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REVIEW ARTICLE

An Updated Insight about Biomarkers of Multiple Sclerosis

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ABSTRACT

Background: Multiple sclerosis (MS) is a heterogeneous neurological disease characterized by progressive neurodegeneration induced by an autoimmune reaction to self-antigens. Clinical symptoms differ according to where neurologic lesions are located and usually occur in conjunction with inflammatory cell invasion across the blood-brain barrier, which causes demyelination and edema. Because MS causes disability and cognitive damage, it is critical to detect it early. This review summarizes current knowledge on the use of biomarkers such as neurofilament light chain, uric acid, interleukins, Tau protein, chitinase 3-like 1 and 2, heat shock proteins, nitric oxide and other potential biomarkers in MS.**Conclusion:** Although numerous investigations on the application of biomarkers in the diagnosis of MS, further investigation is required to identify the clinical value of these markers and to develop diagnostic tools that may be used in daily practice. This, in turn, may lead to earlier MS identification, faster treatment application, and greater therapeutic outcomes.

Keywords: Updated insight, Biomarkers, Multiple Sclerosis

INTRODUCTION

More than 2 million people worldwide are affected by multiple sclerosis (MS), the most common chronic inflammatory illness of the central nervous system (CNS). It is the most prevalent non-traumatic cause of impairment in young people and is most frequently characterized by reduced mobility, delayed cognitive function and/or loss of bladder control. There is currently no treatment that can completely stop or stop the MS-related progressive neurological decline. MS is estimated to cost the US economy 10 billion dollars annually. The precise cause of MS is still unknown. Genetic polymorphisms and environmental exposures are just two of the many interrelated risk factors that can contribute to the chronic nature of MS. Numerous studies have suggested that systemic inflammation is a factor in the development of MS and can be targeted by therapies for the disease [1].

A biomarker can be accurately observed and evaluated as a sign of typical biological

processes, abnormal occurrences or pharmacological reactions to therapy. If the condition worsens or improves, the biomarker level should rise or fall proportionally. A good biomarker should also be safe for an individual and as simple to detect as feasible, ideally using a non-invasive technique. To achieve thorough application, the analytical detection technique must be extremely accurate, reproducible, quick, easy, and cost-effective. As a consequence, the identification method's output should be independent of routine affecting variables including sample gathering, processing and storage [2]. This review summarizes current knowledge on the use of biomarkers such as neurofilament light chain, uric acid, interleukins, Tau protein, chitinase 3-like 1 and 2 heat shock proteins, nitric oxide and other potential biomarkers in MS.

S100β Protein

A subunit of S100 protein present in glial cells is more prevalent in the serum and plasma of MS patients with primary (PPMS) or secondary progressive MS (SPMS). Supporting astrocyte integrity, aiding in neuronal growth and differentiating oligodendrocytes are a few instances of S100β's actions. S100 levels increased in RRMS cases following acute exacerbations; however, the window is small because S100 levels decreased in individuals who had recent acute exacerbations. In addition, alterations in S100 have been seen in cases of ischemic stroke brought on by amyotrophic lateral sclerosis [3]. However, a study comparing clinically isolated syndrome (CIS) patients and healthy volunteers found no marked variance in S100 protein in the CSF or serum. It is possible that some samples were collected more than a week after the acute exacerbation may have affected this conclusion. The expanded disability status scale (EDSS) score and S100 protein concentration did not substantially relate, based on the same study. Furthermore, no variance in S100 protein concentrations among MS major clinical subtypes or CSF S100 protein levels between MS cases and healthy cases [4].

Glial Fibrillary Acidic Protein

Mature astrocytes generate glial fibrillary acidic protein (GFAP), which has been reported to be elevated in the plaques of MS cases and suggests astrocyte injury. CSF GFAP levels were higher in SPMS patients than in RRMS patients. Furthermore, greater impairments and relapse are linked to higher CSF levels of GFAP [5].

Nitric Oxide

Both the serum and CSF of MS cases have been reported to contain higher nitric oxide (NO) levels. Since less energy is produced as a result of the inhibition of cytochrome C oxidase, mitochondrial activity is compromised. Byproducts of NO breakdown have the potential to harm mitochondria, which would significantly worsen MS symptoms. Raising blood-brain barrier's (BBB) permeability, may additionally enhance the impact of apoptosis on glial cells and neurons and enable the pro-inflammatory cells invasion into the CNS [6].

Anti-Aquaporin 4 Antibodies

Aquaporin-4 (AQP4), which helps the CNS achieve homeostasis by allowing water to pass across plasmalemma, is expressed by astrocytes. Nevertheless, research has shown that AQP4 is not present in MS cases. This marker will assist with the challenging task of distinguishing MS from the rare disorder of neuromyelitis optica (NMO), which similarly

causes optic nerve demyelination and spinal cord [7].

Neurofilament

The proteins known as neurofilaments (NF) are the light (NFL), intermediate (NFM) and heavy (NFH) chains of the neuronal cytoskeleton. They control axon diameter and participate in axonal transport. When there is neuronal or axonal injury, NF are released and are seen in the blood and CSF. Single-molecule arrays (SIMOA), a newly discovered ultra-sensitive technology for blood testing, have made it possible to detect NFL in serum for the first time. SIMOA has an analytical sensitivity that is > 25 times higher than detection methods based on ELISA (SIMOA: 0.62 pg/ml, ELISA: 78.0 pg/ml). NFL are also extremely stable and unaffected by standard storage conditions, which strengthens the detection techniques [2]. In an experiment conducted by Disanto et al., NFL concentrations in MS cases were shown to be higher than in normal participants with potential correlations between values assessed sequentially in serum and CSF. Additionally, MRI activity, level of impairment and rate of brain atrophy are all associated with serum NFL levels. NFL is also promising as a predictive biomarker for the transition from CIS to MS [8].

Overall, it appears that several clinical and magnetic resonance imaging (MRI) aspects of MS are correlated with the detection of NFL level, which no longer requires a lumbar puncture but can now be detected in the blood. Thus, a predictive biomarker may someday be used in clinical practice. Although glial fibrillary acid protein (GFAP) is an astroglial marker in the blood, NFL assessment is a biomarker with the potential for neuronal and axonal impairment in MS [9].

Numerous methods have been created to measure NFL levels over the past thirty years. Cytoskeletal proteins called neurofilaments get released into the CSF and circulation by injured axons. Investigations have also discovered a relationship between elevated cNFL levels and elevated CD4+ T cells, which have been linked to MS-related inflammation and RRMS progression to SPMS. Early research has shown that MS cases' cNFL rise during acute relapse and in comparison to normal participants. In MS cases, cNFL and serum NFL (sNFL) levels are positively correlated with cNFL levels 42 times greater than sNFL levels. The ease of obtaining serum is a benefit of employing sNFL concentrations as opposed to cNFL levels. SIMOA has improved the therapeutic relevance of assessing NFL levels over the past few years [10].

Compared to normal, MS patients generally exhibited greater sNfL levels before treatment. In response to disease-modifying therapy, sNfL levels were decreased. It has also been demonstrated that therapies with higher efficacies decrease NfL levels more successfully than conventional therapies. sNFL concentrations have also been linked to the size of T2 lesions. According to certain studies, there is a direct relationship between the quantity of MRI-assessed active lesions and sNFL. Nevertheless, some cases have numerous active lesions with low sNFL, whereas other cases have high sNFL levels despite having no active MRI lesions, suggesting that additional factors can cause elevation of sNFL. Cases will therefore continue to need MRI scans [11]. Additionally, research suggests that sNFL concentrations may be positively associated with the brain and spinal cord atrophy. According to one study, spinal cord and brain

size decreased over five years, while individuals with elevated sNFL experienced a bigger loss [12].

A recent investigation compared long-term clinical outcomes and the predictive significance of sNFL collected shortly after the onset of MS [13]. From specimens obtained during the diagnostic workup, sNFL were examined. sNFL at greater concentrations exhibited a noticeably increased hazard ratio of developing an EDSS ≥ 4 after 15 or more years of follow-up. Although there was a tendency, patients with progressing disease had median sNFL levels that were not statistically significantly higher [12].

At the group level, the sNFL level is an important biomarker. It could be difficult to be utilized clinically to determine if someone had MS. Numerous investigations have shown that there is a remarkable variation between sNFL levels at baseline in MS cases and normal participants, who could have conversion conditions or migraines. NFL is high in infected cases and a variety of neurological and neurodegenerative diseases in addition to MS, therefore using them as an MS relapse maker is imprecise. Due to age-related neuronal degradation, sNFL is positively connected with age [8]. Being older patients tends to be a significant complicating factor for progressing MS cases. Additionally, there is an inverse correlation between blood volume and plasma NFL (pNFL) levels and body mass index (BMI) [14].

As a result, the quest for a biomarker that can both predict and diagnose MS disease activity continues. sNfL cannot be utilized by itself to do so. Monitoring the activity of inflammatory disorder and separating true from false relapses is another use for sNfL. Numerous reports have demonstrated that levels of sNfL are raised in cases with MS during relapse. sNfL levels in RRMS cases overlap significantly, emphasizing the inadequacies of sNfL in relapse detection [15].

Tubulin Beta

One of the tubulins, heterodimeric proteins that form microtubules, is known as tubulin beta (TUBβ). Synthesis of the class II tubulin isotype has been found to rise during neuron growth and regeneration. According to one research, cases with MS had higher levels of CSF TUBβ than those with other neurological conditions [16].

Amyloid-Precursor Protein (APP)

Despite being linked to MS, APP was linked to Alzheimer's disease. Throughout demyelination, astrocyte cells make it and it can be found in active glial cells in both de- and re-myelination processes. APP concentrations are higher in MS cases than in normal cases and APP-positive axons in MS cases have been linked to the development of CNS lesions [17].

Tau Protein

Researchers have found that the tau protein, which has been associated with Alzheimer's disease, is produced during neuronal injury and can therefore be measured in the CSF. Tau protein stabilizes axonal microtubules. There is an association between a greater level of CSF tau protein and a faster progression of the illness as exhibited by an increase of one point in the EDSS score. The CSF tau protein can be used to predict when the next relapse will occur. One report demonstrated a relationship between clinical symptom severity and tau protein levels [10].Respecting demyelination or inflammatory events lasting for a day in one or more sites in CNS, There was no remarkable variation in tau level compared to

normal cases, nor a marked association between EDSS scores and tau concentration [4]. Another investigation, however, discovered that tau protein was associated with EDSS in CIS cases and with CIS progression to MS. They also discovered that tau levels were associated with the number of T2-lesions on MRI [18].

14-3-3 Protein

The 14-3-3 protein is found in neurons and may be detected in the CSF of MS cases. The 14-3-3 protein's role in MS is unclear. According to research, CSF 14-3-3 protein is related to high impairment severity, higher spinal cord involvement and faster development of MS or progression of the disease [19]. Early deposits in the CSF could be associated with slower recovery rates. Several reports have found it difficult to detect 14-3-3 protein in CSF, with one finding that it was in only two cases out of 22 (9.1%) MS cases and another finding it was in 4.7% CIS cases [4].

Neuron Specific Enolase (NSE)

Cases with traumatic brain damage, hypoxic brain injury, or cerebral hemorrhage have been found to have higher levels of NSE, an enzyme present in axons and neurons which may be employed to quantify the density of neurons [20]. A report indicated that cases with CIS had lower levels of serum and CSF NSE than normal individuals [4]. Whereas others reported either no alteration or an inverse relationship between the MS Severity Score (MSSS), EDSS and NSE levels [21].

Myelin Oligodendrocyte Glycoprotein (MOG)

The detection of oligodendrocyte glycoprotein (MOG) antibodies in the serum distinguishes MOG myelin-related disorder, a recently identified disease from NMO and MS. Furthermore, different myeloid cell types in CSF have been identified in individuals with neuro-inflammation, including those with MS and anti-MOG disease [22].

Soluble CD40L (sCD40L)

In an innovative approach, benign MS (BMS) cases with similar age and disease duration were utilized to search for markers of MS progression. When compared to BMS, sCD40L was considerably higher in SPMS. The immunological evaluation revealed no loss of lymphocyte subsets. Instead, a rise in the ratios of CD25+/CD4+ and CD25+/CD3+ and a change in activity ofanti-inflammatory cytokine were detected. The significance of this therapeutic target is further reinforced by the discovery that sCD40L is remarkably increased in SPMS in comparison to BMS. Additionally, they demonstrated that BMS T-cells had increased IL-10 while downregulating neurotensin high-affinity receptor 1 and IL-6 and that retinal nerve fibre layer (RNFL) thickness only slightly deteriorates in BMS [23].

Chitinase-3-Like-1 Precursor (CHI3L1)

CHI3L1, which is produced by astrocytes, white matter, brain lesions and plaques of white matter in MS cases, is reported to be raised in the CSF of individuals with inflammatory conditions. In particular, it was discovered that serum and CSF levels elevated with the disease stage and were linked to CIS cases' quicker transition to RRMS. In addition, cases with progressive MS had lower CSF levels than those with RRMS [24]. Nevertheless, an additional investigation revealed that cases with PMS had higher plasma levels of CH13L1 than RRMS cases and normal cases [25]. In CIS cases, increased levels are linked to a speedier onset of impairment and progression to clinically defined MS (CDMS). In groups of cases who did not respond to IFN-β therapy, it

was revealed that serum levels of CHI3L1 were elevated [26].

Heat Shock Protein (HSP) 70 and 90

HSPs, which are categorized into several molecular weight categories are molecular chaperones that assist in controlling CNS homeostasis. HSP70, a protein found in the cytoplasm, participates in the immune response by fending off stress-related harm to the cell membrane and intracellular area. In MS and during inflammation, extracellular HSP70 can prevent apoptosis in both oligodendrocytes and neurons, but it is involved in triggering an immunological reaction [27]. According to research, HSPA1L gene expression, which produces the HSP70-hom protein, is associated with a higher risk of developing MS. The severity of the disease was also connected with higher levels of the HSP70-hom protein [28].

According to a second study, MS cases exhibited serum levels of HSP70 that were higher than those of normal cases but decreased in cases with other inflammatory neurological conditions. The same report showed that CIS and RRMS had greater HSP70 levels than PPMS or SPMS [29].

Kappa Free Light Chain (KFLC)

Plasma cells synthesize KFLC during antibody production. CSF KFLC was suggested as an alternative marker in the identification of MS with high specificity and sensitivity compared with oligoclonal bands. Particularly, KFLC was shown to be raised in MS cases and linked with future progression of the disease, as CIS individuals with greater KFLC levels converted to clinically diagnosed MS earlier [30].

Human Endogenous Retroviruses (HERVs)

HERVs, which account for approximately 8% of the human genome, are normally inactive within the genome unless activated by an external stimulus. Their activity can cause HERV-W to produce envelope proteins, which seem to be important in MS pathogenesis [10]. They assumed that, while MS cases with HERV at the beginning of the study had similar EDSS scores, the results were substantially varied after six years. Cases in the HERV group also had a greater annual recurrence rate, as well as two cases, experienced a progressive type of MS, whereas none in the MS with HERV group did [31].

Uric Acid

Serum uric acid levels, which have antioxidant capabilities, have been reported to be lower in MS cases. One investigation assessed serum urate concentrations in MS cases and cases with other neurological conditions to see whether this is due to cases being predominantly deficient or to uric acid's peroxynitrite scavenging capacity. They showed that MS cases had much lower urate concentrations compared to cases with other neurological disorders. Nevertheless, no remarkable relationship was identified between urate levels and disability, disease duration or activity, confirming the hypothesis that uric acid is an imprecise marker in MS [32].

Immune Mediators and Cytokines

T helper (Th) 1 and Th17 cells, which are pro-inflammatory, release cytokines including tumor necrosis factor (TNF)-α, interferon (IFN)-γ and interleukin (IL)- which are anti-inflammatory, release IL-10 and IL-4. In an investigation of children with MS, the anti-inflammatory cytokine IL-10 was found to crucial for relapse prediction than other cytokines, demonstrating that assessing them and cellular alterations can detect the type of disease [33]. Furthermore, it has been

identified that C-X-C motif chemokine ligand (CXCL) 13 is associated with a worse prognosis, exacerbations in RRMS, and the transition from CIS to MS. Although cases with infections also exhibited elevated levels, CXCL13 is non-specific. Eotaxin-1 (CCL11) levels in the CSF and plasma were related to the duration of the disease, particularly in SPMS cases. Additionally, they noticed that plasma levels of oncostatin (OSM), hepatocyte growth factor (HGF), macrophage inflammatory protein (MIP)-1a, cluster of differentiation (CD)5, IL-12B and CXCL9 levels in CSF, were all linked to MS [34].

The therapeutic response or prognosis of MS cases may potentially be predicted by immune markers. The majority of biomarker research has concentrated on IFN-β, a medication with a very variable response. Treatment failure is linked to neutralizing antibodies (Nabs) against IFN-β, however, they only partially account for non-responsiveness. Immunologically different subgroups of MS have been identified by serum cytokine profiles and these subgroups may help to determine treatment responsiveness to IFN-β [35].

CONCLUSIONS

Biomarkers support individual choices and are a crucial first step toward individualized treatment. High sensitivity and specificity, as well as an easy, affordable, reproducible, and non-invasive detection method, are characteristics of an ideal biomarker. Currently, various known biomarkers can be used to improve the diagnosis and prognosis of MS, along with the evaluation of therapy response and the evaluation of the risk of adverse effects. However, to encourage the use of potential biomarker candidates in clinical practice, extensive investigations in large cohorts are required. Despite these preliminary achievements, there is still a shortage of biomarkers that allow for an accurate prediction of the response to therapy even before the initiation of treatment. Hence, MS currently requires the development and validation of novel biomarkers.

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