Biomarker efficacy in multiple sclerosis diagnosis and treatment

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ABSTRACT

The goal in studies concerning biomarkers in autoimmune diseases is finding a marker which fluctuates in correlation with the disease’s severity and settles within normal borders after effective treatment. This marker would later be used as an efficient tool in diagnosis and analysis of medicine clout. It seems the most cogent biomarkers are those measurable in serum or plasma.

MS is a neurological disease common within the young adult population with a predictable course, often leading to life-immersing disability. The prognosis, however difficult and limited, is currently possible via diagnostic tests (brain MRI) or clinical information (severity and degrees of disability). Many studies have been conducted in an effort to detect an adequate biomarker. It is still often overlooked that a reliable biomarker should both be clinically applicable and functional is prognosis; as this is what could lead to timely treatment.

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INTRODUCTION

MS (Multiple Sclerosis) is an autoimmune disorder of unknown etiology which affects the central nervous system. This disease is usually observed in persons aged 20-45 [1-3]. The disease is most prevalent in European countries and possesses average prevalence in Iran. There are four separate phenotypes identified for the disease; clinically isolated syndrome (CIS), relapsing-remitting MS (RPMS), primary progressive MS (PPMS), secondary progressive MS (SPMS) [4]. Different treatment programs are available for decelerating the disease’s progress, disabling each attack and alleviating disturbances; namely the prescription of interferon. However, at this point, there is no definite cure for MS [5]. MS is a complex disorder with different factors impinging its mechanism [6].

We have categorized how biomarkers can be beneficial to the MS patient below:

1) Phenotype and severity diagnosis
2) Determining the course of the disease
3) Choosing a treatment method and predicting its success rate
4) Analyzing new treatment options

Studying biomarkers relevant to MS is heavily supported by MS International Federation and the Autoimmune Disease Committee. Many breakthroughs have been made in the field and the positive effects within the aforementioned cate-
Biomarker Types in Multiple Sclerosis
We have grouped MS biomarkers in seven categories:
1. Biomarkers affecting the immune system
   a. Cytokines and their receptors
   b. Chemokines and their receptors
   c. Antibodies
   d. Biomarkers affecting the complement system
   e. adhesion molecules
   f. molecules affecting cell death
2. Biomarkers damaging the blood-brain barrier
3. Biomarkers damaging myelin
4. Stress oxidative biomarkers
5. Biomarkers damaging axon or nerve cells
6. Biomarkers increasing the production of astrocytes in the brain
7. Biomarkers restoring myelin

Alternatively, we can differentiate between the biomarkers by categorizing them into positive biomarkers and negative biomarkers. Positive biomarkers are either not produced in a physiological environment or produced in small amounts and in particular pathological circumstances. Negative biomarkers are produced in a physiological environment in certain tissues and are targeted at the manifestation of the disease. Namely, oligodendrocytes and myelin belong to this category. The aforementioned biomarkers are ideal for analysis before and after the manifestation or before and after treatments[8].

Immunological markers, markers contributing to the destruction and restoration of axon and also stress markers are ideal as well. We can prescribe more specific medical treatments and assess their performance using these biomarkers. Table 1 expands on the prominence of biomarkers [9].

Considerations and Challenges in the Credibility of the Clinical Use of Biomarkers
The biomarker must apply to a set of basic rules when used in a clinical environment. First, it must be reliably and easily measured through a number of precise test. Secondly, the biomarker must be unambiguous and sensitive to the biology of the disease or its pathogenesis such as its inflammatory properties, its degree of nerve damage and ability to damage or restore myelin.

Biomarker Classification
Biomarkers can be classified based on their ability in prediction, diagnosis and correlation with the disease, as elaborated in as elaborated in Tables 1, 2, and 3.

Diagnostic Biomarkers
A diagnostic biomarker makes distinctions between different autoimmune diseases and differentiates between the healthy and unhealthy patient. This biomarker recognizes the probability of occurrence of a destructive nervous disease such as clinically isolated syndromes (CIS) [10] or radiologically isolated syndromes (RTS) [11]. Ideally, assimilated with the clinical disorder scale and radiological examination, the biomarker can contribute to an increase in sensitivity and appropriation. Most studies conducted belong to this class of biomarkers (Table 1). They are especially helpful in patients exposed to CIS which might later transform into MS.

Biomarkers Related to the Course of the Disease
Biomarkers differentiate between relapsing patients and patients diagnosed with secondary-progressive MS (SPMS).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CIS</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
<th>NMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF OCB</td>
<td>Predicts RRMS</td>
<td>Present in 90%</td>
<td>Less frequent</td>
<td>Less frequent</td>
<td></td>
</tr>
<tr>
<td>CD19+ B cells in CSF</td>
<td>Common</td>
<td>Common</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>CD39+ Treg</td>
<td>Elevated</td>
<td>Decreased</td>
<td>Normal</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>CXCL3</td>
<td>Elevated</td>
<td>Normal</td>
<td>Active</td>
<td>SPMS</td>
<td></td>
</tr>
<tr>
<td>BDNF (CSF)</td>
<td>Predicts RRMS</td>
<td>Increased High</td>
<td>Lower</td>
<td>Low if stable</td>
<td></td>
</tr>
<tr>
<td>Fetuin A</td>
<td>N.A.</td>
<td>Increased</td>
<td>High</td>
<td>Lower</td>
<td></td>
</tr>
<tr>
<td>Neurofilament chains (CSF)</td>
<td>N.A.</td>
<td>Increased</td>
<td>High</td>
<td>Lower</td>
<td></td>
</tr>
<tr>
<td>NAA (CSF)</td>
<td>Predicts NMO in LETM</td>
<td>Absent</td>
<td>Present in 60–80%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D binding protein</td>
<td>N.A.</td>
<td>Increased</td>
<td>High</td>
<td>Low if stable</td>
<td></td>
</tr>
<tr>
<td>Neopterin (urine)</td>
<td>N.A.</td>
<td>Increased</td>
<td>High</td>
<td>Low if stable</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Biomarkers associated with disease stages and clinical phenotypes
In MS patients, these biomarkers contribute to the pathophysiological mechanism of the disease.[12] Hitherto, inflammatory and stress oxidative pointers are expected to play a more significant role in the relapse of the disease. On the other hand, inactivity of the glia, the restoration of the myelin and axon damage point to progressive and therefore, destructive syndromes. These biomarkers can otherwise differentiate between an MS patient with moderate symptoms or severe symptoms (Table 2).

### Treatment Response Biomarkers

Treatment response biomarkers evaluate how effective the treatment for the MS patient has been. They also determine the imminence of treatment failure, making it possible to move on to alternative treatment options beforehand. Kinetic and dynamic medical biomarkers also belong to this class of biomarkers, helping the regulation of the patient’s dosage. Another supposed use of these biomarkers is the appraisal of the patient’s response to different personalized medical treatment. With over ten treatment options available for MS, it is crucial that we find the proper blood-based selection to avoid unnecessary side-effects.

### Side-Effect Scrutiny Biomarkers

These biomarkers are advantageous for patients prone to progressive, pathological or inflammatory diseases. For instance, the antibodies for human polyomavirus II, formerly known as John Cunningham virus, is a biomarker convenient for allocating the danger of progressive multifocal leukoencephalopathy (PML) in patients treated with Natalizumab,[13, 14].

### Advantages and Disadvantages of Biomarker Assessment

MS biomarkers are found in different bodily fluids such as urine, blood, cerebrospinal fluid (CSF) and tears. There are advantages and disadvantages to each sample. MS waste products are seldom inspected, making CSF a fitting choice for pathology. There are different approaches to CSF sampling; I. e. markers soluble in both solutions, like cell aggregations. However, CSF sampling is an invasive procedure and only applicable for a limited number of times.

Blood sampling is a fairly simple procedure. However, there is an abundance of biomarkers in the blood which can be a result of different systematic infections in the body, heme catabolism and kidney waste.

Urine is also another non-invasive sampling procedure. The issue with this method is the chronic infections of the urethra and bacterial colonizations of the bladder, common in most disabled MS patients, which manipulates the results.

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**Table 2. Potential biomarkers of disease activity in MS**

<table>
<thead>
<tr>
<th>Correlate</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapse (+), Disability (+)</td>
<td>Cytokines</td>
</tr>
<tr>
<td>Relapse (-), Treatment Response</td>
<td>TNF-α (serum and CSF)</td>
</tr>
<tr>
<td>Relapse (+), Treatment Response</td>
<td>IL-10 (serum and CSF)</td>
</tr>
<tr>
<td>Relapse (+), Treatment Response</td>
<td>IL-12 (serum and CSF)</td>
</tr>
<tr>
<td>Disease activity (+)</td>
<td>IL-17 (serum and CSF)</td>
</tr>
<tr>
<td>NMO severity (+)</td>
<td>IFN-γ</td>
</tr>
<tr>
<td></td>
<td>Osteoponin (CSF)</td>
</tr>
<tr>
<td></td>
<td>IL-6 (CSF)</td>
</tr>
<tr>
<td></td>
<td>IFN=interferon.</td>
</tr>
<tr>
<td></td>
<td>IL=interleukin.</td>
</tr>
<tr>
<td></td>
<td>TNF=tumor necrosis factor.</td>
</tr>
</tbody>
</table>

**Table 3. Recently discovered biomarkers**

<table>
<thead>
<tr>
<th>K2P5.1</th>
<th>Potassium channel affecting T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2</td>
<td>Chemokine</td>
</tr>
<tr>
<td>CXCL10</td>
<td>Chemokine</td>
</tr>
<tr>
<td>IL-8</td>
<td>Chemokine</td>
</tr>
<tr>
<td>Complement C4 fragment</td>
<td>Marker of complement activation</td>
</tr>
<tr>
<td>MMP-8</td>
<td>Affects inflammatory cell migration through tissue</td>
</tr>
<tr>
<td>CD56bright NK cells</td>
<td>Regulate activated T cells</td>
</tr>
<tr>
<td>25-Hydroxy-vitamin D</td>
<td>Has immunomodulatory effects</td>
</tr>
<tr>
<td>Urinary neopterin</td>
<td>Marker of macrophage activity</td>
</tr>
<tr>
<td>ILT3</td>
<td>Downregulates immune activity</td>
</tr>
<tr>
<td>Myoinositol</td>
<td>MR marker of tissue integrity</td>
</tr>
<tr>
<td>Bri2-23</td>
<td>Neuronal protein</td>
</tr>
<tr>
<td>Fetuin-A</td>
<td>Immunoregulatory protease inhibitor</td>
</tr>
<tr>
<td>Pentosidine</td>
<td>Marker of tissue damage and inflammation</td>
</tr>
<tr>
<td>Leptin receptor</td>
<td>Metabolic and immune pathways</td>
</tr>
<tr>
<td>IL=interleukin.</td>
<td></td>
</tr>
<tr>
<td>ILT=immunoglobulin-like transcript.</td>
<td></td>
</tr>
<tr>
<td>MMP=matrix metalloprotease</td>
<td></td>
</tr>
</tbody>
</table>
Plus, some patients with bladder dysfunction use artificial hypo-hydration for ejection, which restricts examination [15, 16].

**Immune Change Biomarker Feedback Cytokines and Their Receptors**

Cytokines are intensely examined in MS. As a result of inflammation in MS waste, there is an increase in different types of cytokines in MS patients. These changes are not exclusive to MS patients and can be detected in other inflammatory CNS disorders. TH1 inflammatory cytokines such as INF Gamma, tumor necrosis factor alpha (TNF alpha) and interleukin 12 increase in MS relapse phases [16], while anti-inflammatory cytokines like TH2, IL-4, IL-10 and TGF Beta correlate with clinical recovery and RRMS patients [17]. TH17 is a proinflammatory cytokine which stands as the gnomon for the IL-17 cell line, able to strongly affect a wide spectrum of cells, subsequently releasing a couple types of inflammatory mediators, the CXCL10 and CXCL1. GN-CSF chemokines, the IL-8 and IL-6[18]. It is reported that there is a connection between MS patients and the level of active plaques. The IL-17 inflammatory cells produce a range of IFN-λ, which makes them pathogenic for MS and EAE [19, 20]. In fact, the IFN-λ produced by the TH1 cells, increase the interference of leukocyte adhesion molecules with the endothelial cells of the blood-brain barrier, making this region more impenetrable [21]. This cytokine also increases the activity of MHCII and MHCII molecules on the surface of astrocytes and oligodendrocytes, enhancing the reactivity of these cells with immunized cells, probably causing cell death [22]. Furthermore, macrophages and microglia cooperate in the destruction of myelin in MS and EAE. We can basically reaffirm that the IFN-λ kills oligodendrocytes using apoptosis or necrosis (depending on the cell’s maturity) [23]. Hence, most MS treatments focus on the suppression of inflammatory responses; I.E. IFN-λ applied for the inhibition of MHCII for its effects on antigen cell surface and the reduction of T cells, via stopping the production of IL-2 [24]. It has been conducted that MS treatments owe part of their competence to the ability of taming the TH17 cells [25, 26]. Sweeney et al. have concluded that the prescription of Interferon Beta for EAE reduces IL-17 while increasing IL-27 [27].

IL-10 operates as a regulatory cytokine, especially valuable in the limitation of inflammatory responses and tissue damage and adjusts the immune system. Studies have shown that CD4-T cells produce IL-10, making them quite profitable in MS treatment [28, 29].

Tumor Necrosis Factor Alpha (TNFα) is another essential cytokine is modifying the immune system for the better[30]. This cytokine is built by TH1, inflammatory macrophages, natural killing cells, mast cells, eosinophils and neurons themselves [31]. The TNFα counts as a pathogen for EAE and MS [32, 33]. This cytokine breaks the blood-brain barrier, activates the microglia cells and inducts apoptosis cells in MS and EAE [32]. There is a direct correlation between TNFα levels and severity of MS symptoms [33, 34]. Ozensy et al. have demonstrated that there is a higher number of cells producing TNFα in MS patients than the average individual. In another research work by Rentzos et al. in 1996, it has been established the TNFα witnesses higher levels in the cerebrospinal fluid in MS patients.

**Chemokines and Their Receptors**

Chemokines are chemotactic cytokines which summon immune cells to the lymphoid organs and inflammation points. The CCR5 chemokine receptor relates to the relapse of the disease accompanied by symptoms [35, 36]; also CXCR3 increases in T lymphocytes during relapse as the immunohistochemistry autopsy report of some brain parts contains active MS waste. This addresses the existence of CXCR3 in all impenetrable T lymphocytes [35, 37].

In brain samples, the CXCR3+ is abnormal. It has been expressed that the retention of T cells and CXCR3+ in MS patients leads to the appearance of its ligands (IP-10) [38]. In the absence of ligands, the CXCR3 cells go back to their normal cycle. Active inflammatory MS waste containing CCR5+/CCR1 is evidence for hematogen monocytes abundantly found in perivascular cell spaces (crowding point for varied leucocytes) and demyelination borders despite their omission in non-inflammatory brain cuts [39]. Chemokines and their receptors might be important in the heterogenous study of the disease, but more research is in order for certainty.

**Oligoclonal Bands and Antibodies**

Recently, the only biomarker accepted apart from MRI in MS diagnosis are called CSF oligoclonal bands (OCB), which occur as isolated immunoglobulins via isoelectric focusing in 90-95% of MS patients [40]. The persistent and remitting presence of OCB in MS patients is a telltale sign of an immune response to B lymphocytes in the spine [41]. The infusion of the oligoclonal IEF immunoglobulin G (IgG) and immune recognition (antibody) of alkali phosphatase is a more sensitive and specific approach to MS symptoms [42]. This method enables the prediction of a second attack in patients with isolated demyelination syndrome and the plausibility of transformation into Clinically Definite Multiple Sclerosis (CDMS). The oligoclonal IgM is similarly detectable in IEF [43]. IgM is the strongest activator for complementation in demyelination areas in MS and NMO patients [44, 45]. Hitherto, the first reports of IgM do not stand as a strong prophecy on early conversion into CDMS and its invasion period [46-51].

The role of auto-antibody pathology in autoimmune disorders is widely affirmed. Specifically, we highlight MBL and glycoprotein oligodendrocyte (MOG) out of the plausible MS autoantigens responsible for experimental autoimmune encephalomyelitis (EAE) because of their positioning in the dense MBP myelin and the outer surface of the myelin sheath of MOG. Recently, it has been suggested that the MBP-exclusive antibodies and IgM antibodies exclusive to the outer cell domain of MOG present in CIS patients’ sera are extremely inclined to turn into CDMS [52]. Anti-
MOG antibodies are more prevalent in MS patients than the healthy individual. Akin to the axon damage perceived in rats after injection, this antibody causes a boost in demyelination and cytotoxicity in the laboratory environment. It is not yet known whether this peculiar production of antibodies in MS patients is feedback to myelin damage or the cause of it [52-54]. More research is needed to actuate the correct relationship between these antibodies and pathological or clinical parameters [55, 56].

Lately, it has become apparent that the Epstein-Barr virus (EBV) is a possible environmental factor contributing to the development of MS [57]. Although the host range specificity of OCB is not yet fully understood and a clear link between OCB and MS’ pathogenesis, EBV seems to be a target of OCB [58]. In an effort to discover OCB’s specificity range, the layout of 37,000 protein expressions were surveyed. Out of these, two highly amenable epitopes composed of EBV proteins were discovered. In blood samples collected before the manifestation of MS, there were higher aggregations of antibodies for EBNA and EBNA1 complexes than that of healthy people and these aggregations maintained their high numbers after MS [59]. There is generally a higher risk for MS with antibody aggregations. Teenagers and young adults with a history of infectious mononucleosis (a fever-inducing illness caused by EBV) are more exposed to MS than individuals who contract this illness in later years of their lives [60]. Apart from EBV [61, 62], HLA-DR15 which is the main genetic component of MS also induce EBNA1 antibody aggregations [63].

Nueromyelitis Optica (NMO) syndrome, is a heterogeneous MS subgroup consisting of demyelination and inflammation in the spinal and optical nerves. Based on serologic and clinical evidence, it has been concluded that antibody autoimmune responses bear greater numbers in NMO patients [64, 65]. Confirmed immunomodulatory MS treatments are ineffective for NMO patients, making treatment plans for these patients tremendously exclusive. Precise primary diagnosis and a strong invasive interference are crucial in order to avoid an NMO relapse, as this increases mortality rates and disability in MS patients [45, 66].

Aquaporin-4 has been identified as the target antigen of NMO-IgG. Aquaporin-4 acts in the elemental astrocytic processes of the blood-brain barrier and is also the main executioner of the brain’s hemostasis [67, 68]. NMO is found in MS waste in various studies done on different patient populations [69, 70]. NMO-IgG antibodies are the most-recently discovered exclusive biomarkers for MS, first distinguished while investigating demyelination diseases affecting CNS. They greatly aid in differentiating between NMO and MS patients [54, 71].

The YKL-40 biomarker is a secreted glycoprotein, also known as chitinase-3-like protein (CHI3L1), is a gliactivation marker mainly produced via astrocyte reaction [72, 73]. It can be alternatively be produced by activated macrophages, vascular cells, epithelial airways and chondrocytes [73]. The serum volume of YKL-14 increases with the occurrence inflammatory conditions such as rheumatoid arthritis [74]. The physiological duty of YKL-40 has not come to light yet, but there is a theory that it helps in the restoration of inflamed tissues [72].

The CXL13 biomarker is a B lymphocyte chemoattractant, making adjustments in secondary lymphoid organs in both homeostatic and inflammatory contingencies [75]. They are produced by B cell follicles and assist in the formation of the germinal center. Its receptor is CXCR5, since it uses active B cells and CD4 T cell follicles in its job [76-78].

Many studies have shown that the expression of CXCL13 witnesses an increase in CIS, RRMS, SPMS and PPMS patients in comparison to the healthy control group and other neurological patients [79]. This increase is often accompanied by the relapse of EDSS and nerve damage [80, 81]. The CXCL13 is likewise more ubiquitous in RRMS patients than other MS patients [81].

**MicroRNAs**

A microRNA is a noncoding RNA molecule containing no more than 22 nucleotides serving in RNA silencing, post-transcriptional gene expression regulation and protein translation [82, 83]. MicroRNAs play an imperative role in many biological processes such as metabolism, apoptosis and angiogenesis[84, 85]. Almost 1/3 of all human genes utilize the indirect encoding of these molecules [86, 87].

MicroRNA profiles have been investigated at length in MS patients in many samples such as the peripheral blood mononuclear cell (PBMC) [88, 89], blood samples [90] and brain damage [91]; all of which have presented the same result: the microRNA profile is correlated with Multiple Sclerosis [89].

A research work was conducted, calculating microRNA expression rates like miR-21, miR-146a, miR-146b, miR-150 and miR155 in MS patients as opposed to that of the healthy control group. The conclusion was that RRMS patients had more notable expression rates of miR-21, miR146a and miR146b [88].

Another study calibrated the expression rate of miR-326 in PBMC and deduced that the rates correlate with the severity of MS and the symptoms of rats suffering from experimental autoimmune encephalomyelitis (EAE) [6].

In the laboratory, containing the miR-126 resulted in lower numbers of TH17 [92].

The microRNA can be present in many bodily fluids including plasma, serum, urine and saliva [93] and is scrutinized as a possible biomarker in many diseases [94, 95].

A recent study conducted by the Gandhi group has assessed the microRNA-320a to be the most significant altering microRNA in MS patients’ serum. They have also found hsa-miR-27a-3p to be the most significant microRNA connected with MS relapses. The microRNA-19 correlates with EDSS, with miR-199-5p correlating with associated disabilities [96].

**KIR4.1 Antibodies**

A detailed study examining various antibody responses in patients with MS [97] identified IgG1 and IgG3 antibo-
ies that bind to glial cells in the brain tissues. This glia-specific immunoreactivity was not found in the sera of patients who had other neurological disorders. KIR4.1, a potassium channel, was the molecular target of these antibodies. This channel helps in maintaining potassium and balancing water levels in the body. Antibodies moving against KIR4.1 were detected in the serum of 186 out of 397 patients with MS (about 46%), whereas they were present in the sera of less than 1% of patients with other neurological disorders (n=329) making it insignificant, and not observed at all in healthy individuals. This study determined that KIR4.1 is the target of an autoantibody response in some patients with MS [97]. KIR4.1 expressed on astrocytes localizes with another channel, the aquaporin-4 (AQP4) which was previously argued to be the target for NMO. NMO-IgGis present in 73% of patients with NMO, but absent in the serum of patients with MS [64]

**TOB1**

Studies have suggested that the polymorphism of TOB1 is an individual supporting the development of CIS into a clinical diagnosis of MS. The TOB1 gene contains the multiplication of T cells. This gene keeps inclined cells in a non-reactive state and its decrease leads to a stringent immune response [98].

**Osteopontin**

OPN, also known as bone sialoprotein I is a protein encoded by the SPP1 gene, expressed from a crowding of cells and tissues. OPN operates in a range of physiopathological duties, as its seen in the nucleus and cytoplasm of many different types of cells [99, 100].

OPN plays a paramount role in nongene affairs; i.e. monocyte adhesion, phagocytosis and cell migration [101, 102]. It also polarizes TH cells into TH1 and TH2, modifying cytokine expression [103].

OPN engages in the advancement of many autoimmune diseases such as MS [104-107], rheumatoid arthritis [108, 109], psoriasis [110] and fungus infections [111]. In MS patients, OPN expression accompanies MS damage [112, 113]. Many studies have also shown that OPN also correlates with severity and relapse of PPMS

**Microbiota-Related Lipoprotein**

Gut microbiota intervenes with many immunological diseases including MS, inflammatory bowel disease (IBD), type 1 diabetes and rheumatoid arthritis [114-116].

When a potent 16s ribosomal RNA was studied with the purpose of analyzing gut microbiota’s association with MS (n=16) and healthy control groups (n=44), how the RNAs change in the presence of MS was revealed. Methanobrevibacter archaea and Akkermansia genera increase and the Butyricimonas genera decrease. These changes in the gut interfere with the expression of other genes involved in dendritic cell maturity, interferon signaling and NF. Patients in treatment present signs of an increase in Prevotella and Sutterella genera and a decrease in Sarcina genera as opposed to patients not being treated. However, in order to confidently comment on how gut microbiota acts in MS pathogenesis, more studies must be in order [117].

**Complement Biomarkers Profile**

There is no shortage of studies on how the complement system could aid in the pathogenesis of MS and therefore, how various proteins can carry out as an MS biomarker. Their conclusions, however, contradict one another. There is a proteomic analysis conducted, scrutinizing complement-exclusive proteins (I Factor, C3 and Clusterin) and their inconstancies, usually occurring in the CSF samples of MS patients. Lately, the H factor, a complement system regulator, is considered a serum biomarker for detection of the disease’s activities. The H factor’s levels are meaningful in progressive diseases and patients with a higher rate of relapse [118].

**Adhesion Molecules as Blood-BARRIER Disorder Biomarkers**

These adhesion molecules exploring the channels for endothelial cells regulating leukocyte transmigration are pathogens for MS [119, 120]. Adhesion molecules can be found in the form of activated endothelial cell solution, coencymes, platelets, serum and CSF [121]. It has been determined that the plasmatic levels of soluble adhesion molecules (sP-Selectin, sPECAM-1, sE-Selectin) have increased in RRMS patients [122], in comparison to chronic progressive patients. Plus, this increase in relapse signifies these molecules as clinical markers for the activity of MS. The escalation of these intercellular molecules’ levels (sICAM-1) has been perceived in MS patients during relapse. A correlation between sICAM-1 in the cerebrovascular fluid and IgG traces and RRMS patients has been addressed [123]. A surge in circulating vascular cell adhesion molecule-1 (SVCAM-1) leads to a decrease in MS waste in patients in treatment, alternatively linked with IFN-β-1a[124]. The first regulation of SVCAM-1 happening in the first one to six months of SPMS clinically interferes with patients being treated with IFN-β-1b in months 19-24. The same thing happened in IFN-β-1a treatments [125, 126].

VLA-4 is more sensitive to predicting treatment results, as compared with the boost in VCAM-1 and overall increases in each relapse period [126, 127].

MMP9 enzymes work in the destruction of the outer cell matrix and the proteolysis of myelin. The MMP9 enzyme around the arteries breaks collagen and opens the blood-brain barrier. This breakage causes inflammatory cells to spill into the white section of CNS. When T cells are distributed in the brain’s white matter, an immune response happens to almost every molecule in the myelin structure. Migrating B cells are discharged against proteins and lipids present in the myelin shell. T cells usually mark regularly-occurring proteins in the myelin, like MOG, MBP and PLP[24]. T cells also secret TNF-α and LT-α cytokines. These cytokines produce nitric oxide and Osteopontin, employing the macrophages, microglia cells and astrocytes
The NO free radical is an important mediator in autoimmune diseases. NO kills the oligodendrocytes via the microglia. iNOS which catalyzes NO production is found in demyelinated areas of the body. TNF-α and IFN-γ encode iNOS in the astrocytes, microglia and macrophages. The final result of antibodies, the complement system, NO and TNF-α is an intensive damage to the myelin and further stimulation of the macrophages moving to the phagocytosis of larger parts of the myelin shell. Osteopontin output by macrophages and T cells regulate the increasing of T-helper-1 cytokines such as IFN-γ and IL-12 and regulate the reduction of IL-10, a T-helper-2. T-helper-1 cytokines worsen MS symptoms, while T-helper-2 cytokines probably stop platelets from growing [128, 129].

Karabudak et al. conducted a study in 2004, investigating the effects of IFN-β on MMP-9 and TIMP-1 in RRMS patients in a one year period. MMP-9 levels did not seem to present anything meaningful, but TIMP-1 rates increased in the course of the disease in a significant manner [130]. Garcia Montoto et al. studied 50 MS patients in 2010 who were being treated with INF-beta within a two-year period. MMP9 and TIMP1 levels almost doubled in this time. It is thus believed that these two are highly inhabitable for INF-β [131].

Liuzzi et al. discerned a reverse meaningful relation between MMP-9 and its containment endogens, TIMP-1 in RRMS. This finding confirms that the increase of MMP-9 and decrease of TIMP-1 in sera levels of MS patients participate in BBB destruction and CNS invasion by lymphocyte T [132].

**CONCLUSION**

Biomarkers present a wide array of biological information concerning MS symptoms. Their analysis acts as a guiding light when looking for the right treatment and investigating its efficiency, in case the researcher is willing to closely examine it before, during and after treatment. We hope molecular techniques result in more specific and sensitive biomarkers. It is certain that with the engagement of biomarkers signifying the disease’s course and activity, we are able to prescribe more peculiar medical treatment and assess the patient’s response adequately.

**CONFLICTS OF INTEREST**

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

**REFERENCES**


