Biomarkers in MS: the current state of play

Abstract
Since the late 80s with the discovery of oligoclonal bands (OCBs) in the CSF of Multiple Sclerosis (MS) patients, scientists have made huge efforts to develop prognostic biomarkers in both the CSF and blood. In general terms, the latter has resulted in the development of either immune system activation/regulation biomarkers, or neurodegenerative biomarkers. Simply put, from a biomarker perspective, disease progression in MS is due not only to the underlying autoimmunity, but neurodegeneration. As there has been resurgence of interest in the OCBs with the 2018 McDonald criteria, we discuss this first in the review.

We then highlight some of the other promising diagnostic and prognostic biomarkers in MS, including osteopontin, microRNAs, neurofilaments, chitinase and chitinase-like proteins.

Introduction
Multiple sclerosis (MS) is a progressive inflammatory demyelinating disease of the central nervous system (CNS). It is now well accepted that Th1 and Th17 cells play an important role in the pathogenesis of MS, but contrary to belief, they are not the only cells involved. A combination of antibody-producing B cells/plasma cells, macrophages, and NK cells are involved in disease pathogenesis, whilst demyelination, inflammation and axonal damage contribute to progressive disability in MS patients. Over the last few years, scientists and clinicians have worked together in order to identify specific biomarkers able to predict the onset and course of the disease. However, despite the dramatic increase in publications, the biomarkers commonly used in clinical practice still remain the cerebrospinal fluid (CSF) oligoclonal bands, and more recently the neurofilament proteins. In this review we summarise the biomarkers that have made waves in MS research over the last ten years, including osteopontin, microRNAs, neurofilaments, chitinase and chitinase-like proteins. We also discuss oligoclonal bands, particularly as these have been reintroduced into the latest diagnostic criteria for MS.

Oligoclonal bands
Immunoglobulin IgG oligoclonal bands (OCB) are detected in about 55% of MS patients and are considered the best diagnostic element supportive of MS diagnosis. Although OCBs are found mainly in CSF of people with MS, they also occur in other inflammatory conditions like paraneoplastic disorders, CNS lupus, neuroarcsidosis, Behçet’s disease and various forms of cerebral angiitis. OCB negative MS patients have been reported to have fewer infratentorial and more juxtacortical lesions compared to OCB-positive patients. OCB negativity is also associated with better prognoses based on physical disability. If we focus on the prognostic significance, Tintore et al. found that those who are OCB positive had a higher risk of conversion from a clinically isolated syndrome (CIS) to clinical definite MS. It therefore adds information to MRI in the first attacks of MS, and henceforth has been re-introduced back into the 2017 McDonald criteria. Several studies have also investigated the correlation between OCBs and cerebral volume. Ferreira et al., studied both grey and white matter volumes, and noted less brain atrophy in those who were OCB negative. While Fenzi et al. found that brain atrophy in OCB positive patients primarily involved the white rather than grey matter.

Osteopontin
Osteopontin (OPN) is a pleiotropic cytokine expressed by immune cells, including T cells, dendritic cells, macrophages and natural killer cells. It is involved in a variety of physiological functions and pathological states such as bone remodelling, wound healing, cancer biology and vascular disorders, and exerts pro-inflammatory and pro-angiogenic effects. OPN is considered to be a pro-inflammatory mediator that amplifies the inflammatory process by enhancing the production of interferon gamma (IFN-γ) and IL-17 from T cells with consequent inhibition of IL-10.

Elevated OPN gene expression was found in MS brain lesions compared to control brain tissue and these findings were also confirmed in analysis of spinal cord tissue in experimental autoimmune encephalomyelitis (EAE). Many studies have reported increased concentration of CSF OPN in relapsing remitting MS (RRMS) patients compared to CIS and secondary progressive MS (SPMS) patients. Similarly, plasma OPN levels have been found to be increased in RRMS compared to healthy controls. However, a raised CSF OPN or blood OPN is not specific for MS, and has also been demonstrated in Alzheimer’s disease and Parkinson’s disease.

MicroRNAs
MicroRNAs (miRNA) are short non-coding RNAs with an important role in post-transcriptional gene expression by silencing via binding of the target messenger (mRNAs) or by degrading the mRNA transcript. MiRNAs play a major role in regulating key processes in immune cells, including Th1, Th17, Tregs, as well as being found in a number of neurological disorders, including traumatic CNS injuries. More recent findings suggest a role for miRNAs as biomarkers in MS. Huang et al. identified a link between dysregulated miRNAs...
and MS. Specifically, higher concentrations of miRNAs were observed in the serum of MS patients compared to controls and among these let-7i miRNA has been found to reduce the number of T-reg IFNγ-IL17A-Fox3P-CD4+ cells, by targeting insulin like growth factor I receptor (IGF1R) and transforming growth factor beta receptor I (TGFBR1). The impairment of T Reg cells, with consequent disruption of the immune homeostasis, is considered crucial in the initiation and perpetuation of autoimmune disease.

**Neurofilaments**

Neurofilaments, an abundant protein in the cytoskeleton neurons are composed of the subunits light (NFL; 60–70 kDa), medium (NFM; 130–170 kDa) and heavy chain (NHF; 180–200 kDa). Although the precise mechanism of axonal loss in MS is still not clear, it has been repeatedly demonstrated that neurofilaments are released into the blood and CSF of MS patients after episodes of relapses and with slow neurodegeneration. They are detectable in most at diagnosis, and even at the early stages of CIS and radiologically isolated syndrome (RIS).

The research has come a long way since MRI was the only tool available at that time to monitor the course of the MS status and there is the need to explore biomarkers that can accurately be detected at a very early stage of the disease. Among these biomarkers, NFL emerged as a promising biomarker candidate. There are many reports backing its usefulness early on in prognosis, with increased levels predicting the development of MS in CIS and RIS.

In MS, NFL levels in CSF and serum increase with EDSS, whilst the incremental rise correlates with lesion load and worsening EDSS. Following natalizumab use, a treatment effect on NFL levels has been demonstrated in the CSF but not in the serum, indicating a relationship between anti-inflammatory therapy and axonal damage resolution. However, with the increased sensitivity of Simoa platform, there has been a recent interest in investigating NFL levels in the serum, raising the possibility of a blood biomarker. To date good correlations have been demonstrated between serum NFL and CSF NFL. MRI activity and disability in CIS patients. At a cohort level serum NFL have definite utility in monitoring treatment effect and reduced levels have been documented with interferon beta and fingolimod, and may be a useful surrogate marker of treatment efficacy in clinical trials. At an individual level, it’s long-term predictive capacity is uncertain.

NHF, like NFL is a bulk biomarker of neuronal damage and has been found to be elevated in optic neuritis, in RRMS and SPMS and correlates with EDSS in cross sectional and longitudinal studies. NHF levels have been demonstrated to improve following lamotrigine treatment in SPMS and phenotypin in optic neuritis; two neuroprotection studies in MS. Antibodies to NFL have also been identified in MS, with elevated levels in CIS, primary progressive MS (PPMS) and RRMS and have been linked with clinical disability and progressive disease course. Their significance in MS is as yet unknown.

**Chitinase and Chitinase-like proteins**

Chitinase (chitinase 1, CHIT1) and chitinase-like protein (chitinase 3-like protein 1, CHI3L1 and 2, CHI3L2) are chitin-binding proteins that belong to the glycohydrolase family 18 and may be indicators of inflammation. Chitinase and Chitinase-like proteins have been demonstrated to be upregulated in optic neuritis, in RRMS and also correlated with MRI activity. CHI3L1, is not specific for MS and has been found to be elevated in cancer and rheumatoid arthritis.

CHI3L2, on the other hand, was originally thought to be a secretory product of chondrocytes, and as such has only recently been studied in MS. Unlike CHI3L1, CSF CHI3L2 decreased in PPMS compared to RRMS patients. A rise in CHI3L2 observed in RRMS and also correlated with other biomarkers of inflammation and tissue damage such as NFL, OPN and MBP, suggesting like the others an association with inflammatory activity.

**Conclusion**

In the past, MS was considered to be an exclusively T-cell mediated-disease, but increasingly it is clear that we are dealing with a multifactorial disease pathogenesis leading to progressive disability. Understanding the role of each of these factors may allow for better definition of the underlying predominant disease process in each patient, permitting more individualised therapeutic strategies. The drive to find new validated biomarkers in MS to facilitate this process has often had unpredictable results. We now understand that the majority of these biomarkers are either indicators of bulk tissue injury i.e. neurodegeneration or inflammation, or impaired immune regulation in MS.