Molecular Biomarkers in Multiple Sclerosis

Brigitte Katrin Paap[1,2]*, Michael Hecker[1], Dirk Koczan[1] and Uwe Klaus Zettl[2]

[1]*Institute of Immunology, University of Rostock, Schillingallee 68,18057 Rostock, Germany*
[2]*Clinic and Policlinic of Neurology, University of Rostock, Gehlsheimer Str. 20, 18147 Rostock, Germany*

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.

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

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 then 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 mRNAs, 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 rheumatiod 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 combinatory 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 biopsies 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 be 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 largest genetic effect on the development of MS is located in the human leukocyte antigen (HLA) class II, first identified 40 years ago. Additional independent risk loci had been identified within the HLA class I regions. However, the mechanisms how these HLA alleles affect 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 CSF 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

Marker category	Gene symbols			
Non-HLA genes with risk SNPs [2]	BACH 2	GALC	MPHOSPH9	SP1 40
	BATF	HHEX	MPV17L2	STAT3
	CBLB	IL12B	MYB	TAGAP
	CD6	IL22RA2	MYC	THEMIS
	CLECL1	IL2RA	NFKB 1	TMEM 39A
	CLECL16A	IRF8	NRM	TNFRSF6B
	CXCR5	KIF2IB	OLIG3	TNFSF14
	CYP24A1	KPNB1/ TBKBP1/ TBX21	PLEK	TNP2
	CYP27B1	MALT1	PTGER 4	TYK2
	DKKL1	MAPK1	PVT1	VCAM 1
	EOMES	MERTK	RGS 1	X
	EPS15L1	MLANA	RPS6KB1	ZFP36L1
	EVI 5	MMEL 1	SCO2	ZNF767
HLA risk alleles [1-3]	HLA C*05	HLA DRB*14	HLA DRB1*07	HLA DRB1*15:01
	HLA DRA*02	HLA DRB1*01:08	HLA DRB1*13:01	HLA DRB5
	HLA DRB*12	HLA DRB1*04:05	HLA DRB1*13:03	HLA G
Genes with with protective SNPs or Alleles [1,2,7,8]	HLA B*44+HLA DRB1*1501 absent			
	HLA A*02			
	NOTCH4			
Diagnostic marker on gene, RNA and/or cell level in PBMCs [1,2,4-8,36]	ANXA (+)	NPEPPS (+)	CD58 (+)	CDK4 (-)
	C7orf54 (+)	TRIB2 (+)	CD40 (+)	GNG2 (-)
	CXCR4 (+)	IL7R (+)	ZMIZ1 (-)	PAK2 (-)
	ITPR1 (+)	TNFAIP3 (+)	TNFRSF1A (-)	TGFBR2 (-)
Genetic marker associated with treatment response [1,2,5-8,36,68,88]	CTSS (IFN b)	LMP7 (IFN b)	MxA (IFN b)	HLA DRB1*04:08 (IFN b)
	IFNAR1 (IFN b)	IL7 (IFN b)	MBP (GA)	HLA DRB1*04:01 (IFN b)
	FAS (GA)	IL12RB2 (GA)	IL1R1 (GA)	

(+)=upregulated mRNA levels and (-)=downregulated mRNA levels in PBMC from MS patients; Genes with SNPs associated with treatment response to GA are marked (GA) and to IFN beta are marked (IFN b)

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 and may indicate a transition from CIS to definite MS [51]. However, OCBs are themselves not specific for MS, but may appear during

Marker category	Sample type	Type of molecule	Name
Diagnosis+Disease activity	CSF [42,38,56]	Antibody	Anti-hnRNPs
		Protein	14-3-3' protein
		Metabolite	Fetuin A
Disease activity	CSF [5,37,38,41,43-47]	Antibody	Anti- Neuro filaments
			Ig kappa light chains
			Oligo Clonal Bands
		Protein	Amyloid beta 42 (+)
			S100 protein (+)
			alpha-Internexin (+)
			Neurofilaments (+)
			p-Tau and Tau (+)
			Gab-43 (+)
			GFAP (+)
			IL6 (+)
			NCAM (+)
			Neurofilaments (+)
		Amino Acid	NAA (+)
		Metabolites and others	Pentosidine (+)
			Bri2-23 (+)
			24S-OH-chol (+)
			Myoinositol (+)
	CSF and brain tissue [27]	Protein and mRNA	Osteopontin (OPN/ETA) (+)
	Brain tissue [22,27]	miRNA and mRNA	hsa-miR-34a (+)
			alpha B-Crystallin (+)
			PGDS (+)
			Leptin (+)
			ACTH receptor (+)
			Prostatic Binding Protein (+)

(+)=Expression upregulated

Table 2: Selection of putative biomarkers within the CNS.

Marker category	Sample type	Type of molecule	Name		
Diagnosis	Serum and CSF [61,62,69]	Antibody	Anti-AQP 4		
			AEF		
Diagnosis and Disease activity	Serum and CSF [25,38,67]	miRNA	hsa-miR-326 (+)		
			hsa-miR-155 (+)		
		Antibody	Anti-NSE		
			Anti-NF-M		
Disease activity	Serum and CSF [5,37,38,41,67-70]	Antibody	Anti-Tubulin	anti-HSP60	
			Anti-NF-H	anti-MOG	
			anti-α crystalline	anti-MBP	
			anti-HSP 70		
		Protein and SNP	IL12 (+)		
		Protein	Tubulin (+)	IL17 (+)	GDNF (-=recov.)
			NF-H (+)	TNF-a (+)	NGF (-=recov.)
			IFN γ (+)	CCL2 (-)	NT3 (-=recov.)
			CCL5 (+)	IP-10 (+)	NT4 (-=recov.)
		Metabolite	Nitric Oxide products (+)		
	CSF and PBMC [5]	Protein	BDNF (-=recov.)		
		cellular marker	CD19+ CD138+ B cells (+)		
	CSF and blood [68,70]	mRNA and protein	Leptin (+)		
			FCER1A (+)		
Disease activity and treatment response	CSF and serum [56,67]	mRNA	BAFF (+)		
		Antibody	Anti-Baff		
		Protein and mRNA	IL10 (-)		
		Protein	CXCL13 (+)		
			sVCAM (+)		
	CNS and blood [6]	mRNA and protein	IL17F (+)		

(+) and (-) are in MS upregulated or downregulated molecules. (-=recov.) are proteins that are downregulated during recovery phases of MS

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

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

Important roles in the pathogenesis of MS play B cells and the presence of autoantibodies. The B cell activating proteins B-cell 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 KIR4.1, 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].

However, for NMO a closely to MS related disease, there is one

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 inverstigated 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. MRNAs 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, mRNA 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

Marker category	Sample type	Type of molecule	Name	
Diagnosis	Serum/plasma [57,91,92]	Antibody	Nuclear antigens	Anti-Neurofascin
			Anti-GAD	anti β-arrestin
			Anti-KIR4.1	
		Protein [37]	pNF-H (+)	
		Cellular [93,94]	plasma endothelial microparticles (+)	
		miRNA [90]	hsa-miR-1826 (+)	hsa-miR-572 (+)
			hsa-miR-1979 (-)	hsa-miR-614 (+)
			hsa-miR-22 (+)	hsa-miR-648 (+)
	Serum and PBMC	miRNA [25,90]	hsa-miR-422a (+)	
	PBMC	miRNA [25-27,71,72,89]	hsa-miR-19b (CD4+CD25+ cells) (+)	hsa-miR-17-5p (+)
			hsa-miR-25 (CD4+CD25+ cells) (+)	hsa-miR-18-5p (+)
			hsa-miR-1275 (+)	hsa-miR-186-5p (-)
			hsa-miR-145-5p (+)	hsa-miR-20a-5p (+)
			hsa-miR-491-5p (+)	hsa-miR-20b-5p (+)
			hsa-miR-584-5p (+)	hsa-miR-223-3p (+)
			hsa-miR-664-3p (+)	hsa-miR-142-3p (+)
		mRNA [1]	ATP7A (-)	OGT (+)
			CCL3 (-)	PLAUR (-)
			EIF4A1 (-)	PTGS2 (+)
			HSPA1A (-)	RBBP6 (+)
			NEAT1 (+)	ZMYND8 (+)
Disease activity and manifestation	Serum	Antibody [5,91]	Anti-EBNA IgG	
			Anti-gangliosides	
			Anti-CD46 and 59	
		Protein [5]	C4 fragment (+)	TIMP1 (-)
			MMP8 (+)	complement factor H (+)
	PBMC	Protein [5]	CCR5 (+)	CXCR3 (+)
			CNTF (+)	ICAM (+)
			CX3CR1 (-)	LFA1 (+)
			CXCL12 (+)	BDNF (+; recov.)
			CXCL8 (+)	
		Cellular [5]	CD56 bright NK cells (+)	K2P5+T cells (+)
			CD8+CD25+FoxP3+Treg cells (+)	PD1/PDL1 (+)
			CD80 (+)	Fas/FasL
		mRNA [5]	ILT3 (-; mono.)	
		miRNA [25,71,72]	hsa-miR-18b (+)	
			hsa-miR-599 (+)	
			hsa-miR-96-5p (+; remission)	
Disease activity and treatment response	Serum	Protein [5]	MMP9 (+)	
	Serum	Protein [5]	IFNR-gb (+)	
			VLA4 (-)	
		Cellular [5]	Survivin (+)	
		cellular and SNP [5]	CD86 (+; GA)	
		miRNA [67]	hsa-miR-146a (+; GA)	
Treatment response to IFN beta, if not stated otherwise:	Serum	Antibody [5,35,84-86]	Anti IFN-Nab or IFN binding Abs	
	PBMC	Protein [35,77]	IFN-γ (-; resp.)	
			p-IFNAR (+; resp.;3 Mo; mono.)	
			p-STAT1 (+; resp.;3 Mo; mono.)	
		Protein and mRNA [5,35]	IL8 (+; resp.)	
		Cellular [5,78,80]	IFNR-a2 (+; generell)	
			PDL2 (+; generell)	
			Th17 cells (+; non-resp.; T0)	
		mRNA [7,8,16,35,68,70,78,87]	CA11 (+; non-resp.; T0)	FADS1 (+; resp.;3 Mo; mono.)
			CA2 (+; non-resp.;T0)	IFI44 (+; resp.;3 Mo; mono.)
			Casp10 (+; non-resp;T0.)	IFIT1 (+; resp.;3 Mo; mono.)
			DNM1 (+; non-resp.;T0)	IFIT2 (+; resp.;3 Mo; mono.)
			Flip (+; non-resp.;T0)	IFIT3 (+; resp;3 Mo; mono..)
			GPR3 (+; non-resp.;T0)	MARCKS (+; resp.;3 Mo; mono.)
			IL17RA (+; non-resp.;T0)	OASL (+; resp.;3 Mo; mono.)
			IL17RC (+; non-resp.;T0)	RASGEF1B (+; resp.;3 Mo; mono.)
			RRN3 (+; non-resp.;T0)	
			YEATS2 (+; non-resp.;T0)	
		mRNA combinations [87]	Casp2+Casp10+FLIP (+; non-resp.)	
			Casp2+Casp10+FLIP (+; non-resp.)	

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

Table 4: Selection of blood biomarkers in MS.

meta-analysis or the analysis of different aspects [10,16,17,77]. We emphasis the need of standardized protocols and the need of large scale, worldwide biomarker studies to which many groups contribute to gain well powered, significant results.

References

1. Kemppinen AK, Kaprio J, Palotie A, Saarela J (2011) Systematic review of genome-wide expression studies in multiple sclerosis. BMJ Open 1: e000053.

2. Gourraud PA, Harbo HF, Hauser SL, Baranzini SE (2012) The genetics of multiple sclerosis: an up-to-date review. Immunol Rev 248: 87-103.

3. Link J, Kockum I, Lorentzen AR, Lie BA, Celius EG, et al. (2012) Importance of human leukocyte antigen (HLA) class I and II alleles on the risk of multiple sclerosis. PLoS One 7: e36779.

4. De Jager PL, Chibnik LB, Cui J, Reischl J, Lehr S, et al. (2009) Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: a weighted genetic risk score. Lancet Neurol 8: 1111-1119.

5. Graber JJ, Dhib-Jalbut S (2011) Biomarkers of disease activity in multiple sclerosis. J Neurol Sci 305: 1-10.

6. Baranzini SE, Nickles D (2012) Genetics of multiple sclerosis: swimming in an ocean of data. Curr Opin Neurol 25: 239-245.

7. Nischwitz S, Müller-Myhsok B, Weber F (2011) Risk conferring genes in multiple sclerosis. FEBS Lett 585: 3789-3797.

8. Grossman I, Avidan N, Singer C, Goldstaub D, Hayardeny L, et al. (2007) Pharmacogenetics of glatiramer acetate therapy for multiple sclerosis reveals drug-response markers. Pharmacogenet Genomics 17: 657-666.

9. Kasper LH, Shoemaker J (2010) Multiple sclerosis immunology: The healthy immune system vs the MS immune system. Neurology 1: S2- S8.

10. Barkhof F, Simon JH, Fazekas F, Rovaris M, Kappos L, et al. (2011) MRI monitoring of immunomodulation in relapse-onset multiple sclerosis trials. Nat Rev Neurol 8: 13-21.

11. Johnson KP (2012) Glatiramer acetate for treatment of relapsing-remitting multiple sclerosis. Expert Rev Neurother 12: 371-384.

12. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, et al. (2007) Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov 6: 975-990.

13. Sanvito L, Constantinescu CS, Gran B (2011) Novel therapeutic approaches to autoimmune demyelinating disorders. Curr Pharm Des 17: 3191-3201.

14. Fox EJ, Rhoades RW (2012) New treatments and treatment goals for patients with relapsing-remitting multiple sclerosis. Curr Opin Neurol : S11-S19.

15. Paap BK, Hundeshagen A, Hecker M, Zettl UK (2012) An inventory of short term and long term changes in gene expression under interferon β treatment of relapsing remitting MS patients. Curr Pharm Des 18: 4475-4484.

16. Hecker M, Paap BK, Goertsches RH, Kandulski O, Fatum C, et al. (2011) Reassessment of blood gene expression markers for the prognosis of relapsing-remitting multiple sclerosis. PLoS One 6: e29648.

17. Correale J (2011) Personalized treatment in multiple sclerosis. Curr Neurol Neurosci Rep 11: 523-525.

18. Paap B, Hecker M, Koszcan D, Thiesen HJ, Zettl U (2012) Analysis of Predictive Biomarkers for Estimation of MS Patients' Long-Term IFN-β Therapy Outcome. Neurology.

19. Martin R, Bielekova B, Hohlfeld R, Utz U (2006) Biomarkers in multiple sclerosis. Dis Markers 22: 183-185.

20. Bielekova B, Martin R (2004) Development of biomarkers in multiple sclerosis. Brain 127: 1463-1478.

21. Lesko LJ, Atkinson AJ Jr (2001) Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. Annu Rev Pharmacol Toxicol 41: 347-366.

22. Steinman L, Zamvil S (2003) Transcriptional analysis of targets in multiple sclerosis. Nat Rev Immunol 3: 483-492.

23. Comabella M, Racke MK (2012) New technologies for biomarker discovery in multiple sclerosis. J Neuroimmunol 248: 1.

24. Villoslada P, Baranzini S (2012) Data integration and systems biology approaches for biomarker discovery: challenges and opportunities for multiple sclerosis. J Neuroimmunol 248: 58-65.

25. Angerstein C, Hecker M, Paap BK, Koczan D, Thamilarasan M, et al. (2012) Integration of MicroRNA databases to study MicroRNAs associated with multiple sclerosis. Mol Neurobiol 45: 520-535.

26. Dai R, Ahmed SA (2011) MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. Transl Res 157: 163-179.

27. Junker A, Hohlfeld R, Meinl E (2011) The emerging role of microRNAs in multiple sclerosis. Nat Rev Neurol 7: 56-59.

28. Thamilarasan M, Koczan D, Hecker M, Paap B, Zettl UK (2012) MicroRNAs in multiple sclerosis and experimental autoimmune encephalomyelitis. Autoimmun Rev 11: 174-179.

29. Eikelenboom MJ, Uitdehaag BM, Petzold A (2011) Blood and CSF Biomarker Dynamics in Multiple Sclerosis: Implications for Data Interpretation. Mult Scler Int 2011: 823176.

30. Teunissen CE, Tumani H, Bennett JL, Berven FS, Brundin L, et al. (2011) Consensus Guidelines for CSF and Blood Biobanking for CNS Biomarker Studies. Mult Scler Int 2011: 246412.

31. Sawcer S (2009) The genetic aspects of multiple sclerosis. Ann Indian Acad Neurol 12: 206-214.

32. Sawcer S, Ban M, Wason J, Dudbridge F (2010) What role for genetics in the prediction of multiple sclerosis? Ann Neurol 67: 3-10.

33. Sadovnick AD (2012) Genetic background of multiple sclerosis. Autoimmun Rev 11: 163-166.

34. Link J, Lorentzen AR, Kockum I, Duvefelt K, Lie BA, et al. (2010) Two HLA class I genes independently associated with multiple sclerosis. J Neuroimmunol 226: 172-176.

35. Killestein J, Polman CH (2011) Determinants of interferon β efficacy in patients with multiple sclerosis. Nat Rev Neurol 7: 221-228.

36. Baranzini SE, Mudge J, van Velkinburgh JC, Khankhanian P, Khrebtukova I, et al. (2010) Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. Nature 464: 1351-1356.

37. Gresle MM, Butzkueven H, Shaw G (2011) Neurofilament proteins as body fluid biomarkers of neurodegeneration in multiple sclerosis. Mult Scler Int 2011: 315406.

38. Dujmovic I (2011) Cerebrospinal fluid and blood biomarkers of neuroaxonal damage in multiple sclerosis. Mult Scler Int 2011: 767083.

39. Tumani H, Brettschneider J (2012) Biochemical markers of autoimmune diseases of the nervous system. Curr Pharm Des 18: 4556-4563.

40. Tumani H, Hartung HP, Hemmer B, Teunissen C, Deisenhammer F, et al. (2009) Cerebrospinal fluid biomarkers in multiple sclerosis. Neurobiol Dis 35: 117-127.

41. Edwards LJ, Sharrack B, Ismail A, Tumani H, Constantinescu CS (2011) Central inflammation versus peripheral regulation in multiple sclerosis. J Neurol 258: 1518-1527.

42. Sueoka E, Yukitake M, Iwanaga K, Sueoka N, Aihara T, et al. (2004) Autoantibodies against heterogeneous nuclear ribonucleoprotein B1 in CSF of MS patients. Ann Neurol 56: 778-786.

43. Mori F, Rossi S, Sancesario G, Codecà C, Mataluni G, et al. (2011) Cognitive and cortical plasticity deficits correlate with altered amyloid-β CSF levels in multiple sclerosis. Neuropsychopharmacology 36: 559-568.

44. Valis M, Talab R, Stourac P, Andrys C, Masopust J (2008) Tau protein, phosphorylated tau protein and beta-amyloid42 in the cerebrospinal fluid of multiple sclerosis patients. Neuro Endocrinol Lett 29: 971-976.

45. Anderson JM, Patani R, Reynolds R, Nicholas R, Compston A, et al. (2010) Abnormal tau phosphorylation in primary progressive multiple sclerosis. Acta Neuropathol 119: 591-600.

46. Rigotti DJ, Gonen O, Grossman RI, Babb JS, Falini A, et al. (2011) Global N-acetylaspartate declines even in benign multiple sclerosis. AJNR Am J Neuroradiol 32: 204-209.

47. Sinclair C, Mirakhur M, Kirk J, Farrell M, McQuaid S (2005) Up-regulation of

osteopontin and alphaBeta-crystallin in the normal-appearing white matter of multiple sclerosis: an immunohistochemical study utilizing tissue microarrays. Neuropathol Appl Neurobiol 31: 292-303.

48. Harris VK, Sadiq SA (2009) Disease biomarkers in multiple sclerosis: potential for use in therapeutic decision making. Mol Diagn Ther 13: 225-244.

49. Salzer J, Svenningsson A, Sundström P (2010) Neurofilament light as a prognostic marker in multiple sclerosis. Mult Scler 16: 287-292.

50. Teunissen CE, Khalil M (2012) Neurofilaments as biomarkers in multiple sclerosis. Mult Scler 18: 552-556.

51. Link H, Huang YM (2006) Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J Neuroimmunol 180: 17-28.

52. Fortini AS, Sanders EL, Weinshenker BG, Katzmann JA (2003) Cerebrospinal fluid oligoclonal bands in the diagnosis of multiple sclerosis. Isoelectric focusing with IgG immunoblotting compared with high-resolution agarose gel electrophoresis and cerebrospinal fluid IgG index. Am J Clin Pathol 120: 672-675.

53. Franciotta D, Di Stefano AL, Jarius S, Zardini E, Tavazzi E, et al. (2011) Cerebrospinal BAFF and Epstein-Barr virus-specific oligoclonal bands in multiple sclerosis and other inflammatory demyelinating neurological diseases. J Neuroimmunol 230: 160-163.

54. Gandhi KS, McKay FC, Schibeci SD, Arthur JW, Heard RN, et al. (2008) BAFF is a biological response marker to IFN-beta treatment in multiple sclerosis. J Interferon Cytokine Res 28: 529-539.

55. Wang H, Wang K, Zhong X, Qiu W, Dai Y, Wu A, et al. (2012) Cerebrospinal Fluid BAFF and APRIL Levels in Neuromyelitis Optica and Multiple Sclerosis Patients During Relapse. J Clin Immunol 32: 1007-1011.

56. Dalakas MC (2008) Invited article: inhibition of B cell functions: implications for neurology. Neurology 70: 2252-2260.

57. Srivastava R, Aslam M, Kalluri SR, Schirmer L, Buck D, et al. (2012) Potassium channel KIR4.1 as an immune target in multiple sclerosis. N Engl J Med 367: 115-123.

58. Libbey JE, Peterson LK, Tsunoda I, Fujinami RS (2006) Monoclonal MOG-reactive autoantibody from progressive EAE has the characteristics of a natural antibody. J Neuroimmunol 173: 135-145.

59. Mayer MC, Meinl E (2012) Glycoproteins as targets of autoantibodies in CNS inflammation: MOG and more. Ther Adv Neurol Disord 5: 147-159.

60. Morimoto AM, Flesher DT, Yang J, Wolslegel K, Wang X, et al. (2011) Association of endogenous anti-interferon-Î± autoantibodies with decreased interferon-pathway and disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 63: 2407-2415.

61. Quintana FJ, Yeste A, Weiner HL, Covacu R (2012) Lipids and lipid-reactive antibodies as biomarkers for multiple sclerosis. J Neuroimmunol 248: 53-57.

62. Diamond B, Huerta PT, Mina-Osorio P, Kowal C, Volpe BT (2009) Losing your nerves? Maybe it's the antibodies. Nat Rev Immunol 9: 449-456.

63. Sato D, Fujihara K (2011) Atypical presentations of neuromyelitis optica. Arq Neuropsiquiatr 69: 824-828.

64. Rossi A, Ratelade J, Papadopoulos MC, Bennett JL, Verkman AS (2012) Consequences of NMO-IgG binding to aquaporin-4 in neuromyelitis optica. Proc Natl Acad Sci USA 109: E1511.

65. Yamasaki M, Matsumoto K, Takahashi Y, Nakanishi H, Kawai Y, et al. (2012) Case of NMO (neuromyelitis optica) spectum disorder triggered by interferon alpha, which involved extensive pyramidal tract lesion of the brain. Rinsho Shinkeigaku 52: 19-24.

66. Tumani H, Deisenhammer F, Giovannoni G, Gold R, Hartung HP, et al. (2011) Revised McDonald criteria: the persisting importance of cerebrospinal fluid analysis. Ann Neurol 70: 520.

67. Waschbisch A, Atiya M, Linker RA, Potapov S, Schwab S, et al. (2011) Glatiramer acetate treatment normalizes deregulated microRNA expression in relapsing remitting multiple sclerosis. PLoS One 6: e24604.

68. Steinman L (2009) A molecular trio in relapse and remission in multiple sclerosis. Nat Rev Immunol 9: 440-447.

69. Zhang H (2011) B-cell activating factor in the cerebrospinal fluid of neuromyelitis optica and multiple sclerosis. Mult Scler 17: 639-640.

70. Goertsches RH, Hecker M, Koczan D, Serrano-Fernandez P, Moeller S, et al. (2010) Long-term genome-wide blood RNA expression profiles yield novel molecular response candidates for IFN-beta-1b treatment in relapsing remitting MS. Pharmacogenomics 11: 147-161.

71. Keller A, Leidinger P, Lange J, Borries A, Schroers H, et al. (2009) Multiple sclerosis: microRNA expression profiles accurately differentiate patients with relapsing-remitting disease from healthy controls. PLoS One 4: e7440.

72. Otaegui D, Baranzini SE, Armañanzas R, Calvo B, Muñoz-Culla M, et al. (2009) Differential micro RNA expression in PBMC from multiple sclerosis patients. PLoS One 4: e6309.

73. Li JS, Yao ZX (2012) MicroRNA patents in demyelinating diseases: a new diagnostic and therapeutic perspective. Recent Pat DNA Gene Seq 6: 47-55.

74. Lange J (2010) microRNA profiling on automated biochip platform reveals biomarker signatures from blood samples. Nature Methods 7: 261-262.

75. Boz C, Oger J, Gibbs E, Grossberg SE (2007) Reduced effectiveness of long-term interferon-beta treatment on relapses in neutralizing antibody-positive multiple sclerosis patients: a Canadian multiple sclerosis clinic-based study. Mult Scler 13: 1127-1137.

76. Vallittu AM, Halminen M, Peltoniemi J, Ilonen J, Julkunen I, et al. (2002) Neutralizing antibodies reduce MxA protein induction in interferon-beta-1a-treated MS patients. Neurology 58: 1786-1790.

77. Comabella M, Lünemann JD, Río J, Sánchez A, López C, et al. (2009) A type I interferon signature in monocytes is associated with poor response to interferon-beta in multiple sclerosis. Brain 132: 3353-3365.

78. Axtell RC, Raman C, Steinman L (2011) Interferon-β exacerbates Th17-mediated inflammatory disease. Trends Immunol 32: 272-277.

79. Bushnell SE, Zhao Z, Stebbins CC, Cadavid D, Buko AM, et al. (2012) Serum IL-17F does not predict poor response to IM IFNβ-1a in relapsing-remitting MS. Neurology 79: 531-537.

80. Axtell RC, de Jong BA, Boniface K, van der Voort LF, Bhat R, et al. (2010) T helper type 1 and 17 cells determine efficacy of interferon-beta in multiple sclerosis and experimental encephalomyelitis. Nat Med 16: 406-412.

81. Axtell RC, Raman C, Steinman L (2012) Type I Interferons: Beneficial in Th1 and Detrimental in Th17 Autoimmunity. Clin Rev Allergy Immunol .

82. Axtell RC, Raman C (2012) Janus-like effects of type I interferon in autoimmune diseases. Immunol Rev 248: 23-35.

83. Lee LF, Axtell R, Tu GH, Logronio K, Dilley J, et al. (2011) IL-7 promotes T(H)1 development and serum IL-7 predicts clinical response to interferon-β in multiple sclerosis. Sci Transl Med 3: 93ra68.

84. van der Voort LF, Vennegoor A, Visser A, Knol DL, Uitdehaag BM, et al. (2010) Spontaneous MxA mRNA level predicts relapses in patients with recently diagnosed MS. Neurology 75: 1228-1233.

85. Hesse D, Sellebjerg F, Sorensen PS (2009) Absence of MxA induction by interferon beta in patients with MS reflects complete loss of bioactivity. Neurology 73: 372-377.

86. Hundeshagen A, Hecker M, Paap BK, Angerstein C, Kandulski O, et al. (2012) Elevated type I interferon-like activity in a subset of multiple sclerosis patients: molecular basis and clinical relevance. J Neuroinflammation 9: 140.

87. Baranzini SE, Mousavi P, Rio J, Caillier SJ, Stillman A, et al. (2005) Transcription-based prediction of response to IFNbeta using supervised computational methods. PLoS Biol 3: e2.

88. Cunninghame Graham DS, Morris DL, Bhangale TR, Criswell LA, Syvänen AC, et al. (2011) Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. PLoS Genet 7: e1002341.

89. Du C, Liu C, Kang J, Zhao G, Ye Z, et al. (2009) MicroRNA miR-326 regulates TH-17 development and is associated with the pathogenesis of multiple sclerosis. Nat Immunol 10: 1252-1259.

90. Siegel SR, Mackenzie J, Chaplin G, Jablonski NG, Griffiths L (2012) Circulating microRNAs involved in multiple sclerosis. Mol Biol Rep 39: 6219-6225.

91. Vyshkina T, Kalman B (2008) Autoantibodies and neurodegeneration in multiple sclerosis. Lab Invest 88: 796-807.

92. Racke MK (2012) Disease mechanisms in MS: The potassium channel KIR4.1—a potential autoantigen in MS. Nat Rev Neurol 8: 595-596.

93. Minagar A, Jy W, Jimenez JJ, Sheremata WA, Mauro LM, et al. (2001) Elevated plasma endothelial microparticles in multiple sclerosis. Neurology 56: 1319-1324.

94. Lowery-Nordberg M, Eaton E, Gonzalez-Toledo E, Harris MK, Chalamidas K, et al. (2011) The effects of high dose interferon-β1a on plasma microparticles: correlation with MRI parameters. J Neuroinflammation 8: 43.