 doi:10.20944/preprints202009.0293.v1

Potential Pathological Biomarkers in Multiple Sclerosis

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Running Title: Pathological Biomarkers in Multiple Sclerosis

 doi:10.20944/preprints202009.0293.v1

Abstract

Multiple Sclerosis (MS) is a complex disease of the central nervous system (CNS) that involves the intricate interplay of different immune cells going awry leading to inflammation, demyelination, and neurodegeneration. Its diagnosis is quite arduous because of the baffling number of symptoms it elicits and the varied clinical manifestation it presents. The simplified criteria (in form of Macdonald's Criteria) which have got modified several times is now the single most important criteria accepted by neurology bodies for diagnosing MS. Biomarkers from time to time have been explored to simplify the diagnosis and prognosticate MS along with a necessity to monitor treatment outcome. In recent years, research on biomarkers has advanced rapidly due to their ability to be easily and rapidly measured, their specificity, safety, and their ability to yield precise results. Biomarkers are classified into various categories including predictive, diagnostic, prognostic, related to disease activity, and monitoring treatment outcome. Each representative of the disease activity category reflects a variety of pathological processes of MS such as neuroaxonal loss, gliosis, demyelination, disability progression, remyelination, etc. This review discusses several promising serum and cerebrospinal fluid biomarkers and imaging biomarkers used in clinical practice. Myelin oligodendrocyte glycoprotein antibody disease which is recently recognized as a definite disease will also be discussed. Furthermore, it sheds light on the criteria and the challenges a biomarker faces to be considered as a standard one.

Keywords: Biomarkers, Multiple Sclerosis, diagnostic, disease activity, Myelin oligodendrocyte glycoprotein antibody (MOG) diseases

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Background

Multiple Sclerosis (MS) is a non-traumatic, disabling, and unpredictable autoimmune neurodegenerative disease of the central nervous system (CNS) whose etiology is unknown. Its pathological hallmarks include the presence of immune infiltrates (plasma cells and lymphocytes), myelin damage, axonal losing the white matter, and neuronal damage along with inflammation in the grey matter. This eventually leads to permanent functional neurological disability which becomes evident during the progressive stage of the disease. It inflicts more than two million people worldwide and 5-20 per100, 000 population in India [139, 333]. Young individuals aged between 20-40 years are mainly affected, although it can develop in children, teenagers, and elderly people [73]. According to Lublin *et al* (2013), MS is categorized into relapsing and progressive diseases, with activity and/or progression [253]. The progressive form of MS comprises secondary progressive MS (SPMS) and primary progressive MS (PPMS).50% of the patients affected with RRMS are eventually transitioned into SPMS which causes further deterioration of neurological disability [50]. Approximately 15% of the subjects are found to be affected with PPMS in which patients are presented with much less inflammation and result in variable brain lesions [338]. After decades of investigation, the origin of MS is unexplained. Accumulating evidence indicates that the source of MS is influenced by the association of several factors like genetic susceptibility, infectious agents, weak immune system, and environment. These are known to evoke immune cells against their tissue and contribute to disease pathology. Among them, environmental factors allegedly act on genetically susceptible individuals and cause the disease. However, the exact cause as to why immune cells are triggered against their myelin sheath is still elusive and hence there is no approved immunomodulatory therapy that can stop the reoccurrence of MS relapse. Amongst all the subtypes of MS, PPMS has the worst prognosis and thus deserves early and decisive diagnosis to achieve the desired treatment and evaluation and proposition of definitive prognostic and therapeutic biomarkers are the need of the hour [292]. Additionally, most of the patients suffering from SPMS do not respond well to new disease-modifying therapies (DMT) that are used to treat MS [132, 266].

Bruck *et al* (2013) studied the safety of disease-modifying drugs by comprehending their mechanism of action, chemical structure, and targets [10]. Neuromyelitis Optica spectrum disorders (NMOSD) is historically considered to be the Asian form of optic-spinal form of MS. However, treatment with interferon- worsens the NMSOD unlikely that of MS. Similarly CSF

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myelin basic protein level (MBP) is compared with patients of MS but CSF- Glial Fibrillary Acidic Protein (GFAP) levels are higher in NMSOD than MS patients. Furthermore, it was a perception earlier that NMO, which is another autoimmune, and demyelinating CNS disease, was an isoform of MS and is not a distinct entity. However, the pivotal discovery of NMO specific IgG antibodies found specifically in the sera of NMO patients, targeting aquaporin 4 proteins expressed on astrocytes allowed the medical community to differentiate MS from NMO patients.

Extensive research and development of highly sensitive and specific methods used to assess myelin oligodendrocyte glycoprotein antibody (MOG) disease have made it possible to diagnose a group of patients with antibodies to MOG who express a clinical phenotype different from MS or NMO. Therefore, MOG antibody disease is now viewed as a separate entity with a specific treatment. MOG ab is also detected in patients suffering from acquired demyelinating syndrome [50, 122, 157], clinically isolated syndrome, optic neuritis, transverse myelitis, NMO spectrum disorders, and MS [30,310]. MOG is a transmembrane glycoprotein that is expressed on the myelin outer surface and oligodendrocytes [238]. It is used to generate experimental autoimmune encephalomyelitis (EAE), a well-characterized and frequently employed animal model of MS [30].

The clinical and radiological features observed in MOG-ab positive cases seem to be uncommon. Although the importance of MOG-Ab for the diagnosis, medications, and prognosis is not yet proved clinically, the findings linked with these MOG abs obtained from radiological testing are not common for patients and studies. It has been seen that astrocytes of patients diagnosed with NMO are damaged, which are seropositive for immunoglobulin autoantibodies against AQP4. However, patients with MOG disease do not have any effect on their astrocytes. Hence it is apparent that MOG antibody-associated disease is a separate CNS demyelinating disease and does not fall under the NMO spectrum of diseases and MS. Serum biomarkers for MOG-ab associated diseases need to be investigated. Thus research on biomarkers is warranted for a better understanding of biological system functioning, various factors that may develop MS, exacerbation, and treatment effects. This review describes different biomarkers available, and their future use for MS.

The criterion to be considered as a standard biomarker

“Biomarkers” are biological molecules that can be calibrated in biofluids. Their measurement and assessment can assist in examining various processes such as pathophysiologic processes, pharmaceutical reactions, and curative interventions. An ideal biomarker is the one, which is seen

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in MS cases, but not in a healthy population, the concentration of the same is proportionate to the disease activity, and it should be obtained with the safe procedure, cost-effective and can be done easily. The characteristics that a biomarker exhibits to be classified as an ideal are, 1) it should have the ability to differentiate between a patient and a healthy individual, 2) it should express at an early stage before the disease advances, 3) it should be easy to evaluate, safe for patients and enables disease management, and, 4) it should render reproducible results. Although conventional magnetic resonance imaging (MRI) is an important tool to diagnose MS, there are certain pitfalls it possesses. For instance, it is not possible to fully capture the affected areas of the brain using conventional MRI. Although, some unconventional MRI like Diffusion-Weighted Imaging (DWI), Susceptibility Weighted Imaging (SWI), Fluid-Attenuated Inversion Recovery (FLIAR) 3d post-contrast, T1 3D-Double Inversion Recovery (DIR), can capture all the pathological situations. However, availability, as well as cost along with qualified observers to read MRI, are the real limitations as far as the role of MRI in MS is concerned. On the contrary, biomarkers evaluation, which is an affordable test, may assist in diagnosing the disease, understanding disease progression and response to therapeutic regimes. Thus there is an essential need to identify biomarkers clinically and scientifically, hence research on biomarkers now a day is an active area of research.

Interrogations faced and approach used for the development of an ideal biomarker

To be considered as an ideal biomarker, they must accomplish some properties such as they should discriminate between a patient and a healthy individual, easy to assess, should give reproducible results, etc. However, there may be some hurdles that can be faced while establishing them as an ideal biomarker and they need to be overcome. For instance, there are different detection strategies and kits available in the market to detect molecular biomarkers. Even a small variation in different detection methods can result in rendering different results for the same biomarker analyzed and thus can greatly influence the significant value of the biomarker. Therefore, it is essential to employ different detection methods to confirm the validity of the biomarker. Furthermore, significant variation in biomarker identification has been detected in patients when they are analyzed for small samples compared to large samples. These are some of the challenges that are met while establishing them as novel biomarkers. Utmost care should be taken while developing them keeping in mind the robustness of the assay used and performing them on large populations to minimize error.

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Classification of Biomarkers

Biomarkers are classified according to personal susceptibility (genetics, virus serology…), diagnoses, prognosis, and response to treatment, immunopathological pathways, pathologic hallmarks, and therapeutic effect on MS (**Table 1 & 2**). Measurement of biomarkers in biofluids enables the diagnostic tests to provide insights about the early development of the disease and hence its management. The biomarkers are classified into A) predictive (**Figure 1a**), B) diagnostic (**Figure 1b**), C) prognostic (**Figure 1c**), D) disease activity (**Figure 1d**), and E) response to treatment in different disease courses of MS (**Figure 1e**).

1. Biomarkers of Susceptibility (Predictive), Diagnosis and Prognosis

CSF

1.1 Predictive biomarkers

Predictive biomarkers are useful to identify particular individuals who are at risk of developing MS. Individuals which express higher risk of MS symptoms include (a) kids and siblings of MS patients; (b) MS patients who have multiple family members; (c) subjects whose white-matter of the brain shows changes; (d) normal healthy netizens diagnosed with CIS and other disorders which are related to the nervous system. The preferred scientific outcome can be obtained if risk factors are identified that correlate to MS. First degree relatives, Epstein – Barr Virus Reaction (EBV), and Human Herpes Virus (HHV)-6 are good examples of predictive biomarkers (**Figure 1a**) [17].

a. First degree relatives

Although MS is not considered a hereditary disease, however, first-degree relatives are more vulnerable to develop MS than healthy individuals. In Sweden, it has been estimated that the chances of MS recurrence are 17.3% for monozygotic twins and 2.6% for siblings [5]. Song *et al* studied whether there is an association between the risk a family of an MS patient possesses and the age of onset [40]. It was a nationwide study conducted in Sweden and the investigators recruited first-degree (parents, offspring, and siblings) and second-degree relatives (grandparents, grandchildren, uncles/aunts, nephews/nieces, and half-siblings) of MS patients (**Figure 1a**). Their findings revealed that family members of MS patients are at strong risk, however, no significant

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difference related to the age of onset (early vs late) was detected suggesting that chance of MS development is more in family members irrespective of age at which MS manifests.

b. Epstein- Barr Virus (EBV)

EBV is a gammaherpesvirus 4 that affects humans. The infection of this virus is very common. EBV was found to develop mononucleosis which is a contagious virulent illness and it also raises the possibility of MS. Through the oral transmission of saliva and genital secretions, most of the EBV infections are found to have occurred. Detection of antibodies to this neurotrophic virus is suggestive of a polyspecific intrathecal B cell response. It is very rare for the disease to be spread through blood and bodily fluids. The implication of this viral infection in MS patients indicates its existence in B cell which gives a clue about the infiltration of meninges and white matter [118]. EBV contributes to MS pathogenesis indirectly by activating silent human endogenous retrovirus – W [96]. Another investigation reveals that IgG antibodies which are considered as a marker for EBV infection also work against EBV early antigen(anti-EA IgG)These antibodies develop in the sharp phase of early infection and eventually inconspicuous after 3-6 months. After 2-4 months of infection, another antibody called anti-EBV nuclear antigen-1(anti-EBNA-1) is also reported to appear [96]. The antibodies may increase inflammation in MS patients. Cepok *et al* (2005) found that IgG antibodies that work against protein epitopes Epstein-Barr Nuclear Antigen-1(EBNA-1) and BRRFF2 increased in MS sampling [21].

There is considerable evidence to suggest the association between EBV and MS because of similarity in the geographic distribution of occurrence of both, more reported cases of the history of infectious mononucleosis in patients of MS and higher titer of EBV specific antibodies associated with increased risk of MS. Anti-EA IgG antibodies and anti-EBNA-1 antibodies have been reported to be apparent many months before clinical manifestation of MS. The evidence of EBV DNA load in blood and CSF is however conflicting [4].

c. Human herpesvirus type- 6 (HHV-6)

Similar to EBV, HHV-6 is a viral contagious marker which enhances the progression of MS in citizens. T cells are highly infected by HHV-6 where the function of T cells is to intermediate as well as monitoring the MS pathophysiology. The presence of increased titers of antibodies IgM and IgG against this marker indicates virus involvement in MS progression [46, 115]. Research shows that high expressions of HHV-6 are found within oligodendrocytes near MS plaques [25].

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There is definitive evidence until now to suggest a causal relationship with MS. Neurotropism pieces of evidence of HHV-6 have been reported in MS in the form of viral DNA in the brain and CSF [**Figure 1a**). Similarly, concomitant studies reported higher levels of HHV-6 expression in case-control studies and increased expression of viral mRNA and protein expression in oligodendrocytes of MS cases [83, 207, 228].

1.2 Diagnostic Biomarkers

The purpose of diagnostic biomarkers is to confirm that a patient has a particular health disorder. Through these biomarkers, clinicians must be able to discriminate between them and normal people or individuals suffering from another disorder. According to recently modified McDonald MS diagnostic criteria, patients with first clinical events are confirmed to be diagnosed with relapsing-remitting MS with inflammation disseminated in space shown in one MRI and immunoglobulin oligoclonal bands detected in their CSF. The modified criteria have included oligoclonal bands as a replacement for the criteria of dissemination in time. So comparing the MS data published in various studies by applying 2010 vs 2017 MS diagnostic criteria, we find that around 37% of patients were diagnosed with MS in 2010. Strikingly, the number increased to 68% when the 2017 criteria were applied [62]. So, the recent criteria provide a quick and cost-effective approach for MS diagnosis. However, certain limitations should be considered (**Figure 1b**). McDonald's2017 criteria apply to patients who present with a typical clinical syndrome. However, patients with a typical clinical presentation or other inflammatory CNS disorders need MS experts for precise diagnosis.

a. Oligoclonal bands (IgG) and (IgM)

Oligoclonal bands (OCB) are immunoglobulins (Ig) that are produced intrathecally and are deemed as an immunological feature of MS [114, 220]. Intrathecal antibodies are mostly found in MS patients [300]. IgG immunoglobulins are present in CSF of more than 95% of MS patients and are absent in their serum and hence serve as an important criterion for MS diagnosis. Oligoclonal bands of the IgG type (OCGB) is the single most important biomarker attributed to the demyelinating spectrum of disorders. The presence of CSF OCGB and not in serum is suggestive of B cell activity intrathecally. The OCB serves as a diagnostic element for MS due to the presence of a higher level of IgG which is detected in CSF of CIS victims and also it is associated with the development of CDMS. The presence of OCB is instrumental in prognosticating the progression of cases from RIS to CIS and CIS to MS [179, 276]. Sensitivity and specificity of CSF OCGB

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is 88% and 86% respectively [35]. The presence of OCGB in the CSF of patients with Clinically Isolated Syndrome (CIS) makes them vulnerable to transition into Clinically Definite Multiple Sclerosis (CDMS) [2, 315]. Tintore and his colleagues found that higher levels of OCGB in CSF influence an increase in the progression of definitive MS patients [269]. The OCB is useful to predict Optic Neuritis (ON) to MS [176, 236], although they do not inform about the intensity of the second relapse. Several lines of evidence indicate that OCGBs are present in CSF and are specifically sensitive to MS [41]. IgG oligoclonal band is associated with demyelinating lesions. Lines of evidence suggested that OCGB MS subjects exhibit a higher level of inflammatory activity which gives evidence of IL-8, IL-10, IL-6, MMP-2 production that leads to tissue damage [42, 43]. According to Rojas *et al* (2012), IgG+ MS patients possess a higher lesion load [253]. It has been approved that MS patients who are IgG+ MS patients have a higher level of disease activity and also have larger brain atrophy [45]. In another study, it was found that like IgG, the prognostic value of IgM works (OCMB) for increasing conversion from CIS to MS [51]. Thompson *et al* (2017) reported that OCB is a substitute for dissemination in time [49] and is the finest markers for MS diagnosis. Similarly, IgM immunoglobulins have been demonstrated to correlate with MS disease activity by a few investigations [149]. OCMB is found in CIS patients which is correlated with brain atrophy, lesion load, and a chemokine named CXCL13 which increased a higher level in CSF that influences the migration of B cells [50, 337]. Several lines of evidence indicate that the NFL is also associated with IgM in CSF, and Retinal Nerve Fiber Layer (RNFL) thinning demonstrates IgM to be co-related with loss of axons in the CNS [50, 116]. According to Villar 22% PPMS subjects who are IgM+ experience more gadolinium-enhancing lesions. The deposition of this immunoglobulin also influences inflammatory activity [48].

b. Immunoglobulin G index (IgG index)

Active plasma B cell produces IgG antibodies and releases them in the CNS. Among all patients with MS, 90% display intrathecal Ig synthesis. The OCBs of IgG and IgM of MS patients indicate clonal extension of B cells and plasma cells in the CNS. The relative amount of IgG in the CSF compared to serum is evaluated by the CSF IgG index. IgG index is the ratio of the CSF IgG to the CSF albumin ratio as compared to the serum IgG to serum albumin ratio [264]. The albumin quotient, albumin in CSF/albumin in serum, indicates disruption of BBB integrity in MS [339]. The patient is said to be suffering from MS when the IgG index value exceeds 0.7 and it signifies an increased synthesis of intrathecal IgG antibodies in the CNS. It has been well documented that

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the IgG index serves as an important biomarker for MS diagnosis and it is routinely determined during the diagnostic procedure.

c. Measles, Rubella, Varicella-zoster Reaction (MRZ)

The presence of antibodies against measles, rubella, and varicella-zoster viruses is a sign of intrathecal B cell response. This response is called "MRZ reaction" which is found mostly in 80% of MS patients [58, 123]. MRZ reaction is used as a marker to detect MS sufferers. Brettschneider *et al* (2009) found that more than two T2-hyperintense lesions in MRI and positive MRZR are observed in 50% of total CIS sufferers and those have a high tendency to get transformed into definitive MS. Thus it is confirmed that B-cells are activated and intrathecal IgG synthesis takes place at the initial progression of MS [57]. Hottenrott *et al* (2018) and his colleagues showed that to distinguish MS from other inflammatory neurological diseases, MRZR could be used as a biomarker because MRZR is found less frequently in other inflammatory diseases [217]. It is not well-known as to why some MS patients don't experience MRZR and others do.

d. Transducer Of ERBB2.1 (TOB1)

TOB1 belongs to the anti-proliferative group of proteins which regulates the development of cell cycle. It is capable of identifying the individuals who are suffering from CIS and are at high risk to develop MS. TOB1 plays a role as a transcription repressor which inhibits T cells from proliferating and this inhibition also decreases the T cell receptor activation [70]. Prevention of TOB1 which is present at higher levels in CD+4 lymphocytes causes to increase CD3response, while overexpression of this marker in T cell gives direction to stop the cell division [70]. Patients suffering from CIS who express decreased TOB1 can inhibit T cell activation whose reaction is to stimulate CD+4 T-cells that migrate in the CNS lesions. This migration occurs because of acytokine named fractalkine (CX3CL1) where they establish inflammatory effects in the target tissue. Thus deficiency of TOB1 facilitates the conversion of CIS patients to MS. Corvol *et al* (2008) reveals that this analysis is detected in patients with CIS [258].

e. Imaging Biomarkers

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is the single most important tool to diagnose the causes of MS because of its distinctive pattern of involvement and resolution of lesions are associated with therapeutic outcomes and measures the worsening too. Distinctive features are also enumerated in

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the literature about the various other components of demyelinating diseases such as CIS, NMO, and MS. MRI scanners assist in taking out pictures of body structures by using a magnetic field, magnetic field gradient, and radio waves. If white matter lesions are found on MRI then it indicates the development of MS from CIS. An MS MRI protocol includes (a) T1 weighted (b) T2 weighted (c) postcontrast scans. T1weighted lesions are used to detect any abnormality in the BBB integrity. Hypointense T1 lesions (also known as black holes) are used as a marker which represents the loss of axons. MRI is classified into conventional and non-conventional techniques (**Figure 2; Table 3, 4 & 5**).

Conventional techniques -

i. T2- weighted MR imaging

MS lesions are typically seen in T2 weighted MRI. The diagnostic significance of hyperintense T2 weighted lesions is high and it measures multiple mechanisms like axon loss, demyelination, and inflammation and edema. Through this method, white (WM) and gray matter (GM) lesions can be identified. WM and GM lesions specify cortical and focal demyelination, and also possess axonal loss. Thus it is an important imaging biomarker for neurodegeneration [62].

ii. Proton density-weighted spin-echo images

Long repetition time (TR) and shorter echo form PD- weighted images. Eco sequences are earlier used in the form of a fast spin-echo (FSE). To get PD and T2 weighted images dual and multi-echo sequences can also be used. According to Chong in 2016, PD-FSE exposes larger lesions which are found in the form of numerous smaller lesions by T2- FSE image. Additionally, 32% extra cervical cord lesions are identified through this procedure than T2- FSE images which give more information about neurodegeneration [210]. Many studies occurred about PD-W and found that this process is capable to give higher quality of anatomical structures by defining them and also individuate between lesions and perivascular spaces. Christoph also figures out periventricular MS lesions by this method [64]. PVL is part of the revised McDonald MS diagnostic criteria which data helps in clinical practice.

iii. T1weighted imaging with gadolinium enhancement

T1weighted lesions are used to detect BBB dysfunction.T1 weighted images also give data about neurodegeneration by analysis of black holes and atrophy. Black holes describe axonal damage

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and tissue destruction while atrophy exposes axonal loss which is believed to happen through tissue damage within lesions. The number and volume of T1 black holes show better co-relation with MS disability and also proposed as a potential biomarker for neurodegeneration [229, 252]. Gd a paramagnetic pass through BBB damage. Gd enhancing MRI is useful to analyze active inflammation [31, 36]. Gd contrast will be detectable within the brain parenchyma as an area of abnormal enhancement.

iv.Fluid attenuated inversion recovery (FLAIR) sequence

Due to the presence of excessive water and high spin density in MS lesions, sometimes it is not possible to get a better figure of parenchymal changes. At this moment FLAIR sequence puts down the liquid signals while MS lesions manage their hyperintense signals for which explanation between the lesion and CSF increases as a result intraparenchymal changes can be detectable [64]. Cortical and juxtacortical lesions are identified through this method [68]. Commonly MS lesions are found in the periventricular region which is also detected by using the T2- FLAIR sequence [64].

Non-conventional techniques

i. The magnetization of transfer imaging (MTI)

MTI imaging is one type of MRI technique that is applied to expose interaction between protons that are present in 3 states (a) bound to macromolecules (b) in free water (c) as water in the hydration layer between macromolecules and free water. MTI detects lesions defining them and also differentiate to know about severity. Various pathological changes which are occurred during MS are also disclosed through this method [68]. Calculations of MTR is obtained by MT image and without MT image.

$$MTR = (S0 – SMT) / S0$$

Where S0 = signals without off resonance pulse

SMT = signals achieved after MT pulse

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MTR is a sensitive method through which MS pathogenesis of white matter lesions and myelinated white matter can be evaluated. Therefore decrease of MTR value is co-related with both demyelination and axonal loss. Changes in MTR of cerebral white matter is occurred because of changes in myelin contents. MTR decreases with acute demyelination and increases with remyelination, thus it works as a remyelinating agent [67, 69]. MTR can provide information regarding the degree of optic nerve demyelination and correlates with axonal loss that is relevant with visual and paraclinical outcomes after optic neuritis [72]. MTR value of optic nerve decreases after optic neuritis is co-related with the thickness of RNFL.

ii. DWI and DTI

DWI is another mode of MRI scan which is established upon the Brownian motion of H2O. DWI technology is unable to measure the volume of tissue loss in MS lesions and can't reveal distinct modifications that occurred in NAWM. It is a method through which pathology and anatomy of white matter are studied. DWI makes an allowance for analyzing the apparent diffusion coefficient (ADC) of water in the brain and if there are any disturbances in cellular structure and white matter tracts then ADC increases [73]. It also distinguishes between a variety of pathologies such as ischemia, infection, tumor, etc. through the calculation of the microscopic motion of water molecules. ADC also exposes the weirdness of diffusion in MS plaques [101]. This process is specific for ischemia stroke but its character as a marker for MS is not well established.

DTI is a refinement method that evaluates motion in multiple directions in the space. DTI characterizes the three –dimensional diffusion of water. DTI has four parameters such as (a) Axial Diffusivity (b) Radial Diffusivity, (c) Mean Diffusivity (d)Fractional Anisotropy [28]. Axial diffusivity demonstrates about axon loss while Radial diffusivity is associated with the status of myelin layer and Mean Diffusivity is average diffusion. DTI uses the FA process that reveals the global direction of water diffusion and it is found increasing in white matter tracts and decreases in CSF and disorganized fibers [75]. T1 and T2 weighted MRI can't give enough information regarding MS pathogenesis like DTI. In the early phases of MS, the value of AD found decreases in NAWM whereas AD value increases in the later phases [185]. A decrease in Fractional Anisotropy is repeatedly observed and found in both regions of the local lesion area and NAWM of MS patients. The presence of higher Mean Diffusivity (MD) and lower FA are observed in T2 weighted lesions. DTI measures evaluated as disease biomarkers. The MD establishes as a predictive biomarker for MS relapse because of changes in MD can be visible before BBB

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disruption at least before five months [328]. AD uses as a potential biomarker for Axonal degeneration and RD for demyelination [10, 130, 223]. DTI technology can describe the changes in cellular and microstructural levels and could be reasoned for this process is increasing.

iii. Magnetic Resonance Spectroscopy (MRS)

MRS is also called Nuclear Magnetic Resonance Spectroscopy (NMRS) which is non-invasive and applies ionizing radiation for measurement of cellular metabolism in the CNS. MRS can describe biochemicals such as N-acetylaspartate, choline, creatine, glutamate, glutamine, etc. which are present in the CNS in a non-invasive way. These biochemicals work as surrogate markers for MS pathogenesis. Lowering the value of N-acetylaspartate is considered as a marker for neuronal/axon loss while choline represents heightened cell membrane turn over which is seen in demyelination, gliosis, etc. Glutamate is used as a biomarker for acute inflammation. Reduced level of N-methyl aspartate gives a signal of decreasing edema in lesions. GABA decreases in the SPMS [81, 325].

iii. Optical Coherence Tomography (OCT)

The OCT is a non-invasive form of technology that allows us to take cross-sectional images of the retina by using low coherence light. This technique allows measuring the thickness of the distinctive layers of the retina. Generally, Optic Neuritis is considered a common syndrome of MS which affects eyes. Maximum ON victim's lesions is found in their optical pathways. Composition of retinal ganglion cells form inner optical pathways, whose somas are found in the ganglion cell layer (GCL) and axons form the RNFL. However, OCT measures the thickness of RNFL. OCT is utilized to take out pictures of axons and its unmyelinated axonal layers of the retina. RNFL reflects neurodegeneration and edema of MS patients. The lower value of RNFL is considered a marker that represents axon loss and is associated with cerebral atrophy [83, 107].

iv. Positron Emission Tomography (PET)

PET scan is a picture model utilized to describe the activities within the tissues and body at the cellular level by using the radioactive process. A PET scan is an effective way to examine the chemical activities in the body. Heterogeneity of the MS lesions and changes in inflammation of normal-appearing white matter (NAWM) and GM can be imaged through PET. The focal point of this technique is to take pictures of the innate immune system of the brain i.e. microglia and macrophages. Activated microglia secretes oxygen species and inflammatory cytokines in

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response to neural injury which causes inflammation. Thus PET scan indicates to imaging neurodegeneration and neuroinflammation of MS patients [212, 308].

1.3 Biomarkers for prognosis

Prognostic biomarkers are biological features that yield insights on the possible outcome of a patient's health (**Figure 1c**).

a. Chitinase-3-Like-1(CHI3L1)

CHI3L1 also called YKL-40, a glycoprotein coded by the CHI3L1 gene. YKL-40 is produced from different cells like macrophages, monocytes, microglia, vascular smooth cells, astrocytes, and chondrocytes. The function of CHI3L1 in CNS is poorly understood. Several lines of evidence have indicated that the expression of CHI3L1 is increased in inflammatory conditions which indicates that it might be associated with the modulation of immune responses. This marker is observed in astroglia, white matter plaques, NAWM, and also lesions of MS brain [87]. Canto and his colleagues found that a higher level of CHI3L1 values is co-related with the rapid development of future disability. During the progression of MS, the concentration of CSF CH13L1 increases. Therefore it is used as a biomarker for the transformation of CIS to CDMS [332]. It has been found that the concentration of CHI3L1 increases in RRMS and SPMS compared to healthy individuals [135]. In a recent study, researchers found that higher levels of CSF CHI3L1 are found in T1 and T2 lesions and brain parenchymal fraction. Thus it is suggested that CHI3L1 indicates inflammation-based damage [135, 281].

b. NEUROFILAMENTS - A promising Biomarker of prognosis, disease activity, tissue damage, and monitoring treatment response

Neurofilaments (NF) belong to type IV intermediate filaments whose role is to provide cytoskeleton to the neurons. In CNS, they are abundantly present in the cytoplasm of neurons and comprises of 4 sub filaments which have different molecular weights: (a) NF-L (light chain of polypeptides) of 68Kda (b) NF-M (mid-sized neurofilament) of 150Kda (c) NF-H (heavy chain) of 190 to 210 kDa and α-internexin [154]. After axonal damage, these filaments are secreted in CSF or blood. Therefore analysis of NFs indicates the loss of axons in the CNS [120]. Each subunit is encoded by a separate gene. All three filaments are double-stranded and have an extremely conserved alpha-helical core region which is lined by amino-terminal at the head region and carboxy-terminal at the tail region [245]. Their main function is to provide a platform, maintain

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shape, and size of the axons [251]. Each disorder which is associated with neuronal and axonal damage shows higher levels of NFs in CSF [64]. NF-L works as a prognostic factor that gives information about the transformation of CIS to RRMS [96, 247]. The higher level of CSF NF-L is considered as a predictive marker for disease severity, progression to SPMS [98] and patients experience disability and cognitive impairment after converting to CDMS [20, 88]. In another investigation, Gunnaarsson *et al* (2011) found that NF-L is associated with CNS damage so it can be used as a biomarker for neurodegeneration [99]. RRMS and SPMS patients display a high level of NF-H. NF-H is used as a prognostic marker to detect MS development and future disability [33, 41, 245]. Recently, Kuhle *et al* (2019) demonstrated NF-L as the marker of tissue damage and disease activity in RRMS patients suggesting it serve as a prognostic biomarker [104]. Thus above data suggest that NF is a good candidate marker for prognosis.

Serum

1.1 Predictive Biomarkers

a. Corona Virus (COVID-19)

In another investigation, it has been found that MS patients also respond to one more virus named “Corona”. Coronavirus also has known as SARS CoV2 belongs to the family coronaviridae. This viral infection is responsible for Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS) and also causes gastrointestinal illness. According to previous literature, the region where these viral activities take place in the MS patient's brain gives evidence that may be coronavirus as MS pathogen through its neurotropism and immune system attack activities [107, 169, 311].

b. Anti- Myelin Antibodies

Oligodendrocytes are the CNS myelin-forming cells that contribute towards cytoplasmic processes to the formation of multiple internodes of central myelin and thus get utmost importance in MS studies. Injury of oligodendrocytes leads to loss of plurality of internodes. Various animal studies have established the role of oligodendrocyte damage with autoantibodies and suggested these autoantibodies as potential biomarkers. The presence of autoantibodies against myelin proteins such as MOG, and MBP in patients with CIS is investigated as predictive biomarkers. The presence of anti-myelin antibodies in the serum reflects an autoimmune attack against CNS myelin. Anti-myelin antibodies are detected in MS lesions. Berger et.al (2003) found antibodies

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against MBP in CIS patients who developed MS [239]. Researchers found that the presence of anti-MBP antibodies in childhood increases the risk of demyelinating encephalomyelitis [102]. Controversially, Kuhle *et al* in 2007 found that there was no connection between anti- MOG and anti-MBP and conversion of CIS to MS [226]. Recent studies revealed that MS patients having positive anti-MOG antibodies express a higher risk of developing MS, and the rate of relapse [111]. MOG which induces EAE both inflammatory and demyelinating components elicits a T-cell mediated immune response and a B cell-mediated demyelinating antibody response [112, 167].

1.2 Diagnostic Biomarkers

a. Kappa Free (KFLC) and Lambda Free Light Chains (LFLC)

KFLC, a biomarker is utilized to detect MS patients. Plasma cells produce KFLC and LFLC during antibody synthesis and these are detected both in serum and CSF [274]. Presslauer *et al* (2008) found that the maximum MS patients having a high amount of KFLC [274]. Rinker *et al* (2006) demonstrate the correlation between the presence of an increasing amount of KFLC and future disabilities in MS patients [268]. Additionally, Villar *et al* (2012) also found that CSF KFLC increases in CIS patients which leads to the conversion of CIS to CDMS. Thus it maybe works as a progressive marker [48]. ɣFLC utilized as a predictive biomarker for immunoglobulins which are produced intrathecally found in inflammatory CNS disorders [118].

b. Anti-Aquaporin-4 antibodies

Aquaporin-4 or AQP4 is expressed on astrocytes of the CNS and functions to provide water transportation as well as maintain homeostatic balance within the CNS. MS patients lack the expression of this protein while 38-75% of the Neuromyelitis Optica (NMO) patients display AQP4 antibodies [205]. NMO is a rare disease in which the body's immune system reacts against myelin that surrounds the optic nerve and spinal cord. Differentiation between NMO and MS is challenging because of both display similar clinical features. NMO presents autoantibodies called NMO specific-IgG which recognizes AQP4 that allows water to move through the cell membrane and bind with it. Thus immunoreactivity of this marker helps to differentiate between NMO and MS and also enhances to determine other immune-related disorders that affect CNS [65, 172].

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c. Antinuclear antibodies (ANA)

Antinuclear antibodies are the autoantibodies against antigens in the cell nucleus. Various proteins or multiprotein complexes are attached to these antibodies inside the nucleus [203]. This marker is observed in different diseases like MS, systemic lupus erythematosus (SLE), etc. The presence of autoantibodies in the patient's blood serum can be analyzed by the ANA test through Indirect Immunofluorescence and Enzyme-Linked Immunosorbent Assay (ELISA) [137]. Becker *et al* (2017) found that SLE is highly associated with anti-double-stranded DNA antibodies works against DNA and is only confirmed by ANA positive test [3]. Thus the illustration of anti-dsDNA antibodies is a specific test for differential diagnosis. Specifically, these antibodies are found at active phases of diseases and also work as an effective marker SLE. Maximum autoimmune disorder patients show positive ANA tests, in that case, the anti-dsDNA test identifies SLE from other impaired immune system disorders possess similar signs and symptoms. This test is used to monitor lupus nephritis, a serious variant of lupus that causes kidney inflammation, which may lead to kidney failure. The other autoimmune disorders like antiphospholipid antibody syndrome, tuberculosis, osteomyelitis, thymoma, lymphoma, etc. are also diagnosed through this specified analysis [126 128, 216]. Susac Syndrome (SS), is another peculiar type of autoimmune disorder obstructs small arteries and capillaries of the brain, retina, and inner ear causing visible disturbances in the brain, eye, and ear functions. Multiple studies suggest that the presence of anti-endothelial cell antibodies (AECA) in SS patients reflect injury in endothelium lining inner walls of blood vessels [232]. Susac and his team suggested that AECA play a crucial role in the pathogenesis of SS [134, 282]. Accumulating evidence indicates that AECA is not a specific marker for SS but SS can be diagnosed through the AECA test. Magro *et al* in 2011 also demonstrated that AECA is an agent which can lead to tissue injury [195].\

1.3 Prognostic Biomarkers

a. miRNAs

miRNAs, noncoding RNA molecules, can be detected both in plasma and CSF. Its stability is more than RNAs. miRNAs can be measured through a different process such as quantitative PCR, miRNAs array analysis, small non-coding RNA cloning, etc. Many studies occurred upon miRNAs and found that increasing or decreasing the level of miRNA in MS is related to the conversion of CIS to MS [132]. For example, a higher level of miRNA -922 found in CIS which is associated

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with the transformation of CIS to RRMS [133]. In another study, Bergmann (2016) found that miRNA- 150 in plasma also works as a prognostic marker which helps to convert CIS to MS [266].

b. Human Leucocyte Antigen (HLA)

The human leucocyte antigen is another type of protein and is composed of major histocompatibility complex (MHC) in humans. It regulates the immune system of a human being. This marker serves as a prognostic marker for MS. According to recent research MS patients, possess both HLA- DRB115 and OCB display faster MS development [92]. HLA-DRB1*1501 and HLA-DQB1*0301 positive MS subjects show worst brain atrophy and also possess T1 and T2 lesions thus progression of disease increases [224]. Lysandropoulous in 2019 revealed that HLA-A*02 is associated with MS progression [139].

2. Biomarkers of treatment

a. Interferon- β (IFN-β)

Interferon- β proteins are produced from fibroblast cells which exerts its therapeutic effect on MS. Interferon- β prohibits the proliferation of T lymphocytes and transfers the inflammatory Th1 type to Th2 form which decreases the production of proinflammatory cytokines [104]. IFN-β reduces gadolinium-enhancing lesions by decreasing the entry of inflammatory cells across BBB that leads to an increase in nerve growth factor production, thus increase in neuronal survival and repair [191]. It also minimizes the relapse rate and disability progression in RRMS patients, improving the condition of subjects [3, 199]. The antiviral activity of IFN-β is stimulated by the production of several proteins like myxovirus resistance protein A (MxA) which have anti-viral, anti-proliferative, and immunological properties [73, 141]. The biological activity of MS patients is estimated by measuring MxA which also gives information about IFN-β. The high level of MxA is found in stable MS patients and also indicates sufficient IFN-β in blood. If an MS patient contains low MxA, it signifies less amount of IFN-β which leads to change in therapy [143]. Casanova *et al* (2018) performed a multicenter study to investigate the association of the presence of oligoclonal M bands with the effectiveness of IFNβ and glatiramer acetate (GA) in the CSF of RRMS patients. They recruited two hundred and fifty-six RRMS patients and treated them with IFNβ or GA. Their findings revealed that more than 50% of patients remained free from further attacks who were kept on GA therapy than the ones undergoing IFNβ treatment. The outcome of the IFNβ therapy was found to be the poorest in patients presented with oligoclonal IgM+ bands

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in their CSF compared to the ones without oligoclonal IgM bands suggesting oligoclonal M bands in CSF may serve as a biomarker of treatment response in MS [194].

b. Natalizumab

Natalizuamb, a monoclonal antibody is used for curing MS. Natalizuamb blocks αintegrin-mediated leukocyte-endothelial interaction and reduces transmission of leukocytes from blood to CNS, and finally reduces the formation of lesions. Christensen *et al* (2014) demonstrated that treating progressive MS patients with natalizuamb therapy reduces inflammation in CSF, axonal damage, and demyelination, thus ameliorating the patient's health [163]. Sometimes, natalizumab interacts with other immune-modulating drugs which results in increasing the risk of progressive multifocal leukoencephalopathy [146].

c. Cladribine

Cladribine inhibits certain types of cells like B and T cells which attack myelin wrapped around the nerves of the brain and spinal cord, thereby stopping the migration of these cells to enter CNS [332, 226]. It is already approved that Cladribine treatment is very effective for the treatment of RRMS patients as it reduces relapse rate by 54.5% annually [102, 206] and the effect of this therapy remains up to 4 years [271]. A plethora of studies have been conducted in the past which describe the effect of cladribine on MS subjects and it has been found that it significantly decreases the relapse rate, disease activities, and risk of disability progression [193].

d. Glatiramer Acetate (GA)

Glatiramer Acetate (GA), an immunomodulatory drug that is composed of alanine, glutamic acid, lysine, and tyrosine is used for the treatment of MS. Studies have revealed that GA treated MS patients indicate less production of inflammatory biomarkers including TNF-α, IL-12, etc. [281]. GA prohibits monocyte reactivity, and in response to that, the body develops its anti-inflammatory type II monocytes which eventually inhibits the myelin antigens [151]. MS patients treated with GA therapy are shown to display a reduction in the activity of MS lesions, a decrease in disability progression, and relapse rate [36, 152, 160]. All these were summarized in **Table 6**.

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3. Biomarkers of Immunological dysfunction

CSF

a. Lipocalin-2

Lipocalin-2 is a type of protein stored in the specific granules of neutrophils and also transports small hydrophobic molecules. It works as an indicator that gives a signal for different mechanisms of the immune system. Khalil et.al (2016) showed that during relapse conditions the level of LCN2 genes are increased in the spinal cord and choroid plexus of the EAE model of MS. LCN2 was expressed by astrocytes and infiltrating neutrophils. Additionally, they also found higher levels of CSF- lipocalin 2 in two different MS cohorts [156]. Berard *et al* (2012) suggests that compared to RRMS lipocalin-2 concentration increases in PPMS which causes various disease activities like demyelination, disorder severity, and proliferation of T- cells in MS patients [157]. Nimer *et al* (2016) found a higher level of NFL found in all phases of MS especially during relapse condition is associated with lipocalin-2 which concentration increases in progressive diseases.

b. Osteopontin

Osteopontin (OPN) is a phosphoprotein which contributes inflammation by producing IL- 12, IL-17, IFN-γ and inhibits IL-10 expression. Chabas *et al* (2016) found increasing amounts of OPN MS brain lesions [158]. In another study Braitch *et al* (2008) found that during relapse condition MS patients showed a higher level of osteopontin whether found in CSF or serum [159]. Different studies have found that osteopontin can be utilized as a progressive biomarker for the development 629 of MS [307].

c. Cytokines

MS patients display inflammatory reactions because of demyelination which releases numerous cytokines and chemokines. CXCL13, a chemokine while interacting with the CXCR5 receptor results in the activation of B and T helper cells towards demyelination lesions. According to khademi higher level of CXCL13 is associated with the conversion of CIS to MS [162]. CSF CXCL8 another type of chemokine which differentiates between MS patients and controls. IL-6, a cytokine which utilizes as an intermediator between T cell and B cells and also having Th-17 responses. The prevalence relapse rate of MS patients is correlated with IL-6 levels in serum [259]. Mouzaki *et al* (2015) found that through cytokines it is easily accessible to separate MS sufferers

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from other inflammatory CNS disorders [90]. Kim *et al* (2012) showed that any imbalance in the IL-1 signaling whether it is increasing or decreasing leads to CNS demyelination [90].

Serum

a. Cytokines

Cytokines are small proteins produced by cells that help in interaction and communications within cells. These markers are glycoproteins that are unable to pass the lipid bilayer of cells for entering the cytoplasm. Cytokines have various forms like lymphokines produced from lymphocytes, monokines originated from monocytes, chemokines appeared through chemotaxis activities and finally interleukins that arise by leucocytes. This marker is generated through a variety of immune cells such as macrophages, B lymphocytes, T lymphocytes, mastocytes, fibrocytes, endothelial cells, and different stromal cells. Cytokines are important for cell signaling including autocrine, aracrine, and endocrine signaling as immunomodulating agents. During demyelination, a huge amount of cytokines are released which works as a biomarker for MS disease activity [224]. Cytokines like TNF-α, IL-10, and IFN-γ, etc. are active players and which are products from different T helper cells. These cytokines are found increasing amounts in MS patients and also represent the pathological process of MS. Intrathecal cytokines with monocytes are produced by B cells reflecting extra immune responses in CSF of RRMS [102]. Ozenci *et al* (2000) found that higher levels of serum TNF-α are found in maximum MS patients [166]. In other studies, researchers showed an increasing amount of TNF-α is also associated progression of MS disease [167].

b. Vascular Endothelial Growth Factor A (VEGF-A)

The vascular endothelial growth factor is an endothelial cell-specific growth factor that stimulates angiogenesis and exhibits neuroprotective properties. The major role of VEGF-A is to stimulate neurons, axons, and macrophage migration. This marker also plays a specific role in different inflammatory disorders by enhancing angiogenesis and vascular permeability. The presence of VEGF in the CNS increases neuron survival and facilitates neurogenesis in a variety of neurodegenerative disorders like MS. Graumann *et al* (2003) found that VEGF-A level increases in serum of MS patients [167]. In another investigation, Iacobaeus *et al* (2011) showed that SPMS patients possess a lower level of VEGF as compared to RRMS patients. This data suggests that VEGF-A may be used as a biomarker for transitioning of RRMS to SPMS [168].

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c. Vitamin-D

Vitamin-D is a neuroprotective agent by activating several neurotrophins and suppressing the immunity of Th-1 in different ways [224]. Several epidemiological studies have revealed that vitamin-D deficiency may increase the risk of MS [75, 171]. MS patients not undergoing treatment possess hydroxyvitamin-D and are inversely associated with radiologic disease activities [312]. In another study, researchers found that the level of vitamin-D is decreased in MS patients in comparison to healthy individuals [153, 306]. Martinelli et *al (*2014) demonstrated that deficiency of vitamin-D in CIS patients enhances the possibility of developing clinically definite MS [306]. Multiple studies have shown the response of vitamin –D on disease activity and demonstrated that the presence of a higher amount of vitamin-D may decrease the chances of relapse rate in subjects [162, 278]. Findings of Mowry *et al* (2016) revealed that vitamin D intake decreases neurodegeneration after CIS and also lowers MS patient's disability [177].

d. B- Cell

B-cells play a vital role in the pathogenesis of MS. B-cells produce antibodies, cytokines that serve as inflammatory mediators and biochemical messengers [179]. Kuenz *et al* (2008) revealed that an increased level of mature B –Cells in the CSF of RRMS patients is co-related with higher disease activity [179]. IgG antibodies an OCB that are produced intrathecally are found in maximum MS patients. The B cells produce oligoclonal bands when Ig transcription of B cell and immunoglobulin proteome overlaps with each other in CSF [44]. Earlier studies demonstrated that MS patients lacking oligoclonal bands have lower immunity which reflects reduced disabilities and risk of converting to SPMS [112]. Joseph *et al* (2009) found that higher CSF- OCB positive MS patients are correlated with disease activity and future disabilities [153]. OCBs are also helpful to evaluate further episodes and progression of MS [185, 324].

e. T-Cell

T-cells enter inside the CNS through the CXCR3 cytokine receptor. This receptor has a weak specificity value for MS due to the presence of a higher level of this receptor in various other inflammatory disorders [213]. CD8 T Cells are expressed at the margin line of MS lesions while CD4 T Cells are recognized at deep lesions of MS patients. Loss of myelin, axonal, and oligodendroglia destruction occurs in MS patients because of these cells [188]. Several lines of evidence indicate that CD4 T lymphocytes stimulate microglia for which it generates several

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cytokines such as IL-4, IL-6, IL-12, and IL-1β [136, 309]. During a relapse, MS patients display a higher level of T lymphocytes [78].

3. Biomarkers of pathology

CSF

1.1 Biomarkers for disease activity

Disease activity has been defined as the occurrence of new neurological symptoms and the recurrence of a past condition. It is measured by the clinicians in terms of a score that predicts whether the symptoms have abridged or stopped and if medication needs to be continued or altered (**Figure 1d**).

a. Demyelination

The MBP is a polypeptide producing myelin sheath around nerves in the CNS. It is the major component surrounding myelin sheath and is attached with cytoplasmic surfaces of cell membranes to maintain the stability of the sheath [178]. Myelin sheath functions like an electrical insulator that provides more accelerated active transmission of the nerve impulse [193]. Positively charged MBP actions are like lipid coupler and they work by interacting with lipids through which myelin layers are closely connected. MBP works as a demyelinating biomarker. MBP levels increase in CSF of MS patients in acute demyelination. However, they are not considered as prognostic biomarkers [18, 164]. This is because the concentration of MBP in CSF displays remyelination of demyelinating lesions but it is such that remyelinating cells may not be demyelinated in the future [190, 197]. Literature reveals that a higher level of MBP was found in the CSF of MS patients [29]. Sellebjerg *et al* (1998) found that MBP levels increased to a great extent during the relapse of MS patients [201].

i. αB-Crystalline

AlphaB-Crystalline is a member of the protein family in humans which is coded through the CRYAB gene [129]. It functions as a molecular chaperon which binds with misfolded protein to avoid apoptosis and protein aggregation. This protein is highly expressed in stress conditions and causes a variety of neurological disorders like MS [201, 202, 203]. The higher level of AlphaB–crystalline of oligodendrocytes and astroglia demyelinated lesions are found in various phases of MS [108, 159]. By stimulating the P38 pathway, CRYAB can be phosphorylated at serine residue which is secreted from astrocytes in demyelinating tissue and supports reactive astrogliosis.

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Therefore this data implies that astrogliosis is essential in primary demyelination [270]. Various cytokines and chemokines such as IL-17, IL-10, IL-13, CCL5, and CCL1 are released by the actions of the CRYAB gene hence it is used as a target molecule for MS [256].

b. Biomarkers of Blood-Brain Barrier (BBB) Dysfunction

The BBB disruption is an important pathological hallmark of MS. BBB has a complex structure composed of endothelial cells comprising of capillary wall astrocytes end-feet enclosing cerebral capillaries and pericytes which are found encapsulated in the basement membrane. BBB disruption is an indication of its permeability through which inflammatory cells enter into the CNS and lesion formation occurs. MS is a cell-mediated autoimmune disorder in which both lymphocytes and leucocytes cross the BBB that causes demyelination and axonal loss and eventually leads to progressive disability. MS biomarkers are used for BBB disruption that helps to monitor disease activity and to understand the MS process. They can also help to guide response to MS therapies [119].

i. Soluble Intercellular Adhesion Molecule sICAM-1

sICAM-1 is called as CD54, a polypeptide that in humans is encoded by the ICAM-1 gene. A small amount of this marker gene is found in the membranes of leukocytes and endothelial cells. Lymphocytes and monocytes enter inside the CNS by the mechanism of adhesion molecules like ICAM-1 and VCAM-1 which are found on the surface of both lymphocytes and endothelial cells including Selectins, integrins, etc. The presence of these adhesion molecules is essential in capturing of leucocytes by L-selectin. After that leucocytes enter into the CNS which influences inflammatory cytokines to be produced thus this data indicates the first features of MS [207]. MS patients show the variable value of sICAM-1. It may be normal [127] or elevated [210]. Dore-Duffy *et al* (1995) found that PPMS patients display a higher concentration of ICAM-1 as compared to RRMS patients [323].

ii. Endothelin System

Endothelins are peptides consisting of 21- amino acids with receptors found in the endothelium. Endothelins play an important role in diseases which are related to the vascular system of various body parts including heart, lungs, brain, etc. and also constricts blood vessel which influence to raise blood pressure [109, 211, 335]. This biomarker has 3 basic elements including Endothelin-1 (ET-1), Endothelin type –B Receptor, and Endothelin Converting Enzyme-1 (ECE-1). Cerebral hypoperfusion and tissue damage of MS patients are detected through the-1 biomarker. These are

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found in most of the patients with MS from the beginning to later stages of diseases. Cerebral hypofusion causes selective damages such as focal lesions, degeneration of axons, cognitive impairment, and fatigue in MS and shows a higher value of CSF ET-1 [214, 216]. The enhanced level of ET-1 in the cerebral circulation may change CBF circulation [77, 215]. In another study, Haufschild et.al found that higher levels of ET-1 in plasma works as biomarkers for Optic Neuritis which is nearly a clue of MS [299]. Hostenbach *et al* (2016) showed that the presence of a higher value of ET-1 which is produced by astroglia subscribe to MS pathogenesis by disruption of BBB and also activates a large number of cytokines, chemokines, etc which allows having more inflammatory responses in MS patients [217]. Thus ET-1 also acts as an inflammatory biomarker.

iii. EBV Infection

Casiraghi *et al* (2011) showed that EBV infection causes BBB damage which allows migration of T cells inside the brain forming lesions in the brain parenchyma [219]. Thus this evidence suggesting EBV antibodies also work as inflammatory biomarkers in MS patients.

c. Biomarkers for axonal damage

Loss of axons, primitive sequence of MS for more than a century. Since 1990 Researchers and scientists have been investigated the disruption of axons in MS patients for exploring the axonal pathology and neurodegeneration due to which subjects suffer from permanent neurological disability [183, 221, 303]. Recently, one study has been found that axonal damage develops at the initial phases of MS [183]. The pathology of axonal loss is still a controversial issue. Axonal damage of MS is a direct attack of inflammatory mediators like CD+8T cells or autoreactive antibodies which could damage axons by invading macrophages, proteolytic enzymes, cytokines, etc. [182, 223, 255]. Because of the damages, there will be an irregularity in calcium homeostasis [229] and glutamate-mediated excitotoxicity occurs which influences to recruit more axonal energy [102, 228]. Another possible mechanism is due to chronic demyelination. The biomarkers for axon loss provide important data like (a) predict more axonal-protective compounds (b) supervise disorder conditions and remedy reactions etc.

i. Neurofilaments (NF)

The phosphorylation of neurofilament influences axonal diameter [81]. It has been shown that MS sufferers display a higher level of NF-H for axonal degeneration especially in progressive MS diseases [232, 250]. Higher levels of NF-H are also found in CIS and RRMS patients [15]. It is co-

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related with the relapse activity of CIS and RRMS patients [230]. NF-L dispersed initially from the parenchyma into CSF due to its low molecular mass or minor phosphorylation rate [129]. Increased levels of this marker are found in MS or CIS patients [93, 235]. During acute relapse or higher relapse rate of MS patients, CSF NF-L levels are increased which enhanced to form lesions. While conversion from RRMS to SPMS higher level of NF-L is also reported [96].

ii. Tau

The tau proteins are a group of six soluble isoforms produced by alternative splicing from the MAPT gene. The major purpose of tau is to keep the stability of axonal microtubules that are essential for axon transport. Tau proteins are associated with microtubules found in neurons of the CNS. Tau used as a biomarker for axonal loss in MS patients [28]. Due to abnormal phosphorylation of tau microtubule instability develops for which neurotoxic insoluble tau produced. The neurotoxic tau results in the formation of common neurodegenerative diseases like MS [175, 236]. After neuronal injury tau protein released to serum or CSF [238]. Anderson et al 2010 suggest that SPMS and PPMS patients show abnormal phosphorylation of tau protein and insoluble tau also leads to disease progression [114, 239]. MS patients possess high levels of Tau protein and elevated levels of tau and NF-L are expressed in CIS victims which values indicate the transformation of CIS to CDMS [15, 114].

iii. 14-3-3 Proteins

4-3-3 proteins are family of highly conserved molecules that are expressed high level in all eukaryotic cells [241] and binds with the number of functionally active molecules that are involved in a mass of cell differentiation, proliferation, and transformation active molecules. Generally, these markers are observed in CSF of MS or CIS patients [242] Colucci *et al* found that an increased level of 14-3-3 levels works as an indicator of short –term conversion to CDMS [174, 189, 243]. 14-3-3 proteins present in MS patients can presume a higher relapse rate as well as patients with EDSS patients [189]. In another study, Satoh showed that 14-3-3 proteins are associated with severe neurological disability and also with disease progression [22].

d. Biomarkers of Glial Activation/ Dysfunction

i. GFAP

GFAP, a class III intermediate filament (IF) protein that is coded via the GFAP gene which is found in various cell types including astroglia and ependyma of CNS. GFAP plays an important

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role in functioning neuro-glia interactions, cell communication, and regulating the BBB mechanism. This marker also plays an essential role in the repairing process after CNS injury. GFAP proteins increased in MS plaques which are related to a high level of astrocyte damage [28, 323]. In another study Researchers' demonstrates that increased levels of GFAP in CSF are found during SPMS than RRMS patients which indicate neurological dysfunction and future disability progression In MS [225]. MS patients with a major disability have an increasing amount o f GFAP in CSF as compared to patients with minor disabilities [251]. During NMO relapse subject has a large amount of CSF-GFAP levels in comparison with MS relapse [12, 320]. Above this data suggesting that GFAP utilizes as a marker to detect the development of MS which indicates a high rate of astrogliosis.

ii. S-100b

S-100b, a glial specific which belongs to a group of S-100 protein found in is astroglia, a small subset of Oligodendroglia and a certain subgroup of neurons. The main function of this protein is to promoting neuronal proliferation, oligodendrocyte differentiation, stimulation of calcium fluxes, maintaining astrocyte morphology, and facilitating astrocyte and microglial activation that occurs intracellularly. Extracellularly, at a lower level of this marker applied to develop neurite outgrowth, the survival of neurons during progression and supports the capacity of chemotaxis neuroglia. During MS relapse, higher CSF-GFAP levels are found and this situation continues over 5 weeks. Petzold *et al* (2002) showed that an increasing amount of S100B levels are found in all MS subgroups which indicate cerebral injury [235].

e. Biomarker for Remyelination

i. Cilliary Neurotrophic Factor (CNTF)

CNTF is a peptide expressed in humans which is coded by the CNTF gene whose actions promote neurotransmitter synthesis, remyelination, and neurite outgrowth. CNTF promotes cholinergic and astrocytic differentiation and enhances the survival of sensorimotor, preganglionic sympathetic, and hippocampal neurons [219, 316]. CNTF is an essential aspect that works for the existence of oligodendrocytes. The responsibility of CNTF is to defend oligodendrocytes from different dead signs as well as work like mediators which helps to maturate OPC into myelin-forming cells and finally supports differentiated oligodendroglia for producing myelin [248, 255]. In MS patients CNTF levels increased which is correlated with disease severity.

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ii. Nerve Growth Factor (NGF)

NGF, a neurotrophin and neuropeptide which participates in activities like development, maintenance, expansion, and existence of sympathetic and embryonic sensory neurons. In MS patients, inflammatory cytokines TNFα, IL-1 to 6, IFNγ, etc. are released from active CD+4 T cells which are co-related with MS disease activity and BBB damage. NGF supervises the collaboration of TNF-α with TNF receptors for which mechanism of TNF-α is affected [7]. Presence of lower concentration of NGF in MS victims which causes inflammatory TNF-α to stimulate its apoptotic effects through collaborating with TNFR1 [258]. The presence of an increased level of NGF in MS patients indicates remyelination via TNFα interact with TNFR2. In addition increased levels also stimulate remyelination by Schwann cells [258, 267]. Higher levels of CSF-NGF are found in MS patients [304].

Serum

a. Biomarkers of BBB Dysfunction

i. Matrix Metalloproteinase Proteins (MMPs)

MMP represent as calcium-dependent zinc-containing endo-peptidase and is a member of proteases family called as metzicin superfamily [262]. MMPs have a primary role in different developmental (reproduction, the progression of the placenta), biological process (morphogenesis), and various neurological disorders like MS. Higher level of MMPs are found in blood and CSF at the time of relapse condition of MS patients [143]. During an acute attack of MS, elevated levels of T- lymphocytes express Gelatinase B (MMPs-9) which disrupts tight junction proteins occluding and claudin -5 and causes BBB permeability [264].

ii. Ninjurin-1

Ninjurin-1 is one type of polypeptide found in humans which is coded by the NINJ1 gene [227]. After nerve damage this marker increases in both dorsal root ganglion neurons and Schwann cells. It demonstrates proteins homophilic adhesion property which promotes axonal growth. Ninjurin-1 works as an intermediator to connect between macrophages and vascular endothelial cells through homophilic interactions [266] and helps myeloid cells to enter through the BBB in EAE [168]. The mechanism of Ninjurin-1 and APC plays an important role in the transmigration and localization of the APCs in the CNS. A higher level of Ninjurin-1 is present in demyelinating lesions of MS patients while a lower level of Ninjurin-1 decrease MS activities [96, 224].

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Biomarkers for axonal damage

N-AcetylAspartate (NAA)

N- AcetylAspartate is an acid derivative of aspartic acid and is detected in neurons, oligodendrocytes, and myelin of CNS [208]. There are several roles performed by NAA including (a) removing water from neurons (b) as a source of acetate for the synthesis of lipid and myelin (c) energy source of mitochondria (d) precursor for the synthesis of N-acetyl aspartylglutamate [76]. Studies have revealed that reduced levels of NAA are found in MS lesions, surrounding NAWM, and cortical gray matter [59]. Disease progression in MS and disability are associated with a low level of NAA [28]. Other studies have shown that a decreased level of NAA is present in CIS patient's gray matter and some white matter lesions [80, 260]. Narayanan *et al* (2001) found that MS patients who are treated with Interferon-beta 1b for one year display a higher concentration of NAA [272]. Through NAA it is easy to distinguish between MS and NMO patients.

ii. Amyloid-Precursor Protein (APP)

APP is an essential protein found in several tissues and its deficiency may affect the development of synapse in neurons. It plays an important role in controlling the structure and function of a synapse and is transported by fast axonal transport [141]. Proteolysis of APP generates β-amyloid protein (Aβ) protein which is detected in Alzheimer's disease (AD). Although the accumulation of Aβ protein is mainly observed in AD, recent studies have indicated that there is a link between Aβ and MS as well. Increasing evidence suggests that myelin damage results in APP proteolytic processing, due to the action of myelin protein. In injured axons, the cytoskeleton causes interruption of axoplasmic flow possibly due to calcium influx [276, 285]. Changes in axoplasmic flow lead to deposition of APP in axons which indicate failure of axonal transport. Mattson *et al* found that the level of α-Sapp and β-Sapp in CSF of MS patients decreases as compared to health individuals [276]. MS patients who possess APP positive axons are associated with lesion development [141]. It is used as a potential biomarker for MS disease progression. APP is not only found induced on reactive glial cells during demyelination but is also found to be expressed uring remyelination. Clarner *et al* (2011) demonstrated that astrocytes are the originator of APP during demyelination [78]. Findings by Gehrmann *et al* (1995) revealed that a high concentration of APP is detectable in MS patients as compared to healthy individuals [273].

iii. Neuron-Specific Enolase (NSE)

NSE is also called as enolase 2 (ENO2), an enzyme found in humans encoded by ENO2 gene. It is an established neuronal marker for all neuroendocrine or perineuronal cells. Three isozymes of

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enolase, expressed by different genes: enolase α is ubiquitous, enolase β is muscle –specific and enolase γ is neuron-specific. NSE is used as a sensitive biomarker for disease progression [39, 139]. Maier *et al* (2008) found that the level of NSE in spinal fluid and serum decreases in CIS patients compared to the healthy individuals for which neuronal activity is reduced at the initial phases of MS. In another study, Forooghian showed that MS sufferers possess higher T-cell responses which work against NSE found in the peripheral blood mononuclear cells [281]. The levels of NSE are commonly found normal in MS patients [304].

iv. Ionic Imbalance

In myelinated axons, sodium channels are bound at the nodes of Ranvier whereas in demyelinated position the distribution of these channels increases all along the axons for which demyelinated axons require ATP. This demand for ATP results in Na+/K+ ATPase failure due to mitochondrial dysfunction which is caused because of higher expression of cytokines, nitric oxides, etc. from MS patients. This enzyme failure collapse ionic gradients for which NA+ ion increases intracellularly. This situation triggers reverse operation Na+ - Ca2+ exchanger that results in higher calcium level within the axoplasm and leads to axonal damage and mitochondrial dysfunction [104, 188, 295]. According to recent research about the impact of metabolic disturbances on MS patients, it hasbeen found that G+/M+ RRMS possess the lower amount of energy to repair axonal injury because of its more aggressive and inflammatory clinical activity as compared to G+/M- RRMS. Oligodendrocyte progenitor cells (OPCs) found in G+/M+ RRMS subjects decrease their capacity for repairing demyelinated regions which leads to poor prognosis in contrast to G+/M- RRMS [85, 284]. Therefore it is concluded that sufficient energy is required for repairing nerve damage and Na+/K+ ATPase is also an essential enzyme to keep an equal level of ions. The enzymatic activity and distribution of Na+/K+ ATPase were found decreased or invisible in MS sufferers [188, 205, 290].

c. Biomarker for Glial activation

i. Nitric Oxide (NO)

Nitric oxide is a molecule that possesses unpaired electrons and also principal oxide of Nitrogen. NO is a signaling molecule in many physiological and pathological processes like MS. The levels of NO increase in CSF and serum of MS sufferers as compared to non-inflammatory neurological disorders [289, 292]. It possesses a deleterious effect on mitochondria by inhibiting cytochromeC oxidase for which mitochondrial energy production affected [197]. NO enhance apoptosis through its extremely toxic effects on neurons, glial cells and also can extending BBB permeability through

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which inflammatory cells enter into the CNS. Sellebjerg et.al demonstrates Nitric Oxide degradation products NO(X) which is seen in large amounts in clinically definite MS patients but not in CIS patients. NO degradation products NO(X) are present in MS patients and are specially related to the destruction of mitochondria, hypoxia in various tissues, and lengthy relapsing condition which causes further damage in MS lesions [225].

ii. Reactive Oxygen Species (ROS)

ROS are oxygen-containing chemical species. Production of this marker is stimulated by different biochemical reactions occurring within cells and also some organelles such as mitochondria, peroxisomes, and endoplasmic reticulum. ROS disrupts oligodendrocytes and myelin through radical-mediated oxidation. Increased levels of ROS are generated in MS pathogenesis which overpowers antioxidant capacity that leads to oxidative stress. As compared to healthy individuals MS sufferers possess higher production of ROS in mononuclear cells due to protein kinase C [297]. Production of this marker in patients induces to generate superoxide and peroxynitrite like higher toxic products which are very noxious for glial and neuronal cells. Dutta *et al* (2006) found that mitochondrial modification and its –derived ROS are responsible for the degeneration of axons in MS patients [283] and also higher levels of CSF- 7-ketocholesterol (breaks down from myelin 966 cholesterol) found in the patients [242].

d. Biomarker for Remyelination

i. Neuronal Cell Adhesion Molecule (N-CAM)

NCAM, a glycoprotein also known as CD56, found on the surface of neurons, glial cells, and voluntary muscles. N-CAM plays an important role in cell-cell interaction, neurite outgrowth, synaptic plasticity, and learning and memory. The higher level of CSF-NCAM are found immediately after relapse and it is associated with an improvement of neurological symptom and also with remyelination [298]. Chipman *et al* (2010) demonstrate that NCAM is involved in contact-mediated axon-glial signaling influencing the survival and outgrowth of oligodendrocytes following contact with the axon during myelination [298]. According to another investigation, Muller (1994) suggests that these proteins are also observed on the surface of regrowing axons [297].

ii. Brain-Derived Neurotrophic Factor (BDNF)

BDNF, a polypeptide that is coded via the BDNF gene and found in humans. The main function of BDNF to play an essential role in the growth and survival of neurons, remyelination, and neuroplasticity which is important for learning and memory. Sarchellis *et al* (2002) reported SPMS patients to have a lower level of BDNF as compared to RRMS patients. The above data suggesting

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reduced levels of BDNF are associated with demyelination and axonal damage progress [298]. In other studies, researchers showed that BDNF in MS lesions is produced from active immune cells thus BDNF receptors are also localized in MS tissue [301, 329]. The level of this marker increases and found in GA – definite T cells that are co-related with clinical improvement [301].

Myelin oligodendrocyte protein (MOG)

MOG, an element of compact myelin is present on the surface of the myelin sheath and 990 oligodendrocyte. It can work as a marker for oligodendrocyte maturation and maintaining the 991 myelin sheath [238]. MOG is found in all ages, and gender but specifically observed at younger ages. Based on clinical manifestation physicians have stated that MOG-abis found higher in younger ages (children) [204, 291] as compared adults [99, 105]. Studies have shown that maximum MS cases exhibit a coalition of MOG antibody with myelin loss and its co-factors like NAA receptor encephalitis, herpes simplex virus, Borrelia, and Epstein virus infections [1270, 298, 302, 326]. Additionally, this antibody has a major connection with optic neuritis than AQP4 NMO. According to Sato (2014), in a Brazilian and Japanese cohort, approximately 72.7% ON cases are MOG+ while24.0% of cases are AQP4+ ON [84]. While another group, Ramanathan *et al* (2014) also found that MOG positive patients are maximum [310]. As per recent research study, most of the MOG+ ON patients display higher average RNFL thickness [315] in contrast to AQP4+ patients; however, on the contrary, diverging facts are also available thus requiring more research on this matter. Biotti (2017) suggested that during MRI of the optic nerve in MOG+ ON patients, the presentation is oedematous, and demonstrates excessive inflammatory lesions as compared to AQP4 + NMOSDrelated ON patients [52]. When comparing with AQP4+ disease, MOG-AD disease possesses more thalamic and pontine lesions [34]. Cobo-Calvo (2018) also reported that MOG+ sufferers possess a lower risk of relapse in comparison to AQP4+ subjects [315]. In addition to this bilateral thalamic and cerebellar peduncle, lesions are found in younger ages with MOG+. This antibody is observed at a higher level in pediatrics which increasing the further risk of relapse while comparing with adults. It has been also approved that maximum MOG-subjects display T2 hyperintense lesions in the thoracic or cervical region of the matters are correlated with this antibody. In addition, Bauman *et al* (2014) have been found that MOG+ children along with ADEM characterized by less intense feelings and possess behavior problems as compared to MOG-patients [13]. It is also revealed that few children along with ADEM having a high concentration of MOG antibodies which decreases quickly to a lower level after the first attack. Di Pauli (2011) stated that these antibodies are observed at a lower level in totally cured of ADEM [317].

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Neuromyelitis optica (NMO)

NMO-IgG/AQP4-AB, a serum marker that is observed mostly in NMO subjects and not in MS patients. These antibodies are found 91% specific and 100% sensitive in NMO disease [26]. It is also approved that this antibody has an association with disease activity. AQP4- AB+ patients possess interrupted BBB, defective glutamate homeostasis, and initiates necrosis. The presence of NMO-IgG in patients can forecast the relapse condition or transformation of NMO [154]. Fluctuating the value of NMO-AB across BBB and different parts of CNS is very much sensitive to Optic tracts [322]. That's why ON patients with AQP4- AB display visual problems [14] and also develop recurrent cases that convert to NMO [150]. As compared to MOG-AB+ subjects, NMO-IgG+ ON patients recover slowly from a visional problem [47]. The commonly chiasmal syndrome is found in the inAQP4-AB+ disease group. It is already shown that these antibodies level will be high in case of full sightless, capacious myelitis and also in cerebral lesions. Again Takahashi (2007) reported that spinal lesions are also associated with these antibodies [320]. Based on scattering NMO-IgG, NMO disease lesions are found exclusively in periventricular and periaqueductal areas and myelitis observed in the gray matter of CNS. According to Jarius (2013), NMO-IgG is also appeared in LETM (longitudinal extensive transverse myelitis) positive patients, brain stem encephalitis as well as diencephalitis subjects [55]. He also reported that the appearance of this antibody with LETM, ON, and brain stem encephalitis also helps to transform to definite NMO. AQP4-AB found in maximum relapsing conditions at a higher level.

 doi:10.20944/preprints202009.0293.v1

Conclusions

The dearth of knowledge regarding the etiology of MS and variation in clinical subtypes makes it implausible to establish one single biomarker that will give assurance of disease evaluation in MS. Since 2009, several investigations have been carried out to identify potential biomarkers and the ones yielding significant information related to treatment response is an evolving area of research. Although progress has been made in the search for an individual biomarker, MS is still a mystery and many unanswered questions persist. To date, there is not one single biomarker that can provide insights about prognostic indication, distinguishing different clinical courses of MS, and esponse to treatment. Due to several challenges that a biomarker faces including lack of sensitivity and reproducibility, it becomes difficult to confirm the reliability of these identified biomarkers. However, continued advancement in biomarker research has resulted in the identification of biomarkers specifically during the early stage of the disease. Although FDA approved immunomodulatory drugs have the potential to reduce the reoccurrence of relapses, however, drugs that can stop progression and disability in primary progressive patients need to be discovered. Therefore, it is necessary to identify validated biomarker panels that are capable of predicting and monitoring the efficiency of the growing number of available treatment strategies.

 doi:10.20944/preprints202009.0293.v1

Abbreviations

AECA, Anti-Endothelial Cell Antibodies; ANA, Antinuclear Antibodies; APP, Amyloid-Precursor Protein;AQP, Aquaporin; BDNF, Brain-Derived Neurotrophic Factor;CBF, Cerebral Blood Flow; CCL, C-C-Motif – Ligand; CHI3L1,Chitinase-3-Like-1; CIS, Clinically Isolated Syndrome; CDMS, Clinically Definite Multiple Sclerosis; CNS, Central Nervous System; CSF, Cerebro Spinal Fluid; CTNF, Ciliary Neurotrophic Factor; DIR, Double Inversion Recovery; DMT, Disease-Modifying Therapies; DWI, Diffusion-Weighted Imaging; EBNA1, Epstein-Barr Nuclear Antigen-1; EBR, Epstein –Barr Virus; EDSS, Expanded Disability Status; EAE, Experimental Autoimmune Encephalomyelitis Scale; ET, Endothelins; FLAIR, Fluid-Attenuated Inversion Recovery; GA, GlatiramerAcetat**e;** GABA, -Amino Butyric Acid; GCL, Ganglion Cell Layer; GFAP, Glial Fibrillary Acidic Protein; GM, Grey Matter; HHV6, Human Herpes Virus 6; HLA, Human Leucocyte Antigen; Ig, Immunoglobulin; IL, Interleukin; IFN- , Interferon- β; KFLC, Kappa Free Light Chain; LFLC, Lambda Free Light Chain; MBP, Myelin Basic Protein; MAPT, Microtubule Associated Protein Tau; MERS, Middle East Respiratory Syndrome; MOG, Myelin Oligodendrocyte Glycoprotein; MMP, Matrix Metalloproteinase Proteins; MRI, Magnetic Resonance Imaging; MRS, Magnetic Resonance Spectroscopy, MRZ, Measles, Rubella, Varicella-Zoster; MS, Multiple Sclerosis (MS); NAA, N-AcetylAspartate; NAWM, Normal-Appearing White Matter; N-CAM, Neuronal Cell Adhesion Molecule**;**NF, Neurofilament; NGF, Nerve Growth Factor; NMO, Neuromyelitis Optica; NOSD, Neuromyelitis Optica spectrum disorders; NMRS, Nuclear Magnetic Resonance Spectroscopy; NSE, Neuron-Specific Enolase**;** OCB, Oligoclonal bands; ON, Optic Neuritis; OPN, Osteopontin; OCT, Optical Coherence Tomography; PET, Positron Emission Tomography; RNFL; *Retinal Nerve Fiber Layer*, TNFR, PPMS, Primary Progressive MS; ROS, Reactive Oxygen Species; RRMS, Relapsing Remitting MS; SARS, Severe Acute Respiratory Syndrome; sICAM, Soluble Intercellular Adhesion Molecule; SLE, Systemic Lupus Erythematosus; SPMS, Secondary Progressive MS; SWI, Susceptibility Weighted Imaging; TNFR1,*TNFα–TNF* receptor-1; Tumor Necrosis Factor Receptor; VCAM-1, Vascular Cell Adhesion Molecule-1; VEGF-A, VascularEndothelialGrowthFactorA;

 doi:10.20944/preprints202009.0293.v1

Declaration

Ethics approval and consent to participate: Not Applicable

Consent for publication*:* Not Applicable

Availability of supporting data: Not applicable

Competing interests: The authors declare that they have no competing interests

Funding: Not Applicable.

Authors' contributions: All authors read and approved the final manuscript. DM was involved in providing concept, writing and editing the manuscript. SR was involved in writing the manuscript, BKM was involved in providing concept and editing the manuscript; GLR was involved in providing concept in manuscript preparation; JV was involved in giving support in manuscript preparation, RK was involved in editing the manuscript and BC was involved in providing concept and editing the manuscript.

Acknowledgements: RK acknowledged DBT-India for awarding Ramalingaswami Re-entry fellowship.

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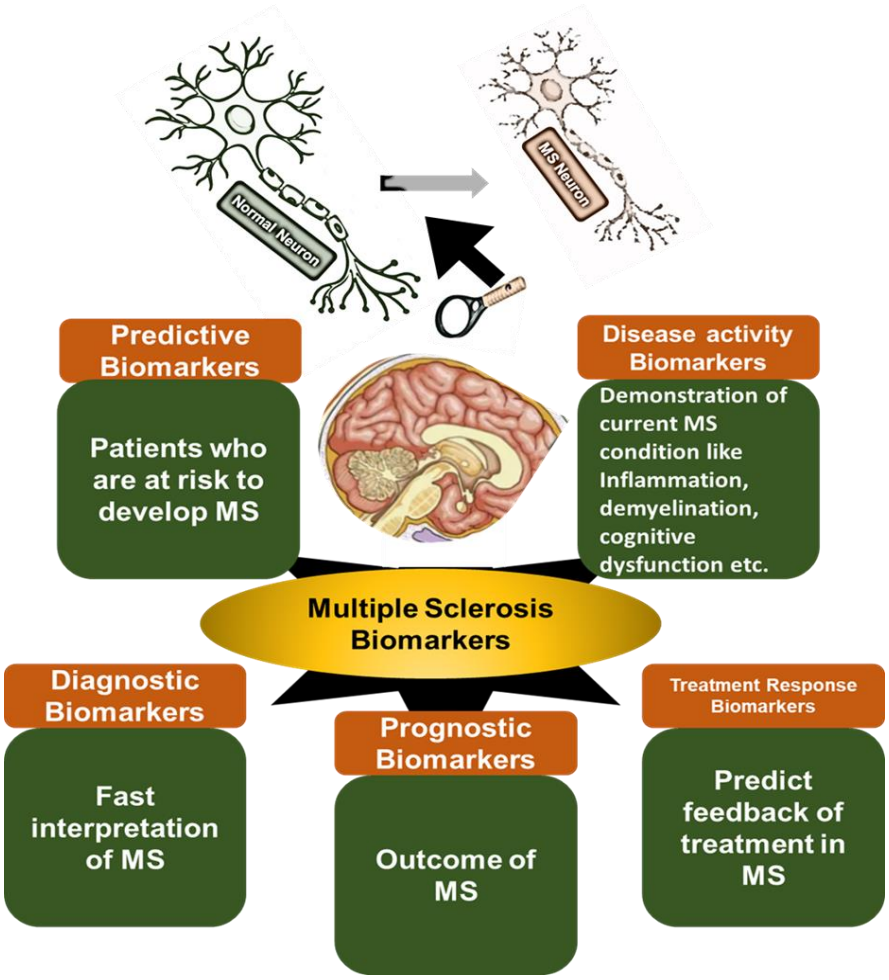

Graphical Abstract

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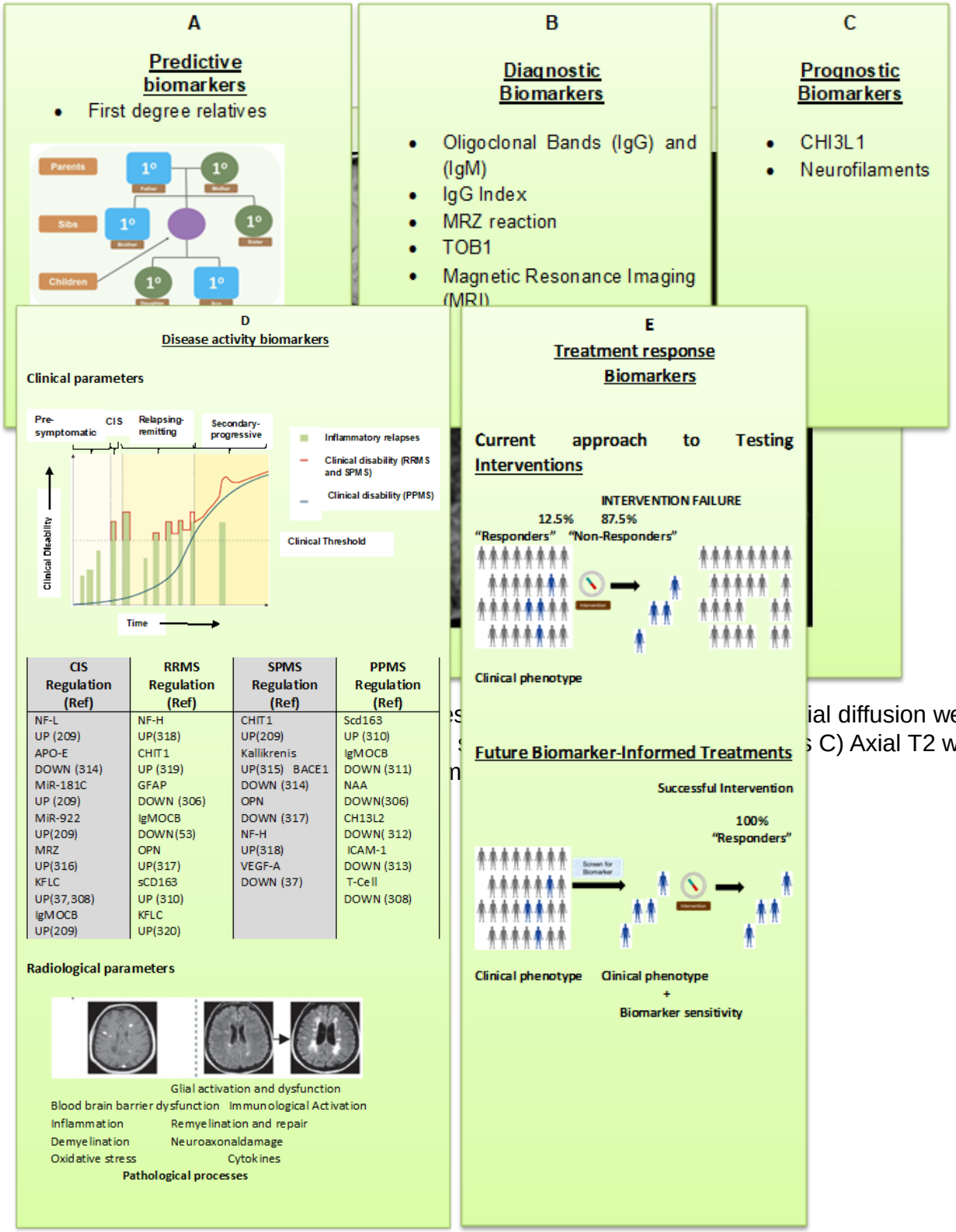

ial diffusion weighted –MRI
s C) Axial T2 weighted MRI of