with decreased ability to control EBV infection may be at most increased risk due to higher chance for EBV to activate the pathologic processes that lead to MS, although this could also be a reflection of a heightened humoral response in PwMS. Consistent with the hypothesis that EBV is an immunological driver of disease, there are possible interactions between HLA and other genes involved with B and T-cell activation, anti-EBNA-1 antibody levels, and MS risk[169–171]. There is high sequence homology between an EBV peptide and the encephalitogenic epitope of myelin basic protein that is presented by a major MS risk allele, HLADRB1*1501[172]. In addition to postulating cell-mediated molecular mimicry, EBV may evoke cross-reactive antibodies produced by clonally expand CSF B-cells in MS bind to EBV EBNA1 and cross-react to the CNS protein GlialCAM[173]. Several additional potential mechanisms by which EBV might alter the host immune system to promote CNS autoimmunity have been postulated and described (reviewed in[174]). Notably, while large and well-designed epidemiologic studies support the causal role of EBV in MS, experimental evidence of causation is lacking.

While the potential causality of EBV in MS engenders thoughts of the potential therapeutic implications, namely would vaccinations or treatments against EBV decrease MS risk or severity, it also suggests that EBV infection and its downstream pathogenic mechanisms

could be useful biomarkers in MS as well as in other diseases that implicate EBV. The high seroprevalence of EBV (~95% in health adults) makes EBV serology or other measures of past-infection of limited utility to rule-in MS, but the absence of evidence of prior EBV infection, particularly if confirmed by multiple methods, could be a red flag against a diagnosis of MS[162–165]. Studies have not found an association between anti-EBNA1 IgG levels and risk of conversion from CIS to MS, but the potential remains for markers of EBV-specific biology to serve as diagnostic, disease activity, and prognostic biomarkers. As more is understood about the immunologic pathomechanisms that EBV exerts on the immune system to potentially cause MS, the field may identify a molecular signature unique to MS pathogenesis that can aid in diagnosis and prognosis.

Conclusion

An ideal biomarker is highly sensitive and specific, reproducible, non-invasive, and easy to interpret. Such biomarkers are currently lacking in MS care, limiting ability to diagnose, prognosticate, and monitor treatment response in PwMS, and also hindering development of new interventions particularly for progressive disease. However, several biomarkers seem poised for integration into routine MS clinical care and interventional trials in the next decade. If ongoing clinical trials affirm the ability of CVS to aid in MS diagnosis this imaging biomarker has the potential to be incorporated rapidly into MS diagnostic work-up. While additional studies are needed to validate appropriate interpretation in various clinical scenarios, tests for NfL levels both in the serum and CSF are clinically available and there is considerable evidence suggesting they may be a useful biomarker of ongoing neuro-axonal injury in MS and may aid in prognosis and treatment decisions. Additional biomarkers that represent cell-specific and pathology-specific processes occurring in various stages of MS, especially progressive disease, are greatly needed, and several candidates have been presented in this review. These biomarkers have the potential both for MS clinical care and in research studies and clinical trials to define and develop novel therapeutic approaches. There are several potential candidate biomarkers and biomarker reservoirs that are promising, and in the future MS clinicians will hopefully have a panel of biomarkers capable of aiding in predicting and monitoring disease activity and treatment response in PwMS.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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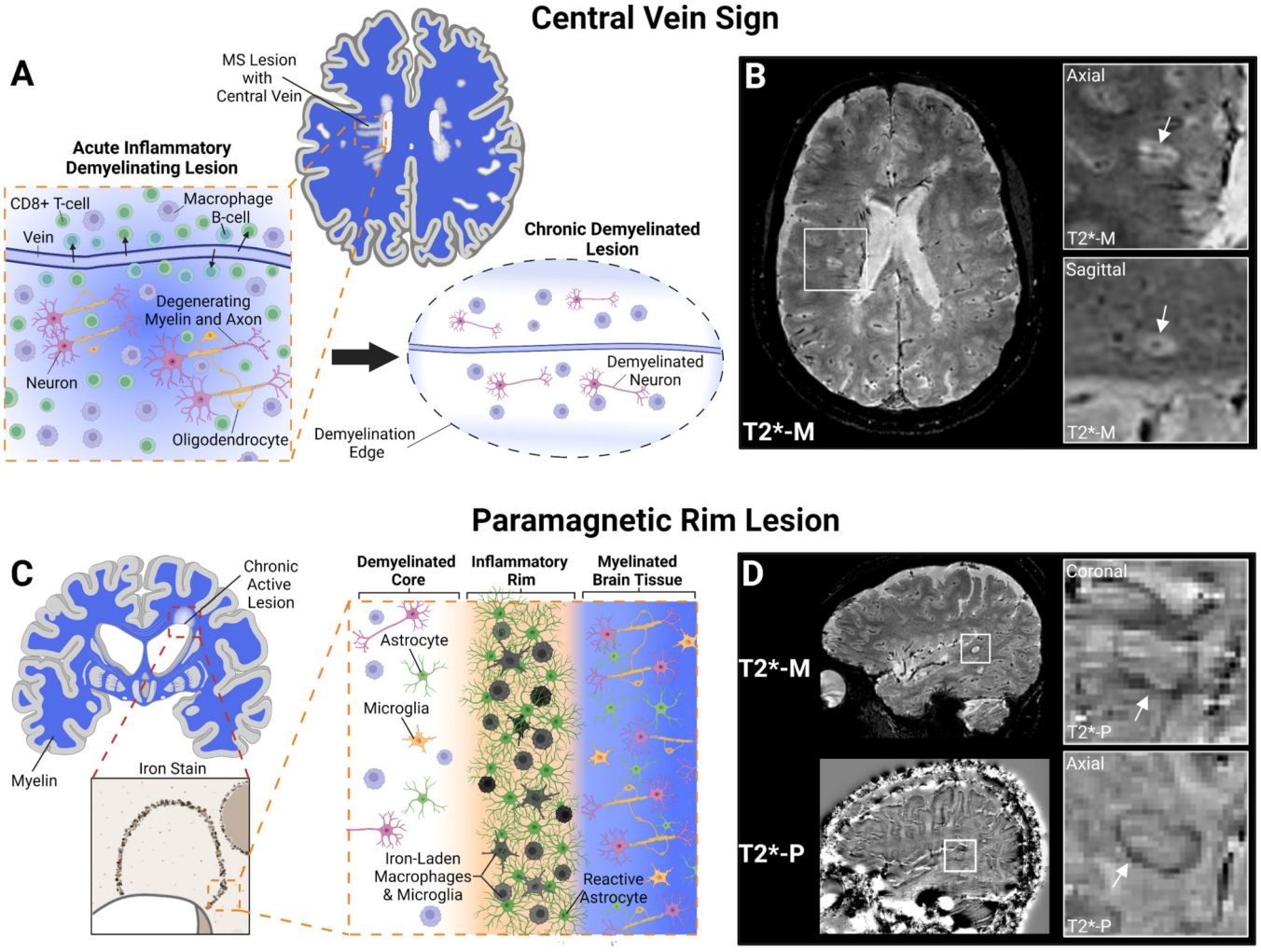

Figure 1: Emerging MRI Biomarkers in MS: Central Vein Sign and Paramagnetic Rim Lesions.

A) White matter lesions in MS are acutely result of immune cell infiltration, particularly $CD8^+$ T-cells, from the periphery into the CNS via small penetrating veins. These inflammatory lesions result in oligodendrocyte and myelin damage as well as neuro-axonal degeneration. After peripheral lymphocyte infiltration resolves a chronic demyelinated lesion centered around a vein remains B) Certain MRI sequences can depict white matter pathology and small CNS vessels simultaneously (e.g., T2*-weighted magnitude reconstruction; T2*-M). These small veins within classic ovoid MS lesions can be visualized and quantified to aid in MS diagnosis. Inserts show confirmation of central vein in two planes. C) Chronic active lesions in MS can be identified pathologically by an iron-rim at the lesion edge that contains iron-laden macrophages and microglia as well as activated astrocytes. D) These iron-rimed chronic active lesions can be visualized on MRI as paramagnetic rim lesions (PRLs) by “unwrapping” the phase reconstruction of the same T2*-weighted imaging (T2*-P)[175]. Paramagnetic rim lesions may represent a biomarker of at least one cause of progressive disease in MS. Created with BioRender.com

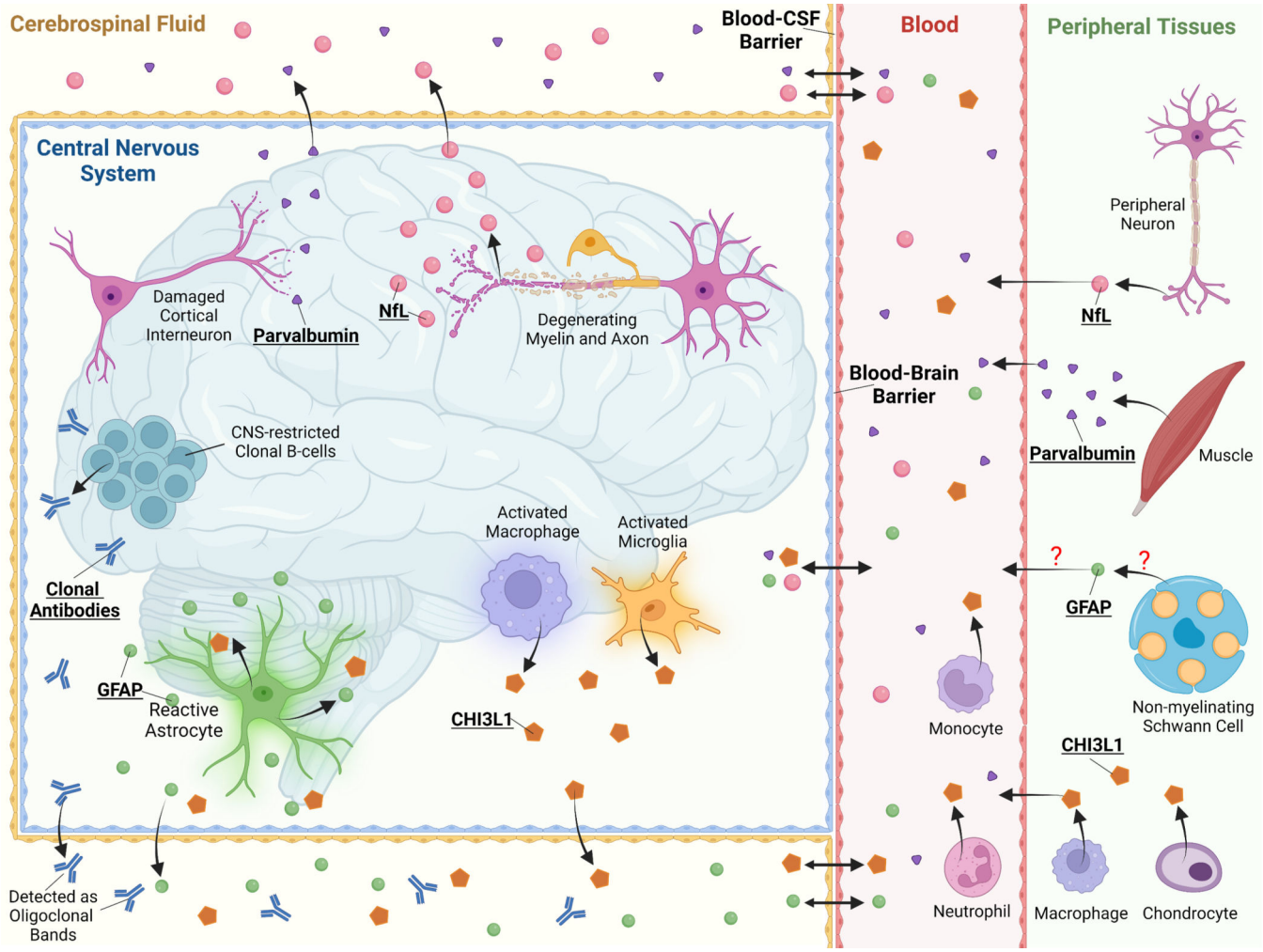

Figure 2: Emerging Neuroglial Biomarkers in MS

Schematic of the CNS, periphery, and blood-brain barrier, and blood-CSF barrier cell types relevant to emerging neuroglial biomarkers and CSF-specific oligoclonal bands. Released neuroglial protein biomarkers are released from one or a select few CNS resident cell types where they can traffic to the CSF and blood. These cell-specific biomarkers may thus reflect cell-type specific pathology, such as axonal damage in the case of Nfl. Many neuroglial biomarkers also have identified or potential peripheral sources that may, if a significant source, limit or prevent the use of blood levels to be a useful as a biomarker, such as the case with parvalbumin and CHI3L1. Many neuroglial biomarkers cross the CSF-blood barrier and even the blood-brain barrier, particularly in the setting of blood-brain barrier injury such as occurs in an active MS lesion. This equilibrium between CSF and serum or plasma levels is important to determine for each biomarker as high peripheral levels from a non-CNS source may impact CSF levels requiring correction of obtained CSF values. Abbreviations: CHI3L1, chitinase-3 like protein 1; GFAP, glial fibrillary acidic protein; Nfl, neurofilament. Created with BioRender.com

Table 1:

Summary of diagnostic and prognostic features of select emerging biomarkers in MS.

Biomarker	Compartment	Diagnosis/Classification	Disease Activity/Prognosis
Neuroglial Proteins			
NfL	CSF, serum, plasma. Good correlation.	CSF & Serum: ↑ in RMS & PMS vs. HC[75–78]; ↑↓ in PMS vs. RMS (conflicting results) [89,176]	Serum: ↑ CIS or RIS that converts to MS[80–83]; ↓ with DMT in RMS or PMS[80,84,177]; ↑ disease activity in RMS[75,76,80,84–86]; ↑ risk of new MRI lesions [76,80,84,86]; ↑ risk of brain / spinal cord atrophy[85]
GFAP	CSF, serum, plasma. Good correlation[104]	Serum: ↑ in active RMS vs. NIND & HC [74,104,110]; ↑↓ in PMS vs. RMS (conflicting results) [78,104,108,111]; ↑ in NMOSD vs. MS or HC [74]	Serum: ↑ risk of worsening EDSS[107,109,111]; ↑ lesion progression on MRI[104,107,111]
CHI3L1/YKL-40	CSF. CSF levels do not correlate with serum	CSF: ↑ in RMS vs. HC; ↑ in PMS vs. RMS & SPMS [101]	CSF: ↑CIS that converts to MS; ↑ likelihood of disability progression; ↓ with DMT; ↑ risk of new MRI lesions [83,95–100]
Parvalbumin	CSF	CSF: ↑ PMS vs. HC[113]	CSF: ↑ increased cortical lesion number; ↑ cortical thinning; ↑ cognitive impairment[113]
BDNF	Serum, plasma	Serum and plasma: ↓ in MS compared to HC[178,179]	Serum: ↓ increased MRI lesions[180]
Osteopontin	CSF, serum. Some correlation between CSF and serum	CSF and serum: ↑ in PMS & RMS vs. NIND & HC[181] Serum: ↑ in RMS vs. CIS & SPMS[181]	CSF: ↑ in active MS vs. stable MS[182]; ↑ risk of brain atrophy[181,183]
Neurogranin	CSF	CSF: conflicting results, no difference or ↓ in MS vs. HC[184,185]	CSF: ↑ in MS with enhancing lesions vs. MS without enhancing lesions[185]
NSE	Serum, plasma, CSF. Good correlation between sources	CSF: ↓ in CIS vs. HC[186]; CSF and plasma: no change between RMS & PMS vs. HC[187,188]	Serum and plasma: conflicting results, no change or negative correlation with EDSS and MSSS[187–189]
KIF5A	CSF	CSF: ↑ in PMS vs. RMS, NIND, HC[190]	CSF: ↑ in RMS correlates with worsened EDSS, MSSS and ARMSSS at 2 year follow up[190]
Cytokines and Chemokines			
TNFα	Serum, CSF	CSF & serum: ↑ MS vs. healthy control [191,192]; CSF: ↑ RMC > PMS > HC[193]	
IL-4	Serum, CSF	Serum and CSF: Conflicting results. Large meta-analysis shows no difference in MS vs. HC[191]	
IL-6	Serum, CSF	Serum and CSF: Conflicting results. Large meta-analysis shows no difference in MS vs. HC[191]	Serum: ↓ with DMT[191]
IL-8 (CXCL8)	Serum, CSF. Serum does not correlate with CSF[194]	Serum: ↑↓ MS vs. healthy control[191,194] (conflicting results) CSF: ↑ RMS & PMS vs. healthy control[193,194]	
IL-10	Serum, CSF	CSF: ↑ in RMS & PMS vs. HC[193]	Serum: ↑ CIS that converts to MS or NMOSD[195]; ↑ with DMT (IFN-b1a)[196]; ↑ in MS remission compared to relapse[197]; ↓ increased risk of relapse in pediatric MS[198]
CCL11 (Eotxain-1)	Plasma, CSF	CSF & plasma: ↑ SPMS vs. RMS[199]	CSF & plasma: ↑ longer duration of disease[199]
IL-12p40	Serum, plasma, CSF	CSF, plasma, serum: ↑ MS vs. healthy control[191,199] & NIND CSF: ↑ in CIS vs. HC[166]	

Biomarker	Compartment	Diagnosis/Classification	Disease Activity/Prognosis
CXCL13	CSF. Serum does not correlate with CSF [128,137]	CSF: ↑RMS and PMS vs. NIND & OIND[122–127]	CSF: ↑CIS that convert to MS[129–131]; ↑ disease activity in RMS[124,127–129] CSF>Serum: ↓ with DMT [127,132,133] Serum: Predict response to fingolimod[134]
IL-17A	Serum, CSF	Serum: ↑ MS vs. healthy control[191,200]; CSF: Large meta-analysis with nearly-significant ↑ in MS vs. HC[191]. ↑ RMS > PMS > HC[196]	Serum: ↓ with DMT (IFN-b1a)[196]
IL-23	Serum	Serum: ↑ MS vs. healthy control[191]	
IL-27	Serum, CSF	Serum: Large meta-analysis shows no difference in MS vs. HC[191]. CSF: ↑ in RMS vs. HC[193,201]	
IL-33	Plasma	Plasma: non-significant ↑ RMS vs. HC[202,203]	Plasma: ↑ decreased lesions number; ↓ with DMT (IFN-b1a)[203]
IL-36	Serum	Serum: ↑ RMS vs. HC[204]	
IL-37	Serum	Serum: ↑ RMS vs. HC[205]	Serum: ↑ with DMT (fingolimod)[206]; ↑ during MS relapse; ↑ increased EDSS[206]
IL-38	Serum		Serum: ↑ in newly diagnosed MS compared to treated[207]
Endothelial Cell-Related Proteins			
Endothelian-1	Plasma	Plasma: RMS or PMS vs. HC[208]	CSF: ↑ increased risk of poor visual recovery from optic neuritis[209]; increased in active vs. stable MS[210]
Endothelian-3	Plasma, CSF	Plasma: ↑ MS vs. HC[211]	Plasma: ↑ MS disease duration
VEGF (total)	Serum	Serum: ↑ RMS vs. HC[212]	Serum: ↑ shortened duration between first and second relapses[212]
VEGF-A	mRNA, CSF	CSF: ↑ in RMS & PMS vs. HC[193]	mRNA (from serum monocytes): ↓ in SPMS vs. RMS[213]
TGF-α / VEGF-β (ratio)	Serum	Serum: ↑ in CIS vs. RMS[214]	Serum: ↓ stable RMS vs. HC; ↑ active RMS vs. HC; ↓ increased EDSS[214]
sNCAM	CSF	CSF: ↓ in MS vs. HC; ↓ in SPMS > RMS > CIS[215–218]	CSF: ↑ after natalizumab or mitoxantrone; ↓ after fingolimod treatment[216]; ↓ increased EDSS[215]
VCAM1	Serum, CSF	Serum and CSF: ↑ in active RMS & PMS vs. HC[219]	Serum and CSF: ↑ during MS relapse vs. remission[219] Serum: ↑ decreased number of MRI lesions. ↑ with DMT (IFNB-1b)[220]
Mitochondria and Autophagy-Related Proteins			
Parkin	Serum, CSF	Serum and CSF: ↑ in RMS & PMS vs. HC[221–223]	Serum and CSF: ↑ in MS with enhancing lesions vs. MS without enhancing lesions[224]
ATG5	Serum, CSF	Serum and CSF: ↑ in RMS vs. HC[222,223]	Serum and CSF: ↑ in MS with enhancing lesions vs. MS without enhancing lesions[224]
ANT1	Serum, CSF	Serum and CSF: ↓ in RMS vs. HC[222,223]	
Other			
Kynurenine	Serum, CSF	CSF: ↓ in remission RMS vs. HC[225]; ↑ in relapsing RMS vs. HC[226] Serum: ↑ in active RMS vs. HC and PMS[226–228]	
CCN3	Plasma, CSF. Good correlation between sources[229]	Plasma: ↑ in PMS vs. RMS[229]	

Biomarker	Compartment	Diagnosis/Classification	Disease Activity/Prognosis
Vitamin D	Serum		Serum: ↑ lower risk of developing MS[152,230]; ↓ neonatal vitamin D increased risk of developing MS[231]; ↑ lower degree of brain atrophy; ↑ less clinical progression at 5 year[232,233]; ↓ increased relapse risk[232]

Up (↑) and down (↓) arrows designate increase or decrease of the biomarker collected from the designated compartment(s). Correlations are statistically significant except where otherwise noted; topics with conflicting results are reported with up/down (↑↓) arrow.

Abbreviations: CSF = cerebrospinal fluid; MS = multiple sclerosis; RMS = relapsing multiple sclerosis; PMS = progressive multiple sclerosis; SPMS = secondarily progressive multiple sclerosis; CIS = clinically isolated syndrome; RIS = radiologically isolated syndrome; HC = healthy control; NIND = non-inflammatory neurologic disorder; EDSS = expanded disability status scale; NMOSD = neuromyelitis optica spectrum disorder; DMT = disease modifying therapy; NfL = neurofilament light chain; GFAP = glial fibrillary acidic protein; CHI3L1 = chitinase-3-like protein 1; BDNF = brain derived neurotrophic factor; NSE = neuron specific enolase; KIF5A = Kinesin family member 5A protein; VEGF = vascular endothelial growth factor; sNCAM = soluble neural cell adhesion molecule; VCAM = vascular cell adhesion molecule; ATG5 = autophagy related 5 protein; ANT1 = adenine nucleoside translocator 1; CCN3 = cellular communication network factor 3