Molecular Biomarkers in Multiple Sclerosis

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Abstract

Multiple Sclerosis (MS) is a chronic immune-modulated disorder of the central nervous system (CNS) affecting mainly young adults. Due to the complexity and heterogenic etiology of this disease diagnosis, treatment, and estimations concerning the future course of the disease for the individual patient are challenging. To encounter the variability in phenotype, disease progression and response to treatments, various new drugs are in development to complement existing treatment options. Since years intensive efforts are directed to identify biomarkers that are associated with various aspects of MS on different levels of the organizational hierarchy of the human body (e.g. DNA, RNA, proteins, cells).

We researched the last ten years of literature to identify those proposed candidates that had been repeatedly published as being associated with MS etiology, clinical manifestation, disease course, and treatment response. Here, we present a categorized overview over molecular biomarkers in MS.

However, despite of the large sum of studies and the long list of candidate markers, today only very few biomarkers are of clinical value. This is mostly due to lack of comparability and statistical power in most studies. However, there are recent advances in the field of applicable molecular biomarkers in MS: For example measurement of anti-AQP4 levels allows differentiation between neuromyelitis optica (NMO) and MS.

Keywords: Multiple sclerosis; Surrogate markers; Biomarkers; Blood; Cerebrospinal fluid; Autoimmune disease; Diagnosis, Disease activity

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system that is characterized by a complex immune response. Its heterogenic etiology translates into complex pathogenesis with variable types of disease manifestations and a miscellaneous range of disease progression. In most cases but not all the clinically isolated syndrome (CIS) as the first single clinical event preludes a clinically definite MS (CDMS). MS is classified into four main types of clinical courses: relapsing-remitting (RR), primary progressive (PP), secondary progressive (SP), and progressive-relapsing (PR). RRMS is the most common type of disease course and is defined by relapses of active disease and phases of remission within which the patient recovers. In most cases RRMS turns at one point into a SPMS form. In this phase of the disease activities progress continuously. However, some patients suffer continued progression of disease activity (PPMS) or suffer relapses of acute disease activities within a progressive type of clinical disease course (PRMS) [1-8].

MS results from a complex interaction between environmental factors, the genetic background of the individual that defines individual susceptibility, and the immunological and physiological setting of the individual. This makes the MS scenario unique for each patient with many molecular pathways involved leading to a multitude of pathological phenotypes. Being able to measure molecular markers for the underlying processes rather than clinical parameters might be the better tool for specifying and monitoring the individual’s MS.

Mechanisms of the pathophysiology of MS involve mainly three physiological compartments: The peripheral blood in which immune processes mainly take place, the blood brain barrier (BBB), which breaks down to a point so that certain immune cells can pass into the brain, and the brain in which lesions mark acute sites of inflammation and neural damage leading to the phenotypic displayed symptoms of disability. In each of these compartments changes in gene expression, a certain set of proteins and cell types, and physiological reactions are characteristic hallmarks of MS pathology like onset of MS, relapses, remission, switches in the type of disease course and lesions [9].

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. Magnetic resonance imaging (MRI) is an important clinical tool in examining disease activity. However, the visualized lesions correlate only partially with clinical endpoints measuring disease progression such as relapse rate or Expanded Disability Status Scale (EDSS) score [10].

Until now there are no clear objective clinical parameters defining or predicting the type of clinical course, important hallmarks of disease progression, such as conversion to a CDMS or the switch from RRMS to SPMS, onset of relapses/remission, the expected malignancy of the individual’s MS, or the patients possible reaction to treatments. Today, most important first-line treatment option for RRMS patients still are therapies with interferon beta based drugs (IFN beta) or with Glatiramer acetate (GA). Both classes of drugs are well proven to reduce disease activity in RRMS and have a good safety profile. However, about one third of RRMS patients show an insufficient response to these drugs [11,12]. Today, several new treatment options are approaching approval [13,14]. Biomarkers that help in the early estimation of the individual

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patients’ treatment response would be a great improvement for patient care [15–17]. Molecular biomarkers that correlate with any of the clinical endpoints may serve as surrogate endpoints. Such markers would be beneficial both in patient care as well as in the drug development [18].

Biomarkers

The terms “Biomarker” and “surrogate marker” are often used interchangeably. At times these terms had been used very loosely. Regulatory agencies and scientist worked on clarifying this terminology: A “biomarker” is a biological characteristic which can be measured objectively, reproducibly and serves as an evaluated indicator of biological, pathogenic or pharmacological processes, responses, changes or conditions. A “surrogate endpoint” is a biomarker which serves as a substitute for a meaningful clinical endpoint. It also is intended for the prediction of a therapeutic effect [19]. That implies that the biomarker provides information about clinical prognosis or therapy efficacy as well as a strong and significant correlation with a clinical disease endpoint [20]. Furthermore, a biomarker is clinically useful when the time, during which the needed information is provided, is shorter than following the clinical course to the clinical endpoint. Thus, waiting for that endpoint can be avoided and an intervention can take place earlier. Also, a biomarker proves usefulness if the measured parameter provides information that is more objective or sensitive than the clinical measures [21]. Biomarkers not only need to show strong and significant correlation to a specific endpoint, but also have to cover the sum of actions that finally leads to the correlated clinical parameter. Both in combination make it difficult to prove surrogacy for a measured molecular or cellular marker [13]. Furthermore, in MS we have a very diverse range of features making the disease individual for each patient. Thus, biomarkers relevant in one group of patients might not account for other patients. Because of the complexity of MS, most likely not single biomarkers but only a panel of biomarkers derived from different platforms will be required to reflect disease-related alterations [22]. All in all, to identify valid biomarkers in MS in general is a challenge.

In MS there are several scenarios where biomarkers could play a role: In diagnosis and for the etiology of the MS, indicating the type of clinical manifestation, giving information about the disease course, or providing evidence about the response to treatments. These scenarios will be discussed later in more detail.

Biomarkers at Different Molecular Levels

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.

On the genetic level single nucleotide polymorphisms (SNPs), allelic variants and Human Leukocyte Antigen (HLA) genotypes of the individual mainly indicate certain susceptibility for developing MS [6].

On mRNA level several studies in transcriptomics were picturing gene expression profiles and changes in gene expression. Topics researches had been focussing on are e.g. differences between healthy subjects and MS patients displaying various clinical manifestations of MS, longitudinal studies in which changes in gene expression under drug therapies had been examined, or differences in the mRNA expression pattern at one time point of measurement between drug responders and non-responders. Changes in the mRNA expression pattern occur rapidly and are due to changes in DNA transcription as well as mRNA stability. SNPs in gene regulatory regions and exons can influence the mRNA amount, lead to truncated miRNAs, or sequence-altered mRNAs. Since mRNA patterns reflect to some degree the set of proteins within cell samples, they indicate differences and changes in biochemical pathways. Thus, measured mRNA pattern reflect to some extent the actual ongoing processes within the sample at the time the sample is taken. Microarray and especially real time PCR technologies are sensitive and not much sample material is needed. However, neither do different levels of gene expression (understood as levels of mRNA transcripts present in the cell) amount automatically lead to more end products (e.g. protein) nor to the affected metabolites (if the protein is an enzyme). Another downfall of mRNA markers is the sensitivity of mRNA to degradation processes. Procedures of cell sampling and mRNA extraction, and differences in handling and other systemic variances in the experiments lead to alterations of mRNA composition and amounts within the samples and thus the results [23,24].

Another RNA type is microRNA (miRNA). miRNAs are small (~22 nt) RNAs that posttranscriptionally regulate gene expression. Assumably, one miRNA regulates hundreds of mRNA targets among, in turn, many mRNAs code for regulatory gene products such as transcription factors or enzyme regulators. Thus, miRNAs may play a role as super regulators in many biological processes. Also in MS miRNAs had been identified to be differentially expressed, and some miRNAs had been postulated to be of significance in MS’ pathology. MiRNAs are a lot more stable than mRNA molecules, which make operation procedures less affected by sample degradation than the handling of mRNA samples [25–28].

Also on protein level using antigen/protein arrays and ELISA techniques several proteins and antibodies had been identified to be altered in MS patients at different stages and scenarios of the disease. Most of the proposed antibody and protein markers are associated with disease activity or treatment response. Although proteins undergo like RNAs a diurnal turnover, proteins and antibodies are more stable than RNA which leads to a greater robustness of the operating procedures. ELISA techniques are already well established in the clinical diagnostics in other diseases, like rheumatoid arthritis. Detection of oligo clonal bands (OCBs) is already used in the context of MS vs. other neuro-inflammatory diseases [19,20].

Immunologically many changes detected on the levels described result into changes in the populations and ratios of immune cells, which can be detected and quantified by FACS analysis or ELI Spot techniques [20]. Slight changes in cell ratios might pivot disease activity or course, or decide treatment response. The cellular composition of the immune cells is very complex and is reflected by RNA or protein markers, whose expression they are the result of Types and subtypes of immune cells are defined by their characteristic sets of membrane and cytoplasmatic proteins and the cytokines and chemokines they release.

Last we want to mention that markers can also be found within the metabolites that are the products of biochemical reactions. The complexity and possible combinatorial effects we observe are increasing from level to level.

Sample Types

Pathological mechanisms of MS mainly take place within the peripheral blood and the CNS. Since biopsy are performed only scarcely, main sample sources are peripheral blood and liquor samples.
as cerebrospinal fluid (CSF), but protein abundances in urine and tears had been investigated as well [20].

CSF may reflect the clinically relevant inflammatory processes best due to its proximity to the lesions within the CNS. CSF is commonly taken by lumbar puncture. Due to the flow pattern and the fact that intraparenchymal extracellular space not necessarily communicates with the free CSF space, the CSF may be similar but not identical to the CSF where the inflammatory plaques occur. One also has to be aware that CSF composition changes occur diurnally. Therefore, a standardized time of collection should be considered. CSF collection is a rather invasive procedure, and sampling should be limited to a minimum number of time points. Currently cell populations and soluble protein, peptide or antibody markers are measured—all of them being associated with disease activity and manifestation [29].

Blood samples are easy to collect and were most commonly used. Blood samples can be subdivided into whole blood samples, peripheral blood mononuclear cells (PBMC), individual cell types, plasma, or serum samples. Biomarkers related to MS relevant processes had been identified in all of these types of blood samples, but the majority of studies focus on soluble serum markers or markers within PBMC samples. Serum markers are not exclusively unique to this sample type. Serum contains also some markers that originally derive from the CNS or endothelium [29].

Whole blood or cellular blood samples reflect the peripheral immune processes of MS. However, levels of measured blood biomarkers are affected by degradation processes during handling of blood samples, extraction procedures and storage. Additionally, artificial alteration of gene expression during blood draw and the handling of blood samples may lead to artificial marker measurements. Due to the system variability molecular studies in blood are difficult to compare and to reproduce [30]. For the evaluation of biomarkers good standard operation procedures to which a broad scientific community agrees on would be very beneficial [6].

Genetic Susceptibility in MS

The latest genome-wide genetic screen identified over 50 non-HLA risk loci. Most of these MS-associated loci are neither necessary nor sufficient to cause or predict MS susceptibility are not clear. Furthermore, the implicated HLA-associated alleles are neither necessary nor sufficient to cause or predict the development of MS [2].

The latest genome-wide genetic screen identified over 50 non-HLA risk loci. Most of these MS-associated loci are located close to or inside genes encoding immune system-related molecules and are associated with other autoimmune diseases, strongly supporting the hypothesis that MS is primarily an immune-mediated disease [2]. Established multiple sclerosis non-MHC risk alleles are e.g. IL7R, IL2R, CD58, and CLEC16A displaying odds ratios between 1.1 and 1.3, while HLA DRB1*15:01 is associated with a MS risk with an OR of 3.08 [31].

Also, none of these non-HLA alleles is sufficient to cause disease or is essential for the development of the disease on its own. Most alleles are common alleles in human populations, of which the vast majority of people do not develop MS [32].

Future studies should consider the emerging significance of interactions between different genetic loci as well as between genes and environmental factors both of which further add to the complexity of disease susceptibility [6].

Susceptibility genes in MS rather indicate the molecular processes that are involved in the etiology of MS. The low odds ratios displaying their modest impact on developing MS show the heterogeneity of the disease, but, so far, are of no value of serving as biomarkers in MS prediction. A list of HLA alleles and non-HLA SNPs mediating a risk for MS development is given in Table 1 [2,3,7,33-35].

Biomarker Candidates in the Central Nervous System

To give an overview over biomarker candidates within the CNS, we sum up markers that had been shown to be associated with MS at least three times. We focus mainly on MS markers but list also some tissue markers. Due to the fact that biopsies are not frequently being performed there are only few tissue markers. Often, studies investigating molecular regulation in lesions are being performed in mice with experimental allergic encephalomyelitis (EAE).

The blood brain barrier allows molecules to passage selectively from CNS to the blood. Due to this circumstance, many but not all antibodies, proteins and peptides that are found in the CSF can also be detected in serum, but, however, at different amounts and often physically altered due to modification and degradation processes in

<table>
<thead>
<tr>
<th>Marker category</th>
<th>Gene symbols</th>
</tr>
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<tbody>
<tr>
<td>Non-HLA genes with risk SNPs [2]</td>
<td>BACH 2, GALC, MPO, SPHIN9, SP1, 40</td>
</tr>
<tr>
<td>BATF, HHEX, MPV17L2, STAT3</td>
<td></td>
</tr>
<tr>
<td>CBLB, IL12B, MYB, TAGAP</td>
<td></td>
</tr>
<tr>
<td>CD6, IL22RA2, MYC, THEMIS</td>
<td></td>
</tr>
<tr>
<td>CLEC1, IL2RA, NFkB 1, TIMEM 39A</td>
<td></td>
</tr>
<tr>
<td>CLEC16A, IRF8, Nfkb 1, TNFRSF6B</td>
<td></td>
</tr>
<tr>
<td>CXCR5, KIF1B, Nfkb 1, TNFRSF14</td>
<td></td>
</tr>
<tr>
<td>CYP2D6A1, KIF1B, TBK1, TBX21, PLEK</td>
<td></td>
</tr>
<tr>
<td>CYP2D6B1, MALT1, PTGER 4, TYK2</td>
<td></td>
</tr>
<tr>
<td>DKKL1, MAP1K1, PVT1, VCAM 1</td>
<td></td>
</tr>
<tr>
<td>EOMES, MERTK, RGS 1, X</td>
<td></td>
</tr>
<tr>
<td>EPS1, IL1, MLANA, RPS6KB1, ZFP36L1</td>
<td></td>
</tr>
<tr>
<td>EVI 5, MME, 1, SOC2, ZNF767</td>
<td></td>
</tr>
<tr>
<td>HLA risk alleles [1-3]</td>
<td>HLA-C05, HLA-D1B14, HLA-D1B17, HLA-D1B115,01</td>
</tr>
<tr>
<td>HLA-DRA1,02, HLA-D1B11,01,08, HLA-D1B113,01</td>
<td></td>
</tr>
<tr>
<td>HLA-D1B11,04,05, HLA-D1B113,03, HLA-D1B115,01</td>
<td></td>
</tr>
<tr>
<td>Genes with protective SNPs or Alleles [1,2,7,8]</td>
<td>HLA B1<em>44, HLA D1B1</em>1501 absent</td>
</tr>
<tr>
<td>HLA A*02, NOTCH4</td>
<td></td>
</tr>
<tr>
<td>Diagnostic marker on gene, RNA and/or cell level in PBMCs [1,2,4,6,36]</td>
<td>ANXA (+), NPEPPS (+), CD58 (+), CD42 (-), C4 (-)</td>
</tr>
<tr>
<td>CCR5 (+), TRB2 (+), CD40 (+), GNG2 (-), CDK4 (-)</td>
<td></td>
</tr>
<tr>
<td>CXCR4 (+), IL7R (+), ZIMZ21 (-), PARK2 (-)</td>
<td></td>
</tr>
<tr>
<td>ITPR1 (+), TNFAIP3 (+), TNFRSF1A (-), TGFBR2 (-)</td>
<td></td>
</tr>
<tr>
<td>Genetic marker associated with treatment response [1,2,5,6,36,68,68]</td>
<td>CTS5 (IFN b), LMP7 (IFN b), MXA (IFN b), HLA DRB1*1504,08</td>
</tr>
<tr>
<td>IFNAR1 (IFN b), IL7 (IFN b), MJP (GA), HLA DRB1*1504,01</td>
<td></td>
</tr>
<tr>
<td>IFNB1 (IFN b), IL12RB2 (GA), IL1R1 (GA)</td>
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</tbody>
</table>

Table 1: Selection of indicative factors on gene level.
blood and liver [29,37,38]. Markers that were found in CSF or brain tissue are summarized in Table 2. Those markers that are shared between CSF and blood are shown in Table 3 [29,39,40].

CSF biomarkers for disease activity may include interleukin-6, nitric oxide and nitric oxide synthase, osteopontin, or fetuin-A [48].

Proteins, peptides or antibodies are directly or indirectly related to nerve tissue degeneration processes and thus, directly or indirectly linked to disease activity, like for example neurofilament proteins or the tau protein. Most often these proteins are not exclusively specific to processes related to MS but to inflammatory processes and neurogenerative diseases in general, like tau, p-tau protein or beta-amyloid 42 [44].

Neurofilament proteins appear to be a promising prognostic marker in early relapsing-remitting MS [48,49]. They may be useful in the clinic for predicting MS onset, monitoring MS progression and response to therapy. Neurofilament subunits and fragments that are released during neuronal damages may even be processed in disease specific ways. However, neurofilament detection assays still have to be refined to increase sensitivity and specificity [6,37,50].

More than 95% of MS patients show OCBs, mainly of immunoglobulin G (IgG), which are not detectable in serum and persisting. The presents of IgGs indicates intrathecal B cell activity. The presence of persisting OCBs provides evidence for the diagnosis of MS persisting. The presents of IgGs indicates intrathecal B cell activity. The immunoglobulin G (IgG), which are not detectable in serum and refined to increase sensitivity and specificity [6,37,50].

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Important roles in the pathogenesis of MS play B cells and the presence of autoantibodies. The B cell activating protein B-activating factor (BAFF), also known as B Lymphocyte Stimulator (BLYS), and a proliferation-inducing ligand (APRIL), also known as tumor necrosis factor ligand superfamily member 13 (TNFSF13) or CD256, and antibody raised against BAFF had been reported to be associated with disease activity. BAFF levels or detection of anti-BAFF Ig had even been discussed to indicate non response to IFN beta therapy [54-56].

Autoantibody against several proteins can be found in most MS patients. However, specific auto-antibodies are not widely shared between patients. The recently identified antibody against the potassium channel protein KIRA1.4, for example, is detectable in not even 50% of MS patients [57]. In summary: No highly MS specific antigen or autoantibody signature could be identified yet [42,58-62].

Table 3: Selection of putative markers shared between CNS and blood.

Infections and inflammatory, cerebrovascular, and paraneoplastic disorders as well [52,53].

However, for NMO a closely to MS related disease, there is one major difference: NMO is a demyelinating disease and not a progressive degenerative disorder. NMO is usually a relapsing-remitting disease, with alternating periods of remission and relapse. In contrast, MS is a progressive disease, with gradual deterioration of function over time.

In summary, MS is a complex disease with multiple risk factors and different etiologies. While the exact cause of MS is still unknown, it is believed to be an autoimmune disease in which the body’s immune system attacks the myelin sheath that protects the nerves. This attack results in inflammation and damage to the nerves, leading to symptoms such as fatigue, weakness, and numbness.

The diagnosis of MS is based on a combination of clinical symptoms, imaging studies, and laboratory tests. MRI scans of the brain and spinal cord are often used to detect lesions that are characteristic of MS. Blood tests may also be used to detect inflammation in the body, which is a hallmark of autoimmune diseases.

Monitoring MS progression and treatment response can be challenging. MRI scans are often used to track the progression of lesions in the brain and spinal cord. However, these scans can be slow and expensive, and may not always provide enough information to make treatment decisions. Alternatives such as biomarkers and functional assessments are being explored as ways to better monitor disease progression and response to treatment.
ELISA test available with which NMO can be differentiated from MS: A test against anti-AQP4 IgG. The identification of an autoantibody exclusively detected in NMO patients against AQP4 has even allowed identification of cases beyond the classical phenotype [17,63-66].

**Blood Biomarker Candidates**

There are hundreds of mRNAs, miRNAs, proteins, and antibodies published to be associated with all important processes involved with MS. For instance Keller et al. and others described several miRNAs that are in MS patients differentially expressed compared to healthy individuals [25,71,72]. MiRNA signatures for MS diagnostics and therapy monitoring are currently investigated in more detail [73,74]. Hsa-miR-146a and hsa-miRNA-miR-142-3p had been shown to be dysregulated in MS patients vs. healthy people, and additionally show response to GA-treatment [67]. However, none of the candidates have been validated in any well-powered study so far.

On the level of antibodies and proteins the occurrence of anti-IFN-beta antibodies in patients is accompanied by a reduction in IFN-beta drug bioavailability. There is evidence that high titers of neutralizing antibodies abolish the in vivo response to IFN beta [75]. Tests for the presence of those anti-IFN-beta antibodies or tests for the levels of IFN responsive gene myxovirus resistance 1 (MX1) as marker for IFN-beta bioactivity can be used as evidence for patients non-response to IFN beta drugs [76]. These tests usually are performed earliest after six months of IFN-beta treatments; if there is other clinical evidence of therapy non-response such as an unaltered, persisting disease activity e.g. in form of relapses. There is still a controversy, if neutralizing antibodies really are a valid biomarker for IFN beta non-response. For sure, other mechanisms that lead to non-responsiveness to IFN beta exist. Comabella et al. proposed a type I interferon signature in monocytes measured after one month of treatment to correlate with a poor response to IFN beta therapy [77]. In 2010, high serum levels of IL17F protein was found to correlate with a poor response to IFN beta [78]. However, these findings could not be confirmed in an independent study [79]. There may be two types of MS defined on cellular/immunological level: One in which inflammatory processes are mainly driven by Th1 cells, and one in which inflammation is driven by Th17 cells. In the first scenario the IFN beta works with a great benefit for the patient, in the latter case IFN beta may not improve the disease course or even may be detrimental. Therefore, Th17 cell and Th1 cytokines in combination are proposed to indicate the immunological type of RRMS and, thus, therapy response [80-83].

MX1, an IFN beta response gene, is a further candidate whose baseline expression on mRNA level is controversially discussed to correlate with the patients’ response to IFN beta therapy. A subgroup of individuals has a relatively high expression level of MX1, without showing signs of any sickness like an active viral infection. In the MS scenario this high MX1 expression is in some publications discussed to may be beneficial for IFN beta treatment response or to may be connected with a poor response to IFN beta [77,84,85]. Our group examined MS patients before IFN beta therapy onset. We could not observe a correlation of MX1 expression and response status of the patients [86].

Our group also analyzed 110 previously published IFN beta response biomarker candidates on mRNA level before therapy onset in our dataset as well as in other independent datasets. Out of all those, we could identify only 13 genes out of those genes whose mRNA level before IFN beta therapy was associated with a poor response to treatment [16]. Hence in our analysis most prognostic biomarker candidates could not be confirmed.

Kemppinen et al. reviewed studies on differential gene expression in MS patients vs. controls and identified 229 genes as being differentially expressed in the same direction in at least two different studies, and only 12 genes occurred as differentially expressed in the same manner in three publications [1]. The differentially expressed genes were significantly associated with immunological pathways e.g. IL-4, IL-6, IL-17, and glucocorticoid receptor signalling pathways, primarily related to Th2 and Th17 cells rather than Th1 cells. This may suggest that Th cell balances play a critical role in etiology and pathology of MS, and may even be being factors influencing responses to treatments as suggested for example by Axtell et al. [81,82]. A list of biomarker candidates in blood is given in Table 4.

However, because of differences in samples, sample sizes, inclusion criteria, as well as platforms used the direct comparison of those expression studies is difficult [1].

Small samples sizes may even be as problematic as in genome wide expression studies than they already are in genetic studies due to expression studies susceptibility to noise introduced by technical and biological factors. Large studies with sufficient statistical power and standardized methods are needed [24].

**Conclusions**

There are recent advances in the field of applicable molecular biomarkers in MS:

AQP4 serum testing can help to make a differential diagnosis between NMO and MS by which misdiagnosis can be avoided and treatment can be guided. CSF analysis may be utilized to increase sensitivity and specificity of MS diagnosis, either by ruling out or by confirming central nervous system inflammation: For instance, measurement of intrathecal OCBs, which are present in more than 95% of patients with clinically definite MS may be beneficial [66].

We introduced different catagories of molecular biomarkers. RNAs and proteins display both the interaction of genetic as environmental influences that play a role the individual clinical course of MS. To find markers of clinical value within these types of appear promising. MRNs are the least stable among those three molecules. This may be overcome by the introductions of good SOPs. With the introduction of new RNA technologies like e.g. whole exon sequencing into the clinical testing in the future, miRNA markers are an important source for biomarkers. However, miRNAs and proteins are far more stable within the samples and promise to be the most reliable source of biomarkers within the types of molecules mentioned here.

An issue is the feasibility and reproducibility of measurement that can be done in a clinical setting: On protein level ELISA or on RNA level RT PCR based diagnostic tests in serum or in minimally processed blood samples may be technically feasible in a diagnostic setting.

However, today despite all efforts the vast majority of biomarker candidates on all molecular levels and different samples could not be confirmed. Reasons why there is a lack of confirmation for the majority of proposed molecular markers in MS can be found in the differences of study designs, definition of endpoints, methodical variations, and a lack of power for most of the studies. One step in the right direction would be, that more researchers would appreciate the possibility to upload their -even though small- data sets with expression data and clinical data. This would enable researchers to utilize these data for
### Table 4: Selection of blood biomarkers in MS.

<table>
<thead>
<tr>
<th>Marker category</th>
<th>Sample type</th>
<th>Type of molecule</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
<td>Serum/plasma [57,91,92]</td>
<td>Antibody</td>
<td>Nuclear antigens Anti-Neurofascin Anti-GAD Anti-β-arrestin Anti-KIR4.1</td>
</tr>
<tr>
<td></td>
<td>Cellular [93,94]</td>
<td>miRNA [90]</td>
<td>hsa-miR-1826 (+) hsa-miR-572 (+)</td>
</tr>
<tr>
<td></td>
<td>Serum and PBMC [25,90]</td>
<td>mRNA [25-27,71,72,89]</td>
<td>hsa-miR-19b (CD4+CD25+ cells) (+) hsa-miR-17-5p (+)</td>
</tr>
<tr>
<td><strong>Disease activity and manifestation</strong></td>
<td>Serum</td>
<td>Antibody [5,91]</td>
<td>Anti-EBNA IgG Anti-gangliosides Anti-CD46 and 59</td>
</tr>
<tr>
<td></td>
<td>Protein [5]</td>
<td>C4 fragment (+) TIMP1 (-) MMP9 (+) complement factor H (+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>Protein [5]</td>
<td>CCR5 (+) CXCR3 (+) CNTF (+) ICAM (+) CX3CR1 (-) LFA1 (-) CXCL12 (+) BDNF (+; recov.) CXCL5 (+)</td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>mRNA [25,71,72]</td>
<td>hsa-miR-18b (+) hsa-miR-599 (+) hsa-miR-96-5p (+; remission)</td>
</tr>
<tr>
<td><strong>Disease activity and treatment response</strong></td>
<td>Serum</td>
<td>Antibody [5,35,84-86]</td>
<td>Anti IFN-Nab or IFN binding Abs</td>
</tr>
<tr>
<td></td>
<td>Protein [5]</td>
<td>IFNR-gb (+) VLA4 (-)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cellular [5]</td>
<td>Survivin (+)</td>
<td></td>
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<tr>
<td></td>
<td>cellular and SNP [5]</td>
<td>LDH6 (+; GA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mRNA [67]</td>
<td>hsa-miR-146a (+; GA)</td>
<td></td>
</tr>
<tr>
<td><strong>Treatment response to IFN beta, if not stated otherwise:</strong></td>
<td>Serum</td>
<td>Antibody [5,35,84-86]</td>
<td>Anti IFN-Nab or IFN binding Abs</td>
</tr>
<tr>
<td></td>
<td>Protein [35,77]</td>
<td>IFN-y (-; resp. p-IFNAR (+; resp.;3 Mo; mono.) p-STAT1 (+; resp.;3 Mo; mono.)</td>
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<tr>
<td></td>
<td>Protein and mRNA [5,35]</td>
<td>IL8 (+; resp.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cellular [5,78,80]</td>
<td>IFNR-a2 (+; generell) PDL2 (+; generell) Th17 cells (+; non-resp.; T0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mRNA [7,8,16,35,68,70,78,87]</td>
<td>CAT1 (+; non-resp.; T0) FADS1 (+; resp.;3 Mo; mono.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>mRNA combinations [87]</td>
<td>Casp2+Casp10+FLIP (+; non-resp.)</td>
</tr>
</tbody>
</table>

(*)=expression upregulated; (−)=expression downregulated; (+; GA)=upregulated in response to GA; (-; resp.)=downregulated to therapy response; (+; non-resp.; T0)=upregulated in non-responders to IFN beta before therapy; (+; resp.;3 Mo; mono.)=upregulated in responders to IFN beta after 3 month of therapy onset in monocytes
References


