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Biomarkers in Multiple Sclerosis: Analysis of the Present Advantages and Look to the Future

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Introduction

MS is a chronic heterogeneous demyelinating disease of the CNS among the young population, manifested by unpredictable attacks and subsequent remissions (McGinley et al., 2021; Lublin et al., 2022). The disease develops as a result of an interaction between genetic and environmental factors (Dobson et al., 2019). The most important genetic risk factor are the alleles of genes encoding human leucocyte antigens (HLAs), especially HLA-DRB1*1501 (Hollenbach et al., 2015). The main exogenous noxes that have the potential to trigger the illness are Epstein Barr Virus (EBV) infection, tobacco use, obesity since childhood, low vitamin D levels. Inflammatory infiltrates within the brain lesions contain CD4 and CD8 T-lymphocytes, activated monocytes and B-lymphocytes which lead to disruption of the myelin sheaths covering the nerves (Housley et al., 2015). It is considered that EBV infection contributes to production of B cells that provokes the activation of CNS inflammatory processes (Leffler et al., 2022). A relationship between gut microbiome-derived short-chain fatty acids (SCFAs) and immune dysfunction in patients with early MS was proposed (Trend et al., 2021). According to a recent hypothesis the EBV infection and B-cell dysfunction connect with gut-associated lymphoid tissue leading to aberrant B-cell responses that guide pathogenic T-cell responses in the CNS (Leffler et al., 2022).

MS usually debut with a single episode with expression of certain neurologic symptoms as optic neuritis, dizziness, numbness or muscle weakness. According to MacDonald criteria for MS diagnosis the dissemination in time and space is a mandatory condition (Thompson et al., 2018). Manifestation of clinical symptoms due to a single brain lesion determines the clinically isolated syndrome (CIS). Such patients are at risk of conversion to MS. Most often the disease begins as relapsing-remitting form and in more than 2/3 of cases it reach to a secondary-progressive course for a different period of time (Katz Sand et al., 2014). The relapsing-remitting phase corresponds with autoimmune inflammation, followed by degeneration and irreversible disability (Filippi et al., 2020).

What are the challenges in diagnosis and treatment of MS? The biggest difficulties include the lack of a pathognomonic test for MS, as well as the absence of reliable predictors of disability (Trojano et al., 2001; Giesser, 2011). The highly variable clinical course of MS further complicates the choice of the appropriate approach. Early prediction of progression will help for selection of the adequate immunomodulatory therapy (IMT) and for timely switching to appropriate drug in case of non-response. All this determines the extremely necessity of reliable MS biomarkers (Dillenseger et al., 2021).

According to the definition of the World Health Organization (WHO) the proper biomarker must be measurable value which points the interplay between the biological system and the factors that are potentially dangerous for a negative impact on it regardless of whether they are chemical, physical, or biological. The registered response could be a functional reaction, biochemical finding at the cellular level or a result from molecular interaction (World Health Organization [WHO], 1993). The way of biomarker development is determined by two main steps: discovery and validation (Paul et al., 2019).

The identification of biomarkers for MS is facilitated by the use of so-called biobanks. The biobank is an entity that collects, processes, stores, and distributes biospecimens, and also records their associated patient information (Liu & Pollard, 2015). Advanced bioinformatical processing is crucial for the analysis and interpretation of the data. The clinical end point is a clinically meaningful measure of how the patient feels, functions, or survives. It could be used in assessment of the effect of IMT (Paul et al., 2019).

The present report summarizes the advantages and disadvantages of current biomarkers used in the process of diagnostic and management of MS and outline the future opportunities.

Routine Biomarkers

The classic biomarkers for MS used routinely nowadays are MRI, spinal fluid analysis, evoked potentials (Harris et al., 2009; Housley et al., 2015).

Routine MRI Techniques

The T2-weighted MRI images can identify demyelination lesions in both white and gray matter as a consequence of inflammation with mixed pathology of neuro-axonal damage and demyelination. Tracking the burden of lesions, their size and localization is an important indicator for the diagnosis and prognosis. The active inflammation in relapsing-remitting MS (RRMS) is confirmed by detecting of gadolinium-enhancing T1 lesions (Yang et al., 2022).

Cerebrospinal Fluid (CSF)

The CSF analysis given its proximity to the CNS would be the preferred body fluid to look for candidate biomarkers. However the CSF sampling is more invasive procedure with potential risks than plasma sampling (Teunissen et al., 2009). Oligoclonal bands (OCBs) are useful diagnostic tool to detect a central humoral response. The CSF-OCB are considered as a hallmark of MS because of their detection in > 90% of cases and in nearly 70% of patients with CIS, however they are not so specific due to their confirmation in other inflammatory and neurodegenerative conditions (Selmaj et al., 2022). The identification of intrathecal IgM, on the other hand, could be prognostic biomarker for more aggressive course of the disease (Carta et al., 2022). The abumin ratio is a measure of impaired blood-CSF barrier. The IgG index is a marker for intrathecal synthesis of immunoglobulins. An IgG index >0.7 is an indicator of an increased intrathecal B-cell response and thus could predict the presence of MS (Feki et al., 2022). CSF OCBs are an independent risk factor for conversion from RIS to CIS and to MS (Lebrun-Frénay et al., 2023). In persons with CIS in who are found both lipid-specific IgM OCB in CSF, as well as high lesion load and brain atrophy on MRI, an aggressive course of the disease is predicted. (Toscano et al., 2021). The periventricularly located lesion load during the first year of the condition is also related with intrathecal IgM synthesis (Durante et al., 2012). OCBs in CIS also predict a more aggressive course of MS and correlate with brain atrophy, lesion load, and elevated CSF levels of CXCL13, a chemokine that directs B cell migration (Harris et al., 2017). The IgM OCB finding in CSF is strongly related with accumulation of higher disability and transition into secondary progressive MS (SPMS). The presence of CSF OCB is one of the mandatory criteria in primary progressive MS (PPMS) (Magliozzi et al., 2020).

Abnormal findings of the evoked potentials (EPs) Test:

The EPs are supporting test for the diagnosis and a useful tool for assessment of prognosis in MS (Grover et al., 2008). The prolonged latencies of the EPs are result of demyelination, and the reduced amplitude and disturbed configuration of the waves point to degeneration. The dynamics of EPs results as well as dynamics of imaging studies support progression in time and space (Ignatova et al., 2014).

Titer againts JC

Persisting antibodies against natalizumab develop at early stage during treatment, persist in about 6% of patients with MS (pwMS) and are associated with a decreased therapeutic efficacy and adverse reactions to natalizumab infusions (Teunissen et al., 2005). Assessment of the risk of progressive multifocal leukoencephalopathy (PML) in pwMS, treated with natalizumab, is still a great challenge for clinicians and scientists. Until recently it was based on clinical data, MRI and presence of JCV-specific antibodies (Housley, 2015). Testing the anti-JCV antibody index in patients without prior use of immunosuppressive drugs to better determine PML risk is now a promising alternative (Antoniol, 2015).

Novel Biomarkers

The novel biomarkers are establishing through improved imaging, neurophysiological and laboratory techniques. It concerns more precise parameters, but they are usually expensive and not yet widely available for practical use. They require further research and validation.

New imaging techniques. 3D double inversion recovery subtraction maps improve the detection of new lesions in a clinical setting both in terms of accuracy and in terms of speed (Eichinger et al., 2017). **Magnetization transfer (MT) MRI** provides an index, called the MT ratio, whose values reflect the efficiency of the magnetization exchange between protons in tissue water and those bound to the macromolecules (Petracca et al., 2018). MT MRI provides information about the changes in both normal-appearing WM and WM lesions, and is sensitive to the course of the disease (Filippi et al., 2000). **The diffusion tensor imaging** (DTI) is based on the principle of water diffusion, and helps to evaluate precisely the microstructure of the brain tissue (Petracca, 2018). The proton magnetic resonance spectroscopy has the ability to quantify the brain concentrations of several metabolites (Tkáč, 2016). Visualization of the leptomeningeal inflammatory infiltrates could be performed during **delayed high-resolution post contrast T2 FLAIR MRI**. Proton Magnetic Resonance Spectroscopy 1 H-MRS allows the quantification of NAA, which is synthesized by neuronal mitochondria, offering the possibility to monitor neuroaxonal dysfunction and quantify diffuse gray matter (GM) involvement (Petracca, 2018). **Positron Emission Tomography** with TSPO radioligands allows in vivo imaging of microglial activation and has proven the persistence of widespread inflammation in cortex and cortical lesions in PMS (Herranz et al., 2016). **Quantitative estimation of brain atrophy**, based on T1W sequences, suggests that progressive loss of brain volume is driven by GM atrophy (Fisher et al., 2008). Greater demyelination, cell loss and possibly disruption of tissue anisotropy in paramagnetic rim lesions (PRLs) are found on 7 T MRI (Choi et al., 2023).

Optical Coherence Tomography (OCT)

OCT is a non-invasive test that uses light to scan the retina and optic disc and evaluates the degeneration of the optic nerve after inflammation (Yang et al., 2022). OCT is proposed as reliable biomarkers of global axonal loss and neurodegeneration in

MS. The thickness of the peripapillary retinal nerve fibre layer (pRNFL) is thought to be a reliable biomarker of long-term axonal degeneration, while the density of the macular ganglion cell layer - ganglion cell inner plexiform layer (GCL-GCIPL) is accepted as an early index of neuronal integrity (Petzold et al., 2017). RNFL loss corresponds with physical disability, cognitive impairments, brain atrophy, as well as with disease duration (Vujosevic et al., 2023).

Molecular Biomarkers

The molecular biomarkers are easily quantifiable parameters, originating from the areas of immunology and neurobiology due to the causal pathomechanisms (Harris et al., 2017). They can excellently complement the clinical and MRI findings (Pachner et al., 2019). Their role has grown significantly in the last decade, but their validation is not complete. This requires multiple studies before their routine application into clinical practice (Ziemssen et al., 2019). According to which component of the CNS is affected and whether inflammation or degeneration will occur, the biomarkers could be divided into biomarkers of neuroaxonal damage, biomarkers of glial damage, biomarkers of myelin destruction and biomarkers of inflammation and immunomodulation.

Biomarkers of neuroaxonal damage

Neurofilaments (NFs): NFs are neuron-specific proteins which are released during neuronal damage (Pérez-Miralles et al., 2020). The availability NF light chain (NFL) in the CSF and then in the blood serum (Soelberg Sorensen et al., 2016) is a consequence of axonal damage even in the early stages of MS (Sapko et al., 2020). NFL level in CSF in patients with CIS and RRMS is thought to be a potential prognostic factor in the assessment of disease activity. The serum NFLs are reliable biochemical magnitude which can be used to evaluate the effects of IMT in pwRRMS and could be an alternative approach to detect subclinical disease activity versus MRI follow-up (Varhaug et al., 2018). NFLs may be used as a predictor of long-term physical disability, as well as indicator of cognitive decline after experience of optic neuritis as a first demyelinating event. They are considered to be an independent risk factor for conversion to CIS and CDMS from RIS (Sapko et al., 2020). The MS patients have higher sNFL levels compared to healthy controls even before the start of treatment with IMT. During the course of such treatment, the sNFL levels become lower (Disanto et al., 2017). The sNFL levels are also associated with T2 lesion volumes (Cantó et al., 2019; Yang et al., 2022). Higher NFL are associated with a higher subsequent rate of whole-brain atrophy, and recent inflammatory activity (new/increasing T2 lesions), as well as T2LV, is associated with higher NFL. NFL concentrations in CSF are reduced when during the second year course of immunosuppressive therapy in persons with active progressive MS and after switching from first-line therapies to fingolimod in those with RRMS (Varhaug et al., 2019); (Håkansson et al, 2018). In patients with RIS, elevated CSF levels NF-L > 619 ng/L have been shown to be an independent risk factor for conversion to CIS and MS (Matute-Blanch et al., 2018).

Phosphorylated Neurofilament H (pNF-H): PNF-H has been proposed as a surrogate marker of axonal injury in many neurodegenerative diseases (Polman et al., 2011). Patients with increased levels of pNF-H are exposed to higher risk of disability progression during the MS course compared to subjects with high TGF (Herrera et al., 2019). After axonal injury in MS, pNF-H release into the extracellular compartment, therefore analysis of its CSF and serum levels may provide valuable information to determine the extent of axonal injury (AbdElghaffar et al., 2022).

Chitinase 3 like Proteins: Chitinases are secreted glycoproteins, which bind and hydrolyze chitin (Kanneganti et al., 2012). Both Chitinase 3 like 1 (CHI3L1, also referred as YKL-40) and CHI3L2 are expressed in the astrocytes in WM plaques and in normal-looking WM in the brain of pwMS (Ignatova, 2022). Although it is not specific for MS, higher CSF CHI3L1 levels are associated with later disease stages and may have a better diagnostic value than OCB (Hinsinger et al., 2015). A threshold of CSF CHI3L1 >189 ng/mL is defined as a predictor for faster conversion form CIS to MS (Hinsinger et al., 2015; Thouvenot et al., 2019). Higher CHI3L1 levels also tended to be associated with a higher risk of disability progression according to EDSS scores (Pérez-Miralles et al., 2020). CHI3L1 levels in CSF correlate with the time of conversion from CIS to MS (Sellebjerg et al., 2019). Increased levels of glial markers which activate YKL-40 and GFAP are predictors of early MS progression with rapid reaching of EDSS 3. The higher concentration of YKL-40 indicated early disability and reaching the EDSS 6 (Magliozzi et al., 2020). CSF CHI3L1 correlates with positive CSF but is not an independent predictor of the risk of conversion from RIS to RRMS (Thouvenot et al., 2019). The Chitinase 3–Like 2 in CSF predicts the occurrence of disability progression within progressive multiple sclerosis (Comabella et al., 2021).

Tau Protein: Tau protein is important for the cytoskeleton of both neurons and oligodendrocytes (Anderson et al., 2009). It interferes in the construction and stabilization of microtubules that are needed for the axonal transport in CNS (Mirzaii-Dizgah et al., 2020). The quality of the synaptic transmission and myelination which are carried out from neurons and oligodendrocytes depend on the efficient intracellular transport. Abnormally phosphorylated (P-) tau is a hallmark of degenerative diseases of CNS and is found in chronic EAE and progressive MS (LoPresti et al., 2022). The serum level of tau protein is lower in pwMS than in healthy controls and may be considered as a potential MS biomarker (Mirzaii-Dizgah et al., 2020).

Amyliod Precursor Protein (APP): The accumulation of APP in damaged axons is known for many years (Mangiardi et al., 2011). The APP is considered as a potential biomarker of axonal demyelination and axonal injury. It is thought that APP modulates nodular formation in axons and is co-expressed with proteins associated with neuroprotective properties. Nowadays it is known that APP is involved in the regulation of inhibitory neurotransmission (Kreis et al., 2021). The metabolism of APP

in pwMS is probably associated with fatigue. APP should be evaluated as a biomarker of the role of structural MS pathology in the development of fatigue in pw MS (Johansson et al., 2022).

Tubilun Beta: Tubulin beta (TUBβ) represents a tubuline's subunit which is involved in the construction of the microtubules. Both the neuron development and regeneration are associated with increased production of the class II tubulin isotype. CSF TUBβ is increased in pwMS compared to patients with other neurological diseases (Yang et al., 2022). The reported results indicate that TUBβ is a promising diagnostic biomarker in MS, but further exploration is needed (Sapko et al., 2020).

Biomarkers of Glial Damage

Trigger receptor expressed on myeloid cells 2 (TREM-2): TREM-2 is found at high levels in CNS microglia (Azzolini et al., 2022). Increased CSF levels of sTREM-2 is a novel biomarker of microglial activation in MS. Its normalization under treatment with natalizumab or mitoxantrone can be used as a measure for the effect of IMT in pwMS. Main disadvantage is its non specifity and detection in other degenerative diseases. (Öhrfelt et al., 2016).

14-3-3 Protein: The availability of 14-3-3 proteins is associated with several neurodegenerative diseases, including MS. Increased expression of 14-3-3 proteins in glial cells in pwMS lesions is found (Kawamoto et al., 2004). The detection of this marker is connected with more severe course of MS and with shorter conversion from RIS and CIS to MS. Future research will confirm whether the CSF values of 14-3-3 proteins indicate axonal damage (Teunissen et al., 2005).

Neuron Specific Enolase: The neuron-specific enolase (NSE) represents a glycolytic enzyme, which is localized primarily in the neuronal cytoplasm. The CSF concentrations of NSE could be used as a biomarkers of neuronal damage in patients with a variety of neurological conditions, what is MS (Lamers et al., 1995; Mitosek-Szewczyk et al., 2011).

Glial Fibrillary Acid Protein (GFAP): GFAP serum level is associated with progression of MS and could serve as an easily measurable biomarker. The serum concentration of both GFAP and NFL are highly elevated in persons with SPMS compared to healthy controls and patients with RRMS (Högel et al., 2020). The GFAP levels are also remarkably higher in the CSF of MS patients compared to HC. Current study revealed that GFAP is slightly more sensitive and specific in predicting the current disease course (RRMS vs SPMS) of the patients than NFL (Högel et al., 2020). A negative correlation between GFAP CSF levels and cerebellar volume is registered in RRMS at diagnosis (Azzolini et al., 2022). Elevated serum NF-L and GFAP levels are registered in pwMS with cognitive impairment in contrast to patients with normal cognitive functioning (van Dam et al., 2023). NFL levels corresponds inversely with speed of information processing – the main vulnerable cognitive domain in MS (Ramani et al., 2021).

Osteopontin (OPN): OPN is thought to facilitate the enhanced regulation of Th1 and Th17 cytokines, especially those of IFN-γ and IL-17. Several receptors of OPN and its fragments are expressed by microglial cells. OPN is significantly upregulated in MS lesions and is elevated in the serum of pwMS (Agah et al., 2018; Rosmus et al., 2022). OPN has an important role in inflammation process and bias the T helper differentiation 1-type and 2-type responses, as well as regulating dendrite cells migration on many levels (Marastoni et al., 2021).

S100 β-protein: The S100 β-protein is an inflammatory molecule and a biomarker of damage of the astrocytes and other glial cells. Its excretion could be triggered by demyelinating damage (Michetti et al., 2019). At higher concentrations, S100 β-protein has a role of damage-associated molecular pattern molecule and supports inflammation and oxidative stress through triggering of microglial and astroglial activation (Momtazmanesh, S., 2021; Fitzner et al., 2015).

Nitric Oxide (NO): NO is a relaxing factor, which is derived from the vessel endothelium. It is produced by a family of NO synthases represents a neurotransmitter with a chemical characteristic of a free radical (abdel Naseer et al., 2020). Under pathophysiological conditions, extreme amounts of NO can be produced for long periods of time (Danilov et al., 2003) It is thought that NO helps to perpetuate the glutamate mediated damage to the oligodendrocytes and neurons during inflammation. The products of NO degradation can destroy mitochondria, leading to damage in MS lesions. It can also strengthen the activation of apoptosis on neurons and glial cells and to facilitate the flow of pro-inflammatory cells into the CNS through increasing the permeability blood-brain barrier. Increased concentration of NO is found both in the serum and CSF of pwMS (Yang et al., 2022). Treatment with Interferon-beta (IFN-β) leads to substantial inhibition of the aroused NO synthase in astrocytes (Sellebjerg et al., 2002).

Biomarkers of Myelin Destruction

Myelin Basic Protein (MBP)

MBP is the second-most abundant protein of the myelin. It reaches 30% of the total CNS myelin protein. MBP is a possible cause for formation of autoantigenic epitopes in MS (Garbay et al., 2000). MBP is engaged in the transmission of the extracellular signal to the cytoskeleton and tight junctions. It is the 'executive' molecule of the myelin membrane regarding its essential role in the composition of the compact myelin sheath (Kister & Kister, 2022). It is assumed that an abnormal isoform of MBP leads to weakening of the membrane interactions and impairment of the stiff myelin structure (Kister & Kister, 2022). Most probably an abnormal isoform composition of MBP causes weakened membrane interactions and loosening of the rigid myelin structure. The anti-MBP immunoactivity leads to appearance of MBP in the CSF. Thus, MBP could be a potential biomarker of myelin destruction (Martinsen et al., 2022).

Matrux Metalloproteinases (MMP)

MMPs may also act in MS by digesting MBP, in addition

to promoting leukocyte leakage into postcapillary venules (Rempe et al., 2016). Significant reductions in serum MMP-9 in patients with RRMS under IFN-β treatment have been observed after 12 months of follow-up. Decreased baseline MMP-9 levels were found in patients treated with NTZ in patients who developed PML (Toscano et al., 2021). MMPs could serve as a surrogate biomarker for assessment of the efficacy and the possible side effects from IMT in pwMS.

Myelin Oligodendrocyte Glycoprotein (MOG)

According to proposed international criteria, the availability of MOG-IGG is the core criterion for MOG-associated diseases (MOGAD). The most frequent MOGAD are acute disseminated encephalomyelitis and optic neuritis, transverse myelitis, and is less commonly cerebral cortical encephalitis, brainstem or cerebellar manifestations (Banwell et al., 2023). In the recent years it became clear that MOG-Ab are exceptional in MS phenotype, suggesting that the MOG-Ab testing should not be performed in typical MS presentation (Cobo-Calvo et al., 2020).

Biomarkers of Inflammation and Immunomodulation

The detection of soluble cell surface biomarkers in CSF could determine the immune phenotype of intrathecal inflammation in MS. The quantification of intrathecal sCD found an increased CSF/serum ratio of sCD163 in subjects with RRMS and PPMS, as well as elevated concentration of other biomarkers of inflammation and neurodegeneration, including increased NF-L in CFS, immune mediators and cytokines (Ignatova, 2022).

Cytokines

Cytokines are indicators for inflammatory state (Herrera et al., 2019). Cytokine/chemokine profiling can help for better understanding of the MS pathogenesis and adequate monitoring of both inflammation processes and treatment response (Kothur et al., 2016).Th1 and Th17 cytokines are more commonly elevated in pwMS. The increase in CSF IFN-c inducible protein (IP-10 or CXCL10) and the decrease in macrophage chemoattractant protein CCL2 (MCP-1) levels in pwMS is a evidence for IFN-c mediated processes. Decreased levels of CCL2 (MCP-1) - a chemokine regulated by IL-4, correspond with experience of relapses and and gadolinium-enhancing lesions on brain MRI, suggesting active phase of MS.

Interleukins

CSF IL-8 levels in persons with RIS are associated with higher risk of conversion to RRMS (Rossi et al., 2015). Increased levels of CSF Th17-related cytokines (IL-6, IL-17, IL-23), involved in the regulation of autoimmunity, is a typical finding (Kothur et al., 2016). IL-12p40, CXCL13 and IL-8 are most commonly expressed in untreated pwMS with active intrathecal inflammation (Pranzatelli et al., 2008). The enhanced expression of proinflammatory molecules asIL-1β, IL-2, IL-6, and IL-8 is predictor of both disease activity and neurodegeneration in MS (Stampanoni Bassi et al., 2018; Ignatova, 2022). Significant positive correlation between IL-9 and TREM-2 CSF levels was found (Azzolini et al., 2022).

CXCL13

The conventional lymphoid chemokine CXCL13 is aberrantly elevated in CSF of pwMS, so its intrathecal production obtained diagnostic and prognostic value in MS (DiSano et al., 2020). CXCL13 has been found to be overexpressed in active MS lesions and in intrameningeal B-cell follicles of chronic WM lesions, maintaining humoral autoimmunity and disease activity (Serafini et al., 2004).

Neutrophil-to-lymphocyte ratio (NLR) and monocyte to lymphocyte ratio (MLR)

NLRs appear to reflect better systemic inflammation than specific neutrophil and lymphocyte counts alone (Min et al., 2017). NLR and MLR are also proposed as biomarkers in MS. NLR is a potential biomarker of disability progression in MS. There is a trend of higher NLR and MLR in relatively severe or acute course of MS and lower NLR and MLR in chronic phase. Higher percentage of pwMS with relapse has increased NLR or MLR than patients without relapse. NLR and MLR obtained from routine blood tests could be a hopeful, easy and cheap biomarkers for evaluation of the disease activity in pwMS (Olsson, A., 2021; Huang et al., 2022).

Soluble CD40L (sCD40L)

The sCD40L promotes the lymphocyte proinflammatory activity. The interaction between CD40 and CD40L has a crucial role for the pathogenesis of MS. IL-31 triggers the JAK-STAT pathway in several different cell types, to induce proliferation and tissue remodeling in fibroblasts, epithelial cells, and endothelial cells. Some studies have described a correlation between these two cytokines and decreased serum levels of sCD40L and IL-31 after MS treatment, accompanied by a lower inflammatory response. The possible interaction between IL31 and sCD40L in the proinflammatory state in MS is discussed. We also describe the justification for this hypothesis and whether it is possible to investigate these cytokines as biomarkers of MS (Pastor Bandeira et al., 2022). Increased EBV-encoded nuclear antigen-1 (EBNA1)-specific IgG responses can predict conversion from CIS to MS. The EBNA1- IgG titers is thought to be a prognostic biomarker for MS conversion and disability progression (Lünemann et al., 2010).

The MRZ reaction (MRZR)

The MSZ includes the antibody indices against measles, rubella, and varicella zoster virus. This parameter reflects the intrathecal polyspecific B cell response which is very specific for MS. Its positivity could be associated with more obvious neuroaxonal damage, represented by higher NFL levels in the CSF. The MRZR could be used as a diagnostic biomarker for confirmation of the diagnosis of primary progressive MS (PPMS). Unfortunately it's pathophysiological and clinical relevance is not yet clear (Robinson et al., 2020).

Heat shock protein 70 and 90 (HSP70 and HSP90): HSP70 and HSP90 are proposed to be hopeful biomarker to monitor the inflammation processes in MS (Zimmermann et al., 2020). They modulate inflammatory processes by producing anti-

inflammatory cytokines and modulating the response with toll-like receptor 2 and 4 (TLR2 and TLR4) (Sapko et al., 2020).

Kappa Free Light Chains

Kappa free light chain (KFLC) index is a measure for intrathecal production of free kappa chains. It is increasingly recognized for its diagnostic potential in MS as a quantitative alternative to IgG OCBs. KFLC index is not affected by DMTS, used to treat MS. This indicator has some advantages as a MS biomarker versus OCB (Rosenstein et al., 2021). An international panel of experts in MS and CSF diagnostics recommended to include intrathecal kappa-Free Light Chain (κ-FLC) synthesis in the next revision of MS diagnostic criteria as an additional tool to measure intrathecal immunoglobulin synthesis (Hegen et al., 2023).

Human Endogenous Retroviruses (HERVS)

The alteration of transcription and expression of HERV derived proteins is linked with several diseases, including MS (Kristensen et al., 2021). An EBV-triggered transactivation of HERVs with differential expression of ERVMER61-1, ERV3-1, and copies from the HERV-K (HML-2) family in LCL from individuals with MS is suspected (Wieland et al., 2022).

Uric Acid

It is proposed, that the assessment of NLR as a new marker for inflammation in MS, together with uric acid value, is a protective measure in pwMS and might be more effective than evaluating these parameters alone in prediction of long term disability (Gokce et al., 2021).

Look To The Future

Extracellular vesicles (EVs)

EVs of brain origin, isolated from blood and their protein cargoes, could function as a biomarker of pathological conditions (Lizarraga-Valderrama et al., 2021). MBP concentration in oligodendrocyte EVs (EDEV) is significantly increased in CIS, RRMS and PPMS, compared to HC and correlates with disease severity measured by EDSS and Multiple Sclerosis Severity Score. The MBP concentration in ODEVs is significantly augmented in PPMS compared to RRMS and CIS (Agliardi et al., 2023). The proteomic studies are beginning to discover cell type-specific EV cargo signatures which for the future could allow us to target specific neuronal or glial cell populations during the treatment of MS and other degenerative diseases (Lizarraga-Valderrama et al., 2021).

Circulating microRNA (miRNAs)

Circulating microRNA is a class of small noncoding RNAs consisting of 17–25 nucleotides, whose main role is gene regulation by mediating mRNA degradation, as well as by regulating transcription and translation (Melo et al., 2014). The miRNAs form up to 1% of the human genome. Circulating miRNAs, usually packaged in microvesicles or exosomes, are relatively stable, which promote them as potential biomarker (Gandhi et al., 2013). They are contained in the most biofluids as CSF, serum and peripheral blood mononuclear cells (PBMCs). Impaired regulation of miRNAs may play a key role in the MS pathogenesis and is a potential indicator for assessment of the disease progression. Results from serum samples of persons with RRMS showed that miR-146a and miR-155a are upregulated, while miR-34a, miR-143a, and miR-373a are downregulated. Fingolimod treatment decreased miRNA 150 plasma levels and did not affect CSF levels, while natalizumab treatment increased miRNA-150 plasma levels and decreased CSF levels (Chen et al., 2016). Hence, the microRNAs are potential diagnostic and prognostic biomarkers for MS. Their restoration or inhibition may be a possible therapeutic approach for pwMS. Future research is needed to establish their routine application in the management of MS (Saeidi et al., 2023).

Pharmacogenetic Biomarkers

Genes implicated in mechanisms that alter the pharmacokinetics of small-molecule therapeutics have historically been the focus of pharmacogenetic research (Coyle et al., 2017). Genomics research proposed that TNFSF13B is associated with increased risk of MS and higher level of soluble BAFF in the blood, which is already a target of belimumab, a monoclonal antibody used in clinical trials for MS (Steri et al., 2017). FDA allowed new protein therapies developed on the pharmacogenetics basis. It is a time-old knowledge that genetic polymorphisms affect the efficacy and toxicity of an approved drug. The modern approaches use pharmacogenetic information about the protein therapeutic candidates and their protein receptors can facilitate the development of successful drugs, based on protein content. The immunogenicity of protein therapies is a great opportunity in the development of biologics, so the pharmacogenetic strategy is crucial (Swaminathan et al., 2023).

Transcriptomics

Transcriptomics throws light to our understanding of how genes are expressed and interconnected (Anthony et al., 2023). Modern transcriptomics uses high-throughput sequencing methods to analyze the expression of multiple transcripts in different physiological or pathological conditions, and this should rapidly expand our knowledge of the relations between the transcriptome and the clinical phenotype.

Proteomics

The proteome includes set of expressed proteins in a particular type of cell or organism, at a certain time and according to the defined conditions. The study of the proteome is named proteomics (Yang et al., 2021). The most notable change in the CSF proteome in RRMS is the oligomerization of TTR in high molecular weight species which occur in approximately 70% of the tested persons (Salazar et al., 2022). The levels of alpha-1-antichymotrypsin in CSF of subjects with RRMS is lower compared to patients with other inflammatory diseases of the CNS.

Metabolomics and Lipidomics

Specificity of mass spectrometry to survey metabolomics patterns and lipidomics unique signatures in MS is a promising technique for searching of reliable MS biomarkers. In a recent study on tear lipidomics 30 significantly modulated phospholipids were revealed and a lot of sphingomyelins were

lower in pwMS. The metabolomics approach, applied in both tears and serum, stressed on the diagnostic potential of specific aminoacids and acylcarnitines and avoids the invasiveness of CSF assessment. The metabolic profiling of tears indicates the pathological processes of the CNS, suggesting that the molecular repository of tears can be established as a source of potential biomarkers for MS (Cicalini et al., 2019).

Gut Microbiome

Gut micribiome influences on the state of immune system and thus becomes involved in the pathogenesis of MS. It is a part of gut-brain axis, which is impaired in this disease. In pwMS the gut microbiome is altered with predominance of short-chain fatty acid (SCFA) producers which could explain the specific for the disease chronic inflammation (Ordoñez-Rodriguez et al., 2023). Alpha diversity inversely correlates with a CXCR3þ Th1 phenotype in MS (Choileáin et al., 2020). The gut microbiota composition could be a predictive factor of the disease course in patients with active RRMS. Significant positive correlation between the number of sequences in the RRMS and the levels of the Ezakiella and Bilophila genera was found (Navarro-López et al., 2022). IL-17A-associated bacterial gut microbiota enhances the disease activity. Two antiinflammatory bacterial species are found - Faecalibacterium prausnitzii and Gordonibacter urolithinfaciens whose metabolites, butyrate, and urolithin, are known to counteract immune disruption MS (Thirion et al., 2023).

A relationship between the dysbiotic, inflammation-associated Bact2 enterotype with disability progression in patients with RRMS over 5 year period of follow-up is found (Devolder et al., 2023), so the gut microbiota characteristics could be a potential biomarker for worsening of the disease. The need of large-cohort studies in this regard in the future is essential.

Leucocytes Telomere Length

The measurement of telomers (TL) is proposed as a potential biomarker for assessment and prediction of clinical phenotypes of MS. Even more, the blood sample drawing is more safe and convenient and is appropriate for repeated testing in longitudinal research. Telomerase activity correlates with telomerase reverse transcriptase (TERT) expression, and significantly reduced TERT. mRNA levels were detected in stimulated blood cells of pwMS. In this way, the dysregulation of TERT is probably associated with deviated immune responses in MS (Bellon et al., 2017).

Shorter TL could also be predictor of increased disability and brains atrophy which is independent of the chronological age. This propose that biological aging can also influence the processes of inflammation and neurodegeneration in MS, so the assessment of TL could be used as a biomarker of immunosenescence, providing useful information about the individual course of disease (Bühring et al., 2021).

A recent study of López-Armas et al. is the first work, which confirms the correlation between leukocyte telomere length (LTL) and mitochondrial DNA-copy number (mtDNA-CN) in RRMS patients with mild to moderate grade of disability. These biomarkers may be useful to unravel the molecular mechanisms involved in the process of neuroinflammation, neurodegeneration and aging, as well as their relationship with the structure and function of telomeres and mitochondria in MS. Conducting longitudinal studies with large cohorts is extremely necessary to successfully differentiate the individual MS phenotypes, which will help to identify the clinical utility of LTL and mtDNA-CN for timely assessment and prediction of the course of the disease (López-Armas et al., 2023). Until now, no research was conducted on TL through brain tissues or cerebrospinal fluid cells from pwMS. Hence, the impact of the telomeres length of the CNS cells on the pathobiology of MS remains to be further investigated (Bühring et al., 2021).

Discussion

The essential need for reliable biomarkers in MS is determined by the lack of a pathognomonic diagnostic test, the wide variability of the disease manifestation and difficulties in the course prediction. The major advances in imaging technology, molecular biology and immunology undoubtedly contribute to the progress of science. Both clinical heterogeneity and biological complexity of MS make difficult identification of a single biomarker. The novel approach proposes integrative analytical methods that combine clinical characteristics, MRI findings and information from different omics approaches. The omics sciences include genomics, transcriptomics, proteomics and metabolomics and their joined use are promising direction for future research on potential biomarkers for diagnosis, prediction of disability and disease progression, as well as follow up the efficacy and safety of administered IMT in pwMS (Lorena et al., 2023).

Given the pathophysiology of MS, the synaptic dysfunction and neurodegeneration should be measured for adequate choise of IMT and proper management of the disease. Since tau protein is presented at the synapses, a specific change in tau posttranslational modification might indicate synaptic dysfunction. Accumulation of a large amount of tau could assume neurodegeneration. In persons with different MS phenotypes are supposed distinct biomarker profiles. Furthermore, analysis of the blood samples in pwMS will show that each subject is presented with an unique pattern, independently from their emphatic clinical type, which suggests a choice of selective therapy for modifying the synaptic functioning and process of neurodegeneration (LoPresti et al., 2022).

Biomarkers represent characteristics that can be objectively measured and can assess the magnitude of biological or pathogenic processes, as well as the responses to treatment. Currently, several biomarkers with satisfactory degree of relevance in a clinical setting are applied. They include MRI and OCBs as diagnostic tests. The development of biomarkers is carried out in two phases: discovery and validation. Recent studies indicated that other tests as MRZ reaction, NFL chitinases etc. are on standby to be used now and in the future. Although, there is no a single biomarker which is effective for

diagnosis and prediction of prognosis of MS with respect to sensitivity and specificity (Arneth et al., 2022).

Measuring the reduction of antigen-specific T cells, the changes of natural- and induced Tregs and pharmacodynamic biomarkers such as IL-10 and markers reflecting damage of the target tissue as for example NFL are potential MS biomarkers (Docampo et al., 2022). Regardless of the unquestionable immunopathogenic role of cytokines in the pathogenesis of RRMS, their availability fluctuates according to inflammatory exacerbations and remissions (Herrera et al., 2019).

The sNFL level is an important biomarker at group level. However, it is not enough to differentiate whether the persons suffers from MS. Many studies confirmed a wide overlap between the baseline sNFL level of patients with MS and their controls who complaints from other conditions as migraine or conversion disorder (Cantó et al., 2019). Using NFL levels as a biomarker for MS relapse is not specific, as NFL levels are elevated in infections and many neurodegenerative (Wang et al., 2012) and neurological disorders in addition to MS. sNFL correlate with advanced age due to age dependent neuronal degeneration and therefore it is found in increased concentration in older patients during MS relapse (Disanto et al., 2017). This may confound the clinical assessment since the persons with progressive MS usually are at older age. Therefore, the search for a predictive and diagnostic biomarker for MS continues because sNFL cannot, itself, be individually used to determine MS disease activity (Yang et al., 2022).

Several modern immunological biomarkers could help to identify patients with a high risk of PML more accurately. They include selectin-positive circulating T-lymphocytes, JCV effectors memory T-cells and miRNA levels (Antoniol et al., 2015). Quantitative MRI parameters might be more sensitive towards regional cortical pathology compared with the isolated use of conventional biomarkers and may help for early detection of tissue impairment in MS in the future (Straub et al., 2023).

The main limitations in application of biomarkers are lack of specify and validity. The majority of biomarkers are found not only in MS, but also in other degenerative and inflammatory diseases of the CNS, such as Alzheimer's disease, Parkinson's disease, neuromyelitis optica, inflammatory polyneuropathies etc. Extensive studies with sufficient numbers of participants over a long period of time should be performed and standardized procedures for exploration is needed to be introduced (Docampo et al., 2022).

Conclusion

Biomarkers are essential for the timely diagnosis and prognosis of MS, as well as for the development of adequate immunomodulating therapy which will improve the quality of life of the pwMS and of the percent general population at risk. Defining of clear cut-off values for different biomarkers in diagnosing MS and determining its prognosis will be a revolutionary advance in the management of MS. To achieve to this point, process for biomarker validation and qualification is needed to be passed. The reliable biomarkers should be rapid, relatively inexpensive, and uniformly administered across multiple centres and clinicians.

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No Conflict of Interests

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