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Review Article

Some Serologic Biomarkers of Multiple Sclerosis Activity: A Narrative Review

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Abstract

Interferon-beta (IFN-β) preparations represent a well-established cohort of pharmaceutical agents employed for immunomodulation in individuals diagnosed with multiple sclerosis (MS). The clinical courses and manifestations of MS exhibit considerable variability, ranging from mild forms to progressive stages characterized by the development of irreversible clinical deficits with limited responsiveness to standard therapeutic interventions. Notably, highly effective treatments have been developed and have become readily accessible in recent years. The imperative for reliable markers for disease detection, staging, and prognosis prediction arises. This review presents some serologic biomarkers of MS activity, such as antibodies to IFN-β, MxA, viperin, NfL, and IL-17 , which are of interest for predicting MS activity and may contribute to informed decisions regarding optimal therapeutic strategies.

Keywords: multiple sclerosis, serologic biomarkers, disease activity, neuroimmunology, treatment

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1. Introduction

Multiple sclerosis (MS) stands as a chronic, immune-mediated inflammatory demyelinating disorder of the central nervous system (CNS), impacting over 2 million individuals globally. Its highest prevalence is observed among individuals aged between 20 and 40 years [1]. There is currently no pathognomonic serological diagnostic test for MS. When faced with symptoms specific to MS, patients undergo testing to exclude alternative pathologies, including acute disseminated encephalomyelitis, cerebral autosomal dominant idiopathic leukoencephalopathy (CADASIL), posterior reversible encephalopathy syndrome [2], Moyamoya angiopathy [3], neuromyelitis optica, antiphospholipid syndrome, systemic lupus erythematosus, primary angiitis of the CNS, and mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes [4]. Furthermore, distinguishing between a genuine MS relapse and a pseudo-relapse, complicated by concurrent infections and comorbidities, can pose a challenge in clinical assessment [5]. In addition to the imperative of identifying markers for MS diagnosing and confirming disease exacerbations, there is an equally crucial demand for markers facilitating the monitoring of therapy. Timely recognition of inadequate response to the treatment enables

prompt transitioning to alternative drugs [6]. Interferon-beta (IFN-β) is one of the first disease-modifying drugs for MS. Their effectiveness and safety have been proven by numerous studies. These agents were able to significantly reduce relapse rates by 40% and slow the progression of disability in patients with relapsing-remitting MS [7]. The early initiation of IFN-β treatment in clinically isolated syndrome patients not only extends the time to the first relapse but also the progression to established MS [8, 9]. Moreover, they have demonstrated effectiveness in secondary progressive MS with relapses [10]. However, despite such therapeutic measures, a subset of patients persists with both clinical and radiologic disease activity [11]. Thus, there is a pressing need to identify sensitive biomarkers capable of predicting disease progression in MS.

2. What is biomarker

A biomarker is characterized as an objectively quantifiable and assessable trait, functioning as an indicative measure of normal biological processes, pathological deviations, or pharmacological responses to therapeutic interventions [12].

To assess the effectiveness of MS treatment along with clinical parameters, magnetic resonance imaging (MRI) is very important. MRI provides insight into the size, number, age, and progression of CNS lesions, playing a pivotal role in both diagnosis and therapeutic monitoring [13]. MRI monitoring in MS is based on assessing the impact of IFN-β on the pathogenetic links of the disease development. The fact that one of the directions of IFN-β action is the effect on adhesion molecules and matrix metalloproteases, contributing to the decrease in the permeability of the blood-brain barrier (BBB), and preventing the appearance of new foci of demyelination. If IFN-β is ineffective, demyelination processes will progress and be reflected in the MRI picture in the form of new active foci [14].

Within the domain of MS, extant molecular biomarkers predominantly comprise proteins, a majority of which are antibodies [15], obtained from blood or cerebrospinal fluid (CSF). This article introduces clinically relevant and promising biomarkers derived from blood such as antibodies to IFN-β, Myxovirus resistance protein A (MxA), viperin, neurofilaments (NfL), and IL-17 demonstrating their usefulness in the MS prognosis, as well as in evaluating therapy response and potential side effects (Figure **1** and Table 1).

3. Anti-drug Antibody

IFN-β, like other drugs of protein nature (interferon-α, erythropoietin, clotting factor VIII, and human insulin) obtained by gene recombination, can be immunogenic. The therapeutic impact of IFN-β can be significantly compromised by the presence of neutralizing antibodies (NAbs) [32]. The production of antibodies to IFN-β is associated with impaired immune tolerance to its antigens [33–35]. Disturbance of immune tolerance, in turn, is induced when an autoantigen is repeatedly presented to the immune system over several months. The most likely cause of induction of tolerance to IFN-β may be the features of genetic background: HLA-DRB1*04:01, *04:08, *16:01 were identified as genetic markers that are associated with an increased risk of anti-IFN-β antibody development [36].

Among many other reasons for the development of IFN-β immunogenicity, the following factors can be emphasized: patient's condition, dosage and method of drug administration, duration of treatment, and combination with other drugs. Thus, the presence of concomitant diseases can cause

Table 1: Selected markers of response to IFN-β therapy in MS patients.

Name	Function	Reference
NAbs	Disrupt receptor-mediated cellular signaling and transcriptional processes associated with IFN-β-inducible genes, ultimately diminishing the bioactivity of IFN-β.	[9], [16], [17]
NfL	These proteins constitute components of the cytoskeleton, released from damaged axons into cerebrospinal fluid and blood.	[1], [18], [19]
IL-17	Production of proinflammatory cytokines	[20], [21], [22], [23]
MxA	Inhibiting transcriptional and replicative function of viruses	[24], [25], [26]
Viperin (RSAD2)	Antiviral activity against a wide variety of viruses mediates signaling pathways and modulates cellular metabolism by binding to the viral N protein.	[27], [28], [29],[30], [31]

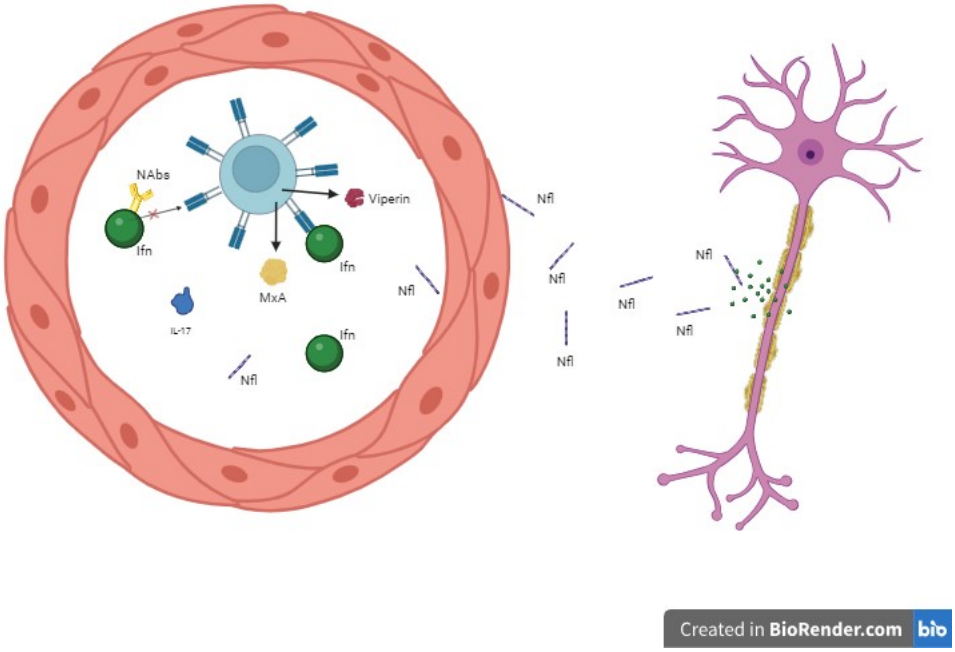

Figure 1: Serologic biomarkers of MS activity (Created with BioRender.com).
Inf– interferon; NAbs - neutralizing antibodies; NfL – neurofilaments; MxA - myxovirus resistance protein A.
Interferon binds to the receptor and activates the expression of antiviral proteins: MxA, and viperin. NAbs can bind to the interferon and block its activity. Axonal damage leads to the expression of NfL. IL-17 is a proinflammatory cytokine.

posttranslational modification of IFN-β molecule, changing its quality in a more or less immunogenic direction. The higher the dose of the drug and longer the treatment, the greater the probability of immune response development [37]; pre-treatment with hydrocortisone can reduce antibody formation [38, 39].

NAbs typically emerge between 9 and 18 months into IFN-β therapy. The incidence and titers of NAbs exhibit variations depending on the specific IFN-β preparation utilized. Generally, the prevalence is around 2–6% for intramuscular IFN-β-1a, 15–30% for subcutaneous IFN-β-1a, and 27–47% for subcutaneous IFN-β-1b. It is noteworthy that individuals who initially test positive for NAbs with low titers may eventually transition to a Nab-negative status over time. Numerous trials have demonstrated that patients who test positive

for antibodies experience elevated relapse rates, increased lesion activity on MRI, and a higher incidence of disease progression. The clinical impact of NAbs has been observed to manifest with a delay following their initial detection, becoming evident only after 12 to 24 months of the treatment [40]. In the case of Glatiramer acetate treatment NAbs are frequent; however, they do not negatively impact treatment efficacy or result in adverse events. On the other hand, NAbs occur in approximately 5% of natalizumab-treated patients within the first 6 months of therapy. Persistent NAbs in this context are linked to both a lack of efficacy and acute infusion-related reactions, warranting consideration for a change in therapy [35].

Some studies have investigated whether gender influences the response to immunomodulatory therapy. Men and women responded equally to interferon therapy in the context of exacerbation frequency, and NAbs reduced the efficacy of IFN-β to the same extent in both sexes [41]. In another study, the response to therapy was significantly higher in men than in women. The most likely explanation for this difference is the effect of sex hormones on the immune system [42].

To identify patients who respond to IFN-β, it is reasonable to use biomarkers that are induced in response to the administration of IFN-β and reflect their activity. According to Comabella et al., low level of interferon-inducible genes in monocytes is associated with poor response to IFN-β therapy [43].

4. Myxovirus resistance protein A

The biological activity of IFN-β can be assessed through the measurement of specific biomarkers that are recognized as downstream elements of IFN-β signaling. MxA is categorized among IFN-β-induced proteins, and the expression of its corresponding gene has consistently proven to be one of the most dependable biomarkers for assessing IFN-β bioactivity. The mRNA levels of MxA experience a significant surge following the initiation of IFN-β treatment [44]. Baseline MxA mRNA levels might serve as a valuable predictor for determining the responsiveness of patients to IFN-β [45]. The interval until the occurrence of the subsequent relapse and progression was significantly prolonged in patients exhibiting MxA induction [46]. The reduction in MxA mRNA is considered a more reliable marker of the efficacy of IFN-β treatment compared to the sole presence of positive results for NAbs. The decrease in MxA mRNA levels may precede the detection of NAbs by several months [47].

A correlation between low spontaneous levels of MxA mRNA and an increased incidence of new T2 lesions was identified. Lower baseline MxA mRNA levels were also linked to a greater occurrence and frequency of relapses during the follow-up period. However, no significant associations were observed between spontaneous MxA mRNA levels and clinical scores (expanded disability status scale, timed-25-foot walk, 9-hole-peg test) [48].

5. Viperin

Viperin (RSAD2) - is an antiviral protein identified in the macrophages of patients undergoing IFN-β therapy [49]. The literature reports its antiviral activity against various viruses, including human immunodeficiency virus, hepatitis C virus, West Nile virus, dengue fever virus, Chikungunya virus, influenza A virus, and cytomegalovirus (40). Monitoring biomarkers such as MxA and viperin contributes to the early detection of NAbs positivity in MS patients receiving IFN-β therapy. Individuals with BAbs+/NAbs+ MS exhibited low levels of MxA and viperin, both at the study's initiation and 6 months, thereafter (41). The baseline

concentrations of viperin were elevated in patients exhibiting no disease activity. However, these baseline values were lower in individuals who did not experience subsequent progression. After 24 months, the levels of viperin were notably higher in those who remained free from relapses in the ensuing years [50]. Thus, a reduced viperin level may be associated with a higher disease activity.

6. Neurofilaments

NfL - structural proteins of axons and dendrites are reliable markers of their damage. There is a theory that neuronal tissue undergoes damage long before the onset of clinical symptoms. This has been substantiated by studies demonstrating elevated levels of NfL, indicating axonal damage well before the diagnosis is established [19]. High concentrations of NfL in CSF are a predictor of progression within 2-3 years. The authors proposed to use the assessment of NfL content in CSF, in clinical trials as a surrogate endpoint to assess the efficacy of therapy: a decrease in the level of NfL in CSF, as evidence of reduced axonal degeneration, would indicate a good response to treatment [51]. Hence, NfL appears to lack utility in diagnosing MS due to the considerable overlap in results between patients and controls, coupled with the absence of a well-defined and accurate cutoff point. Furthermore, its elevation may be observed in other diseases that are part of the MS differential diagnosis, such as neuromyelitis optica spectrum disorder [52]. A recent study has shown worse MRI outcomes, T2LV and BPF were associated with higher sNfL levels [53]. Elevated levels of NfL after treatment discontinuation may serve as a potential means to identify patients susceptible to future MS disease activity [54]. There is a theory that neuronal tissue undergoes damage long before the onset of clinical symptoms. This has been substantiated by studies demonstrating elevated levels of NfL, indicating axonal damage well before the diagnosis is established. Future comparative studies are still imperative to determine whether NfL levels can effectively differentiate between MS and other potentially confounding diseases. However, it is crucial to note that, at present, NfL cannot be regarded as a robust candidate for a biomarker in the MS diagnosis. While the NfL level appears promising as a predictor of future disease activity when considered in conjunction with clinical and radiological data, further refinement is essential to better define its capacity to independently measure disease activity and evaluate prognosis. Studies have shown CSF NfL levels exhibit elevation in patients experiencing clinical relapse compared to those in clinical remission [55]. Other studies have demonstrated an association between NfL levels and progression of disability, as well as the predictive value of initial NfL levels in the transition to secondary progressive MS [56] and in brain atrophy. A subsequent investigation revealed a notable decrease in NfL levels when transitioning from medications with lower efficacy to those with higher efficacy [51]. However, other investigations have concluded that associations with current or future disability are inconsistent, and there is a lack of evidence supporting NfL as a responsive marker for purportedly neuroprotective treatments [57]. More and longer studies are needed examining the evolution and significance of NfL as the disease progresses.

7. IL-17

Research on MS pathogenesis has unveiled a significant role for interleukin-17 (IL-17) in the development of this disease. IL-17, a cytokine

produced in response to inflammation, is considered a key player in the inflammatory processes in MS. Th17 cells exhibit an elevated production of proinflammatory cytokines, predominantly comprising IL-17A, IL-17F, TNF-α, IL-21, IL-22, CCL20, and granulocyte-macrophage colony-stimulating factor. Certain cytokines associated with Th17 lymphocytes cause activation of matrix metalloproteinases, potentially playing a key role in the disruption of the BBB [20]. IL-17 members stimulate the synthesis of local chemokines to attract monocytes and neutrophils to inflammatory sites, which worsens the disease. According to results from a previous study, blood levels of IL-17A and IL-17F were significantly elevated in MS patients compared to healthy individuals and serum levels of IL-17F closely correlated with the frequency of relapses [58].

Several authors believe that the expression levels of interferon-inducible genes in the peripheral blood of MS patients before treatment can serve as a biomarker for assessing the clinical efficacy of therapy [59]. Axtell et al., observed elevated IL-17F in MS patients before the start of interferon therapy, and its high concentrations correlated with poor response to interferon therapy [60]. During a study on experimental animals, a decrease in process activity was observed at low levels of IL-17 [61]. Elevated concentrations of IL-17 were observed in the serum of MS patients when compared to those in healthy controls. Furthermore, male patients with MS exhibited higher IL-17 concentrations compared to their female counterparts [62].

Another investigation demonstrated that the IL-17F concentration on its own does not function as an indicator of the responsiveness to IFN-β-1b treatment in individuals with relapsing-remitting MS [63]. The concept of the role of T cells in the pathogenesis of MS has recently been brought into question with the introduction of monoclonal antibodies targeting CD20 (predominantly present in B cells), which have demonstrated remarkable clinical efficacy in MS. However, B cells engage with their T cell counterparts, contributing to inflammatory cascades. It has become evident that the inflammatory mechanisms in MS display a complex interplay among diverse immune cell subsets, as well as resident cells of the CNS [22]. Thus, the regulation of IL-17 and its impact on various cellular populations play a crucial role in the pathogenesis of MS, making it a potential target for therapeutic intervention in the treatment of this disease.

8. Conclusion

This review provides an overview of the prognostic role of some markers in monitoring MS activity and the effectiveness of MS therapy. More and more evidence shows the validity of biomarkers in identifying MS patients with disease progression, and patient adherence to indications of the urgent need for marker panels. The absence or insufficient amount of data on the reference values of specific markers limits their use in clinical practice. Further studies are needed to identify sets of markers that can provide absolute confirmation of disease activity, as well as the effectiveness of therapy.

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