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Increasing Prevalence of Multiple Sclerosis: Biomarkers Theranostic Strategy from Bench to Bedside- A Review

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ABSTRACT

The Biomarker is a measurable indicator that can be used for a particular disease state or some other physiological state of an organism. Either molecular change in the DNA, RNA, peptides, proteins, lipid metabolites, and other small molecules can also serve as molecular diagnostic biomarkers. Molecular biomarkers have been used for evaluating and monitoring the clinical response to a therapeutic intervention. Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS with unknown etiology, which leads to myelin disruption in which the insulating covers of nerve cells in the brain and spinal cord are damaged. This damage disrupts the ability of parts of the nervous system to communicate. To enhance the sensitivity and specificity of a biomarker for diagnosis and prognosis predictions of MS disease it is important that laboratory testing and cellular medical imaging be incorporated. Therefore, biomarkers aimed to determine the prognosis, diagnosis, monitoring, and pharmacologic responses to a therapeutic intervention. As a result, the biomarkers should have a high sensitivity and specificity index, as well as having clinical applications.

KEY WORDS: Multiple Sclerosis, Neurodegenerative Diseases, Molecular Biomarkers, Diagnosis, Cell Imaging, Imaging Biomarker.

1. INTRODUCTION

Biomarker characterization and its types: The terms "biomarker" (or biological marker) and "surrogate marker" are often used interchangeably. Supervisory agencies and scientist worked on clarifying this vocabulary: a biomarker is an organic constituent, characteristics, or images that can be objectively measured and assessed as a scale of biological state of an organism such as physiologic procedures, pathologic procedures or a biologic answer to a specific drug in pharmacologic interventions (Lesko and Atkinson Jr, 2001). A "surrogate endpoint" is a biomarker, which serves as a replacement for a relevant clinical end. It also is envisioned for the conjecture of a medical result. Medical signs stand in contrast to medical symptoms, which are limited to those indications of health or illness perceived by patients themselves. Also, a biomarker is clinically valuable when the required data is delivered in the time which is shorter than following the clinical course to the clinical endpoint. Thus, expecting that endpoint could be procrastinated and an intervention can occur initially. Similarly, a biomarker proves beneficial if the measured parameter provides information that is more objective or sensitive than the clinical measures.

Therefore, biomarkers may involve physiological indicators, such as blood pressure; molecular markers, such as liver enzymes and prostate-specific antigen; and imaging biomarkers, such as those derived from magnetic resonance imaging (MRI) and angiography. In the research context, biomarkers can deliver indications of both the potential effectiveness and the potential dangers related to a medical intervention. They can be used to understand the mechanism by which a drug works, to make decisions about whether to develop a drug, to screen compounds for toxicity before they enter clinical trials, to monitor the development of toxicity during clinical trials, and to forecast adverse events resulting from wider exposure. In addition, biomarkers can decrease the costs of developing drugs, improve the safety of drugs, and hustle the drugs to the marketplace. In another definition, any specific molecular change at the RNA level, DNA level, metabolite, or a specific cell protein can be considered as a molecular biomarker. From clinical point of view, biomarker can reflect the state of a disease, specifically and sensitively, and could be used for diagnosis and disease monitoring during the therapy. To identify biomarkers as surrogate endpoints requires the determination of relevance and validity. Relevance refers to a biomarker's ability to

appropriately provide clinically relevant information on questions of interest to the public, healthcare providers, or health policy officials. Validity refers to the requirement to characterize a biomarker's effectiveness or utility as a surrogate endpoint. Unluckily, validity is not characteristically completely recognized, but alternatively it has a spectrum. Some investigators actually banned the term validation as "unsuitable" to the study of biomarkers since it suggests that there can be a complete biological relationship between a given biomarker and a clinical endpoint, an assumption they reject. Instead, an alternate term that has been offered is "evaluation" to refer to the ongoing process of studying biomarker's success at acting as surrogates for individual clinical endpoints. According to this stringent view, treatment development using biomarkers does not ever prove conclusively that treatments result in particular clinical endpoints. Rather, the clinical trial process is one of progressively reducing uncertainty about the relationship between an intervention, a biomarker, and a clinical endpoint.

In the cellular and molecular biology era, biomarkers are usually classified into the following groups (Chen, 2015):

a) First group are those showing the disease progression with the time passage and are known by clinical measurements.

b) Second group are those monitoring the mechanism and effect of a drug in a specific tissue.

c) Third group are those used as surrogate endpoints in clinical trials Surrogate endpoint is a biomarker considered as a substitute for a clinically meaning end and is expected to monitor the effect of a therapeutic intervention (Chen, 2015).

Overall, a biomarker should have the following criteria (Bielekova and Martin, 2004):

a) Biologic sense: there has to be a correlation between the biomarker and the pathologic mechanism of the concerned impairment.

b) Clinical sense: it has to picture the clinical state of the patient accurately and clearly.

c) The ability of prediction and identification of the disease prognosis: the biomarker has to be able to identify the disease onset, disease ending, activation, or relapse of the disease, progression course, and differentiation of the concerned disease from other neurodemyelinating diseases.

d) Sensitivity and specificity: it has to demonstrate the pseudo-positive or pseudo-negative results correctly and in proper timing.

e) The repeatability of a result and correspondence with the first time

f) Practicality of the detection and measurement approach for clinical use.

Biomarkers can be classified whether by their application or their source and detection method (Akbarian Firozabadi, 2015; Katsavos and Anagnostouli, 2013):

Biomarkers are categorized in the three following groups based on their application:

a) Prognostic biomarkers: biomarkers that predict the probability of a lesion formation or the onset of disease symptoms

b) Diagnostic biomarkers: biomarkers that estimate involvement to the systems and organs of the patient toward the lesion and the progression course of the disease. They predict the recovery based on specific parameters.

c) Therapeutic biomarkers: These markers applied to evaluate the response of the damaged area to a specific drug or an intervention procedure like stem cells injection (Baumann, 2008; Rostamzadeh, 2014).

Biomarkers are categorized in the two following groups based on their source and detection method that any biomarker of these groups can be prognostic, diagnostic, or therapeutic:

a) Laboratory (or Genetic) biomarkers: biomarkers from variant sources like cerebrospinal fluid, peripheral blood, urine, tears, etc. that are measured by different laboratory techniques i.e. ELISA, immunofluorescence, proteomics and genomics techniques, flow cytometry, polymerase chain reaction (PCR), and western blotting.

b) Imaging (or structural) biomarkers: biomarkers that are recognized toward different imaging techniques like magnetic resonance imaging-MRI, positron emission tomography-PET, and computed tomography-CT scan

Multiple sclerosis (MS): Multiple sclerosis is a chronic inflammatory disease of the central nervous system and is known with the body's complex immune response. Historically, MS was first termed and described in detail by the French neurologist Jean-Martin Charcot, who followed patients with varying neurological symptoms, such as muscle spasms and walking difficulties after pathological examinations revealed "multiple plaques" or scars along the nerves, which correlated to the clinical features of the disease. Followed in 1868, he named the condition la sclerose en plaques, i.e. multiple sclerosis. On the molecular level of genomics, transcriptomics, proteomics, metabolics, and immunology, various changes and differences that head or accompany clinical processes in MS have been identified. The different molecular and biological levels display an interactive network that in its sum leads to the displayed clinical features. The heterogeneous etiology of MS leads to a complex pathogenicity with variable demonstrations and diverse progressions of the disease (Gourraud, 2012). MS is the most common disabling neurologic disease among young adults, particularly in females and has broad-spectrum clinical manifestations (Pittock, 2006).

Myelin sheaths of the nerves fibers are damaged and lesions take place in axons of the white matter in MS process. Determination and identification of the sequence of the events that result in formation and development of inflammatory lesions in the brain is one of the main goals of research about multiple sclerosis. In general, pathologic mechanisms of the disease consist of infiltration of immune cells through blood brain barrier (BBB), disruption in BBB integrity, inflammation, and neural lesions in genetically susceptible people (Muldoon, 2013; Menbari, 2013). There has been a theory that proposed some of systemic infections increase adhesion molecules in brain and spinal cord endothelium and that leads leukocytes to migrate to the damaged tissue, pass through vessel walls, and enter the central nervous system and eventually forming demyelinated lesions. These lesions usually occur in the white matter, where myelin sheath and its producing cellules, namely oligodendrocytes, are the primary targets of the immune response (Pittock, 2006). Myelin reactive T cells are present in peripheral blood of whether diseased or healthy individuals, but the immune response in these two groups has differences. Myelin reactive T cells in MS patients and healthy individuals are respectively active and primary in phenotype (McFarland and Martin, 2007). As noted above, multiple sclerosis is the most common etiology for neurologic disabilities in middle aged and young people. Identification of pathologic mechanisms and understanding the courses of the disease progression is the first step to a rational treatment choice. Therefore, the main target for researches in the present time is identification of independent and reliable biomarkers for any pathogenic factors of multiple sclerosis (Katsavos and Anagnostouli, 2013). MS is divided in the following categories based on its clinical course: Relapsing-remitting MS (RRMS), primary-progressive MS (PPMS) secondary-progressive MS (SPMS) and progressive-relapsing MS (PRMS).RRMS is the most common clinical course that is recognized with its relapsing and remitting phases that happen alternatively. In most cases RRMS turns into SPMS in an interval.MS emanates from complicated interactions between environmental factors, genetic basis that determines susceptibility for the disease and the immunologic and physiologic settings of the patient. This matter causes MS to represent exclusively in every single patient and its numerous molecular pathways lead to different pathological phenotypes. Measurement of molecular markers in lieu of clinical parameters can be a better method for identifying and monitoring MS patients in the future. There has never been any specific clinical parameter to define or predict an index for the progression of disease like turning into SPMS from RRMS, relapsing/remitting onset, expectable malignancy for MS patient, or their probable reaction to treatment to date. Nowadays, the most important first line therapy for RRMS patients is treatment with drugs based on Interferon beta or Glatirmer acetate (also known as Copolymer 1). However, about one-third of RRMS patients manifest at least one insufficient response to these drugs (Akbarian Firozabadi, 2015; Johnson, 2012; Borden, 2007). Biomarkers that are helpful in primary evaluation of treatment response can be and impressive advance in taking care of the patients (Hecker, 2011). The management of such a complex disease requires meaningful information about the underlying physiological processes to assist the clinical decision process or to identify, investigate, and evaluate novel therapeutic targets. Currently, MRI is an important clinical tool and most comprehensive as well as accurate imaging technique has been considered for prognosis evaluation, MS progression determination, in examining disease activity and recently for monitoring the treatment status (Akbarian Firozabadi, 2015). MRI because of its high sensitivity, desirable spatial resolution, high contrast, and also possessing novel techniques like diffusion-weighted imaging (DWI), perfusion weighted imaging (PWI), magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI) and diffusion tensor tractography (DTT), turned into a basic and efficient option in prognosis determination, disease diagnosis, monitoring the progression course, and even MS therapy (McDonald criteria) (Akbarian Firozabadi, 2015). However, the visualized lesions correlate only partially with clinical endpoints measuring disease progression such as relapse rate or Expanded Disability Status Scale (EDSS) score. Nowadays, because of advances in cellular and molecular biology (Ahmadi, 2014) and nanotechnology genetic, immunologic, and hematologic and biochemical achievements have made progress in field of biomarkers. Based on these progresses, classifications of molecular and structural biomarkers are listed below:

a) Biomarkers reflecting alteration of the immune system that itself divides into different subgroups as Cytokines and chemokine's, Antibodies, Cell adhesion molecules (CAMs), Biomarkers of neuroprotection, Biomarkers of cellular subpopulations (Pawelec, 2007).
b) Biomarkers of blood-brain barrier disruption
c) Biomarkers of demyelination
d) Biomarkers of oxidative stress and cell toxicity
e) Biomarkers of axonal/neuronal damage
f) Biomarkers of dysfunction in glial cells

Detection source for molecular biomarkers: MS biomarkers can be detected in different sources i.e. peripheral blood, urine, tear and CSF and they are explained below.

CSF: CSF can reflect clinically related inflammatory processes perfectly because CSF is proximate to the central nervous system, that being so detection of MS biomarkers, which are directly involved or excreted during tissue damage and inflammation, is more sensitive in CNS rather than blood. The other advantage of CSF as a source of

biomarkers like antibodies and immune cells specific protein is high amount of activated immune cells that are selectively involved in MS pathophysiology. One of main characteristics of the CSF that turned it into an important source for MS disease course estimation is that the amount of CSF biomarker cannot be affected by the function of liver or kidney. On the other hand, invasiveness of the procedure of collecting samples, limits multiple sampling. A circadian fluctuation in biomarkers concentration in CSF is still unclear (Akbarian Firozabadi, 2015). Based on experiences obtained in other aspects of neurology these fluctuations might be individual and exclusive in every single patient (the same as circadian fluctuations in amounts of hypo keratin, dopamine, and tryptophan) (Grady, 2006; Poceta, 2009; Kennedy, 2002). Most researches propose that CSF sampling by lumbar puncture better be done in the morning and with fasting (Akbarian Firozabadi, 2015).

Peripheral blood: blood sampling is easier and has fewer limitations about patient immunity in comparison with CSF; nevertheless, it has limitations due to circadian changes. For instance, IL-6 has its maximum concentration at 8 a.m. and its minimum concentration at 10 p.m. the way liver, kidney, and spleen works and blood related infections can interfere with the measured amount of biomarkers and processed results (Kennedy, 2002; Bielekova and Martin, 2004).

Urine: It is the easiest sample to collect. Even in a 24-hourperiod, that it overcomes the fluctuation issues mentioned earlier. However, bacterial colonization in the urine tract can affect the measurement.MS patients with bladder dysfunction might adjust their fluid intake that affects their urine output (Bielekova and Martin, 2004).

Tears: specificity and sensitivity of oligoclonal bands (OCB) in the tears of MS patients is similar to that of CSF. However, tears sample as a valuable biologic material in MS patients should be investigated further for other biomarkers. Furthermore, tears sampling needs high sensitivity and precision for investigation of respective biomarkers due to particular set up of the visual system (Bielekova and Martin, 2004).

Biomarkers reflecting alterations of the immune system:

Cytokines and chemokines: Cytokines and chemokines are secretory proteins with different functions such as growth, differentiation, and activation that regulate and determine the nature of immune responses of the body. They control immune cell movements and the cellular arrangement of immune organs. Some cytokines have pro-inflammatory and some other has anti-inflammatory activities. CXCL13 chemokine, which is a diagnostic biomarker, lead B cells and T helpers to the active demyelinating lesions by interaction with the CXCR5 receptor. Researches show that there is a close correlation between levels of CXCL13 in the CSF with B cells of the CSF, plasma blasts, and intrathecal immunoglobulin synthesis. In addition, high levels of CXCL13 in patients with clinically isolated syndrome (CIS) and clinically definite MS-CDMS has been detected. On the other hand, the protective role for CXCL12 chemokine against CNS inflammation in experimental autoimmune encephalomyelitis (EAE) has been recognized (Bielekova and Martin, 2004).it has been shown that level of CCL2 chemokine that normally is induced by immune response of Th2 cells decreases a short time after treatment with methylprednisolone due to the relapse of the MS (Bielekova and Martin, 2004). Inflammatory actions in active demyelinating lesions lead to secretion of many cytokines that can analyses and report the disease progression course as immune system biomarkers. Pro-inflammatory cytokines that are present in the margin of the lesion primarily originate from B and T cells, while it seems that B cells and monocytes that have more immune adjusting role amongst other cellules in the CSF are mainly in charge of production of intrathecal pro inflammatory cytokines in RRMS (Bielekova and Martin, 2004). Interferon-gamma and TNF-alpha are the main products of Th1 immune response.IL-6 as a connector between B and T cell immune response and also as the initiator factor of Th17 immune response can be beneficial as a diagnostic biomarker in the disease phenotype expression. Studies demonstrate that serum level of IL-6 strongly correlates with relapse abundance in MS female patients and also it is related to the age of initiation of the MS in all patients (Chen, 2012). In addition, experimental studies propose that any imbalance in IL-1 signaling (increase or decrease) in female mice causes demyelination of nerve fibers in white matter (Chen, 2012). Flowcytometry analyses indicate that B cells and monocytes in MS patients cause an increase in IL-15 expression and this increase itself leads to provocation of $CD8^+$ T cells that renders them to kill glial cells and cross the blood-brain barrier. Studies show that IL-15 level in the serum and CSF of MS patients is higher in comparison with other neurological disorders (Chen, 2012). In addition, osteopontin, which is a phosphoprotein, derived from macrophage, increases the INF-gamma and IL-12 level and decreases the IL-10 neuroprotector level. Level of this phosphoprotein increase in CSF and serum during MS relapse, but this increase can be seen in many other inflammatory disorders (Braitch, 2008). T cells in CSF that express CCR2 and CCR5 receptors have individual ability for osteopontin production during the disease relapse (Sato, 2012).

Antibodies: As mentioned before MS is a disease due to immune system of the body and many antibodies that are produced unnaturally in CSF have been recognized. It is believed that antibodies play an important role in MS pathophysiology. One of the MS characteristics is OCB presentation in CSF. Although OCB appears in the CSF of 95% of the MS patients, but it has not been recognized in MS, specifically and exclusively yet (Link and Huang, 2006). Presentation of OCB in the CSF based on a specific clinical course is still the strongest parameter to confirm

MS diagnosis (Link and Huang, 2006). In addition, OCB presentation in the CSF of CIS patients is a risk factor for CDMS (Link and Huang, 2006; Dobson, 2013; Fortini, 2003). More than 95 % of MS patients manifest antibody against OCB that is mostly from the IgG class and is not detectable in the serum constantly. Presence of IgG antibodies is an indicator for intrathecal activation of B cells. The constant presence of OCBs provides evidences for MS diagnosis and can be a sign of transition from CIS to definite MS (Link and Huang, 2006).However, OCBs themselves are not specific for MS and they can appear in infections and inflammations, cerebral vessels dilations and Para neoplastic disorders (Fortini, 2003). Their diagnostic sensitivity is high (more than 90%) but they lack specificity among CNS inflammatory disorders (almost 30%). Studies demonstrate that intrathecal IgM synthesis against OCB worsens MS progression and can be a prognostic factor (Mandrioli, 2008). On the other hand association of IgM antibody against OCB and MS progression has not been confirmed in another study that can be due to the differences in study designing and experimental techniques (Schneider, 2007). High levels of free kappa light chains-FKLC in CSF have been reported repetitively in MS that owns higher sensitivity but rather lower specificity in comparison with IgM antibody against OCB (Presslauer, 2008). High levels of FKLC in CSF are considered a strong predictor factor for CIS conversion into CDMS (Villar, 2012). Additionally, FKLC displays a sensitive index of intrathecal synthesis in CNS inflammatory disorders (Arneth and Birklein, 2009). Information about self-antigens like light, moderate, and heavy chain neurofilament (NF) might give us more details about MS pathogenesis mechanism and can be helpful in treatment approach. However, data about anti NF antibodies is heterogeneous overall. Some studies have shown a correlation between intrathecal anti NF antibodies production and neuronal destruction (Arneth and Birklein, 2009; Eikelenboom, 2003). A recent study indicated intrathecal synthesized anti NFL antibodies could be predictive for primary transformation of CIS into CDMS (Eikelenboom, 2003). Researches demonstrate that some of the antibodies take actions against different proteins from heat shock protein-HSP family and are often detectable in the CSF of MS patients, so that amount of IgG antibody against HSP 70 and HSC 70 was considerably higher in MS patients versus patients suffering from other motor neuron disease (MND) (Chiba, 2006). Antibodies against various viruses like Epstein-Barr virus (EBV), Measles, Rubella, and Zoster (MRZ) are usually detectable in the CSF of 90% of MS patients and these antibodies are more specific for MS than antibodies against OCB (Owens, 2011; Cepok, 2005). Comparing to IgG against OCB, IgG reaction against MRZ in MS patients CSF owns higher specificity as a prognostic, diagnostic, and therapeutic factor for MS diagnosis and CIS transition into CDMS (Cepok, 2005). Additionally, studies show that there is high percent of IgG antibodies against EBNA-1 and BRRF2 protein epitopes of the virus and EBNA-1 specific T cells in CSF and serum samples of MS patients (Cepok, 2005; Lünemann, 2006). Recently there have been some reports of BBB endothelial cells' contamination that is the trigger for dysfunction mechanisms and pro-inflammatory cytokines production (Casiraghi, 2011). EBV antibodies are considered as a sign of more intense inflammatory activation and the onset of primary disease (Casiraghi, 2011; Katsavos and Anagnostouli, 2013).

Cellular adhesion molecules: CAMs belong to proteins that mediate the cell-cell adhesion and appear as 2 forms of membrane-bound and free in extra cellular matrix. These proteins are important for tissue formation and tissue infiltration by blood cells. Impairment in the expression of these proteins in MS causes the increased BBB permeability and eases the immune cell migration into CNS. Pro-inflammatory cytokines result in increased expression of soluble intercellular CAM-SICAM. It has been shown that soluble E-selectin protein and Vascular CAM1-VCAM1 levels in CSG are higher in MS patients that OND patients (Correale, 2003). Higher levels of sICAM-1 and sVCAM-1 in CSF of NMO patients rather than MS patients has been reported and it has been proposed that BBB in neuromyelitisoptica (NMO) show more intense changes. Therefore, cellular adhesion molecules can be a proper option as biomarkers for monitoring of the disease progression (Iacobaeus, 2011).

Biomarkers of neuroprotection:

Vascular endothelial growth factor-A (VEGF-A): This is an angiogenesis factor with a neuroprotection feature. This factor's decreased mRNA expression has been reported in the monocytes of blood sample of MS patients with SPMS grade in comparison with RRMS grade patients. Therefore, VEGF-A can manifest the disease progression from RRMS to SPMS grade as a diagnostic biomarker (Iacobaeus, 2011).

Vitamin D: Many epidemiologic studies revealed the probable role of vitamin D deficiency in MS as in different geographical regions and regions with different climates the course of progression and relapse of the disease is variable. Vitamin D suppresses the Th1 immune response in many levels and stimulates multiple neutrophic factors production. The level of 25-hydroxy vitamin D in non-treated patients has an inverse relation with the radiologic results of the disease (Løken-Amsrud, 2012). It has recently been shown that part of INF-beta therapeutic effect in MS relapses may be due to higher production of vitamin D (Løken-Amsrud, 2012). Obtained evidences from animal studies demonstrate that A and E fat soluble vitamins can perform as disease activation modulators (Torkildsen, 2013).

Biomarkers of cellular subpopulations

B cells: Mature B cells and plasmablasts aggregation has been detected in the CSF of patients with RRMS and MRI results also confirm that (Kuenz, 2008). Centroblasts have usually been recognized in lymphoid tissue germinal centers and in the CSF of MS patients as well that is an evidence of its intrathecal production (Kuenz, 2008). Transient changes of B cells in primary stages of MS like increased expression ofCD4, α4 integrin and α1 integrin has a direct relationship with their ability to cross the BBB (Lee-Chang, 2011). The α4 expression in B cells has been associated with some gadolinium enhances lesions that are visible in MRI in people suffering from CIS (Katsavos and Anagnostouli, 2013).

T cells: Autoreactive memory T cells enter the CNS with via CXCR3 chemokine receptor. CXCR3 has high levels in many other inflammatory disorders, so has a weak diagnostic specificity for prediction of the course of progression and treatment of MS (Liu, 2005). Recent studies display increased $CD4^+$ and $CD28^-$ T cells in MS patients' serum. Level of T cells present in the CSF that express CCR2 and CCR5 receptor increase considerably during MS relapse (Broux, 2012).

NK cells: MS patients with RRMS demonstrate high level of CD56 surface antigen on their NK cells in their remitting phase it has been revealed that NK cells with $CD56^+$ markers may regulate T cells survival in MS (Bielekova, 2006).

Biomarkers of blood-brain barrier disruption: BBB disruption is one of the early features of the MS plaques formation that cause edema, excitotoxicity, and penetration of serum proteins and inflammatory cellules into CNS tissue. Disintegration of the tight junctions between endothelial cells owns a primary role among the incidents that lead to the disruption of BBB and blood-cerebrospinal fluid barrier; (BCB) (Bennett, 2010). Experimental researches on mice model with BBB damage shows those tight junction proteins, including occludin and claudin-1 decrease after treatment with myelin basic protein (MBP) (D'Aversa, 2013). If choroid plexus, which enroll in CSF formation, and is known as the main CNS autoimmune regulator, involves in a primary BCB dysfunction. T helper cells can easily enter the non-inflammatory areas (Engelhardt and Ransohoff, 2012). Expression of different adhesion molecules in vascular endothelium has a key role in passing inflammatory factors through BCB.

Matrix metalloproteinase protein (MMPs): MMPs are a family of endopeptidases that destroy extracellular proteins. Additionally, they control cell migration through BBB with damaging sub-endothelial basement membrane and eventually lead to tissue damage in MS patients. MMPs of serum and CSF have raised during MS relapse constantly. It has been recognized that MMP-9 levels in RRMS patients have been lifted. Cell crowds of T, CCR2+, CCR5+, and CCR6- express high levels of MMMP-9 during relapses (Sato, 2012).

Nerve injury induced protein 1(NINJURIN-1): this factor is a cell surface protein that is produced because of nerve damage and triggers axonal growth in peripheral nervous system. However, NINJURIN 1 function has not been clearly known in vascular system and central nervous system (Lee, 2010). Proteomics screening of cells forming human BBB demonstrate that NINJURIN-1 protein expression extent by BBB endothelial cells and myeloid antigen presenting cells play an important role in migration transition and following setting of inflammatory antigen presenting cells in the CNS. NINJURIN-1 is found up-regulated in active demyelinating lesions (Ifergan, 2011).

Biomarkers of demyelination

Myelin basic protein (MBP): this protein the second most important protein in central nervous system myelin is responsible for adhesion of the cytosolic surfaces of the present myelin in adjacent axons. Although MPB and its fragments are found extensively in the CSF of most of the MS patients, but it also has been seen in many other neurological diseases, as a result it has low specificity (Sellebjerg, 1998). Considerable relation and congruity between reduction of MBP present in CSF, contrast enhancement in MRI images and MS clinical inability to respond to methylprednisolone is an indicator of connection between inflammation and myelin sheath damage in primary stages of disease progression (Barkhof, 1992).

Alpha-beta crystallin: Immunohistochemical analysis of demyelinating lesions revealed raised alpha-beta crystallin expression, which was not found in unaffected myelin. Alpha-beta crystallin is a heat shock protein that forms cellular accumulations of brain white matter cells during stress. This protein is considered as a primary target molecule for T cells in MS patients, and its action mechanism includes activation of TNF, IL-13, IL-10, IL-17, CCL5, and CCL1 chemokines (Stoevring, 2005; Van Noort, 2010).

Biomarkers of oxidative stress and cellular toxicity:

Nitric oxide (NO) and its metabolites: This factor can cause mitochondrial damage and tissue hypoxia that the process itself leads to more destruction in MS lesions. There have been reports displaying high nitric oxide levels in serum and CSF of other neurological inflammatory disorders as well, therefore it could not be accounted a specific marker (Rejdak, 2004).

Reactive oxygen species (ROS): Reactive oxygen species are oxygen containing chemical compounds (examples include peroxides, oxide ion) that are highly reactive due to the presence of unpaired valence shell electrons. Reactive

oxygen species production that has an operator role in demyelination and axonal damage in MS exceeds the anti-oxidant capacity. That brings antioxidant capacities and their defensive potential to an end and thus oxidative stress incidence at the lesion area. Reactive oxygen species also can damage protein, lipids, nucleic acids, and eventually cause cell death. Studies show that ROS has a significant role in reduction of cytokine-induced expression of myelin genes in human primary oligodendrocytes. In addition, ROS can help monocytes to cross BBB via adhesive molecules increased expression (Van Horssen, 2011). Myelin cholesterol disintegrates into 7-ketocholesterol that its level rises in MS patients CSF (Van Horssen, 2011).

Glutamate: extracellular glutamate levels are naturally adjusted by its active reuptake in oligodendrocytes. In active demyelinating lesions, impairment of hemostatic mechanisms causes extracellular glutamate aggregation and thus more axonal damage (Srinivasan, 2005). It seems that active lesions lead to an increase in cystine-glutamate antiporter expression with the ultimate goal of intracellular cystine aggregation in order to produce glutathione, which is a kind of antioxidant (Pampliega, 2011). This biomarker is the critical and key factor for evaluation of disease duration and progression of MS in metabolically imaging by MRS.

Biomarkers of axonal/neuronal damage: CNS destruction is one of the MS characteristics that disable patients. So, tissue damage reflecting proteins can be evaluated as a diagnostic factor for the disease grade perusal. Since the tissue damage is not exclusive in MS, it also happens in other neuron damaging diseases. Generally, neuron damage proteins including NF, Tau protein, N-acetyl aspartate (NAA), neuron-specific enolase (NSE), Amyloid precursor protein (APP) and Fetuin-A can give a clue over activity and severity of MS.

NFs: NFs are a major component of axonal cytoskeleton and neuronalintracellular proteins that release into CSF after cellular damage. Therefore, neurofilament level measurement in body fluids can evaluate the extent of CNS axonal damage not only in MS, but also in other neural tissue damaging diseases. Three subunits constitute neurofilament include light (NF-L), medium (NF-M) and heavy (NF-H) subunits. Different studies indicate that NF-L and NF-H CSF levels in MS patients are higher compared to healthy controls (Teunissen and Khalil, 2012). Generally, NF-L has it maximum CSF level during the acute remitting phase of RRMS patients and is considered a poor prognosis factor. Nevertheless, in SPMS patients NF-H level rises. Diagnostic and therapeutic values of NF subunits as molecular biomarkers for MS onset prediction, assessment of disease stage characteristics, progression course, and response to treatment should undergo more evaluations and studies (Teunissen, 2009).

Tau protein: Tau is a cytoskeleton protein whose basic responsibility is stabilization of cellular microtubules. Reports show that Tau P CSF level increase in MS patients. Simultaneous elevation in Tau and NF-H CSF levels in CIS patients has a 70% predictive value of conversion to CDMS that has a higher predictive value comparing to MRI (Gresle, 2011).

N-acetyl aspartate (NAA): NAA is an aminoacid highly expressed in neurons that transfer extra cellular water molecules against concentration gradient actively. Magnetic resonance spectroscopy (MRS) technique indicates that NAA level decrease in not only MS lesions, but also in normal appearing white matter-NAWM. Decrease in CSF NAA level is an indicator of patients' disability (Teunissen, 2005). On the contrary, NAA level in CSF and serum is considerably higher in RRMS patients compared to healthy controls and NMO patients. Subsequently, NAA could be helpful for differentiating between MS and NMO whether as a laboratory marker or as an imaging marker (Tortorella, 2011).

Neuron specific enolase (NSE): NSE is a glycolytic dimer in neural and astrocytesglial cells. NSE-γ isoform is specific for neurons and is present in cell bodies and in axons. Neuronal damage causes increased NSE CSF level, which is a characteristic for many neural damaging disorders. However, no difference has been detected among NSE mean concentration CSF levels of MS patients and healthy individuals or patients with different MS subtypes which indicates low specificity of this biomarker in diagnosis and monitoring of MS grades and progression course of the disease (Royds, 1983).

Amyloid protein precursor (APP): APP accumulation in the axonal hillock is a sensitive biomarker for acute axonal damage in MS lesions (Gehrmann, 1995). APP accumulation has been shown in all kind of MS lesions, but it has higher density in active primary and terminal lesions compared to inactive and re-myelinating lesions (Kuhlmann, 2002). Since APP, accumulation happens in primary stages of lesion formation, it can be considered as a key factor for prognosis determination during primary processes of the disease. Few researches have been done respecting APP and APP derivative proteins in MS patients' serum and CSF that indicate equal CSF APP levels in MS patients, neuromyelitisoptica patients, and healthy individuals. As a result, this factor has low specificity and sensitivity diagnostic value (Kuhlmann, 2002).

Fetuin-A: Fetuin-A or alpha-2 Hermans-schmid glycoprotein belongs to a group of binding proteins mediating the transport of substances like calcium in the bloodstream and is located in CSF. Protein's coding mRNA is overexpressed in MS patients' CNS, resulting in its high concentration in active demyelinating lesions. Accordingly, different studies recommend this protein as a key biomarker for

differential diagnosis, prognosis determination and response to therapy thus decreasing of this protein's CSF level in RRMS patients compared to healthy individual and increasing of it in SPMS patients compared to healthy people (Ottervald, 2010). Fetuin-A seems to antagonize anti-inflammatory TGF- β since the patients who have responded to Natalizumab, present a considerable reduction in their Fetuin-A CSF level (Yan, 2008).

Biomarkers of dysfunction in glial cells

Protein S100B: S100B is a calcium binding protein primarily expressed in astrocytes, whose CSF elevated levels have been previously correlated with cerebral trauma. There are reports of S100B CSF increase in RRMS patients but information on this protein is not thorough and outright yet (Petzold, 2002).

Glial fibrillary acidic protein (GFAP): bGFAP is a structural protein of the astrocytes whose CSF level increase is in association with gliosis-astrocytosis. GFAP levels of in CSF are considerably higher in NMO patients in comparison with MS patients. On that account, GFAP levels estimation is not suitable as a diagnostic-differentiating marker for MS progression course monitoring (Misu, 2009). However, CSF-GFAP amount in SPMS patients displays its maximum level which indicates the correlation with neurologic disability degree, but the protein's CSF level rarely increases in RRMS patients. Subsequently, CSF-GFAP level can be helpful in determining the RRMS conversion into SPMS (Axelsson, 2011).

2. CONCLUSION

MS is an idiopathic and progressive disease causing problems in diagnosis and treatment for neurologists. In MS, there are several description aspects where biomarkers could play a role: diagnosis and etiology of the MS, type of clinical manifestation, prognosis determination, disease stage identification, and disease course monitoring and therapeutic responses in pharmaceutical interventions. Correspondence and agreement between laboratory results and cellular and structural imaging, indicates the amount sensitivity and specificity of a biomarker that modifies the diagnostic-therapeutic approach. The important issue in biomarkers discussion is their sensitivity, specificity and the ability of being applicable clinically. No biomarker is likely to have all the characteristics necessary to provide a robust understanding of response. As a result, future use of combinations of multiple biomarkers is likely. Yet the use of such combinations of biomarkers may introduce its own challenges, including technical issues of how to combine results, how to control quality, and how to interpret results in different clinical contexts. Biomarkers could only serve as true replacements for clinical relevant endpoints if we completely understood the normal physiology of a biological process, the pathophysiology of that process in the disease state, and effects of an intervention– pharmacological, device, or otherwise–on these processes. Since we rarely if ever have the full picture of those types of processes, since there are always more details, we do not know or understand, biomarkers as surrogate endpoints need constant reevaluation. Studies using biomarkers should always have as ultimate measures clinical outcomes, at least for retrospective analysis of biomarker correlation success. Although the process of discovery and development of MS biomarkers is an encouraging approach for diagnosis, treatment, but there still is a considerable gap between candidate biomarkers, validated biomarkers, and clinically useful biomarkers, as and researches are still continued to confirm a unique and basic collection of biomarkers for evaluation and monitoring the MS disease.

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