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Review

IgM to phosphatidylcholine in multiple sclerosis patients: from the diagnosis to the treatment

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***Abstract*:** Multiple sclerosis (MS) is a demyelinating and neurodegenerative disease of the central nervous system. It affects young people, and a considerable percentage of patients need the help of a wheelchair in 15years of evolution. Currently, there is not a specific technique for the diagnosis of MS. The detection of oligoclonal IgG bands (OIgGBs) is the most sensitive assay for it, but it is not standardizable, only reference laboratories develop it, and uses cerebrospinal fluid. To obtain this sample, a lumbar puncture is necessary, an invasive proceeding with important side effects. It is important to develop and implement standard assays to obtain a rapid diagnosis because the earlier the treatment, the better the evolution of the disease. There are numerous modifying disease therapies, which delay the progression of the disease, but they have important side effects, and a considerable percentage of patients give up the treatment. In addition, around 40% of MS patients do not respond to the therapy and the disease progresses. Numerous researches have been focused on the characterization of predictive biomarkers of response to treatment, in order to help physicians to decide when to change to a second-line treatment, and then the best therapeutic option. Here, we review the new biomarkers for the diagnosis and response to treatment in MS. We draw attention in a new assay, the detection of serum IgM to phosphatidylcholine, that showed a similar sensitivity as OIgGBs and predicts the response to disease modifying treatments.

Keywords: biomarkers, diagnosis, prognosis, IgM, interferon-β, multiple sclerosis, phosphatidylcholine, Tysabri®

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, characterized by demyelination and axonal loss.[1] It is estimated that more than 2.8 million people are diagnosed around the world, and the incidence is increasing.[2] It usually presents between the third and fourth decade of the life and has a high social impact.[3–5]

The ethology is unknow,[6] and it is hypothesized that autoreactive lymphocytes migrate to the central nervous and would be the responsible of the demyelinating plaques.[7]

The lesions can affect all the areas of the central nervous system, the histopathology is different among patients,[8–11] and for these reasons, the clinical manifestations and the evolution of the patients are very variable.[12,13]

Consequently, the diagnosis is complex, and it is based on criteria diagnosis which include the assertion of symptoms related with demyelination and laboratory test.[14–16]

Regarding this, the hallmark of the disease is the presence of intrathecal IgG synthesis,[14,17–19] demonstrating the aberrant activation of B-lymphocytes in the central nervous system. Intrathecal IgG synthesis can be observed in approximately 70% of MS patients using quantitative methods.[17] These assays are based on the quantification of IgG and

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albumin in the cerebrospinal fluid (CSF) and serum using nephelometry.[20]

Nevertheless, the sensitivity of these procedures is lower compared to qualitative techniques. The later, based on isoelectric focusing and immunodetection assays demonstrated the presence of oligoclonal bands in approximately 90% of MS patients.[18,21–23]

Currently, the detection of oligoclonal IgG bands (OIgGBs) is the gold standard for the diagnosis of MS.[14,17,21–24] The pattern of OIgGBs is variable among MS patients, but it remains unchanged in the same patient.[23,25,26] Moreover, the IgG obtained extracted from autopsy brain plaques also showed and oligoclonal pattern.[27]

These evidences indicate that different antigens can be involved in the activation of the humoral immune response in MS patients. It was observed that IgG from the CSF target myelin and neuron antigens, such as, myelin basic protein,[28–31] myelin oligodendrocyte glycoprotein,[32] proteolipid lipoprotein,[31] myelin-associated glycoprotein,[33] glycolipids, fatty acids, and neurofilaments.[34] Other authors demonstrated that antibodies from MS patients bind to oligodendroglial and neuronal cell-lines.[35] Recently, is was demonstrated that OgIgGBs target debris.[21] These data indicate that IgG also plays a pathogenic role.

Unfortunately, the detection of OIgGBs is cumbersome and the sensitivity between laboratories is very variable.[36–38] Moreover, it is necessary to perform a lumbar puncture to obtain CSF, an invasive technique with important side effects.

An ideal biomarker must be specific of the disease, safe for the patient, easy to detect, and in the best case, the proceeding must be noninvasive. Blood samples fulfill these requirements, the obtention of the sample is safe, quick, and easy, and it could be performed at different timepoints.[39]

Regarding this, the presence in blood of B cell against brain antigens is related to disease relapses in MS.[40] The number of CD5+ B lymphocytes are also related with the activity of the disease.[41] These cells produce natural antibodies, most of which recognize lipids,[42–44] and it was described that serum antibodies to this antigens correlate with the damage of the brain tissue.[45] These data indicate that peripheral lymphocytes play a main role in myelin destruction and are in agreement with the mechanisms occurring in the central nervous during the progressive phase of MS.[7,46]

High levels of S100β in serum are detected during exacerbations in remittent recurrent MS patients.[47,48] There is a correlation between the levels of neurofilament light chain (NfL) in CSF and those observed in serum.[6,49,50] The levels of NfL associate with the activity of the disease,[51] number of lesions observed in magnetic resonance imaging (MRI)[50,52–54] and disability.[55] However, other authors indicate that there is no correlation between the levels of NfL and long-term disability or number of relapses overtime.[52]

In summary, there is not a universally accepted serum biomarker for the diagnosis or predicts the prognosis of MS up today. Therefore, it is necessary to determine new diagnosis and prognosis biomarkers in MS.

Here, we demonstrate that serum IgM to phosphatidylcholine (IgMPC) is a new diagnosis biomarker and also predicts the response to disease modifying therapies.

Antibodies to lipids are a main characteristic of MS patients

Myelin consists mainly of lipids, being the most abundant, cholesterol, PC, sphingomyelin, cerebrosides, and sulfatide.[56,57] Ganglioside (GD) are also main components of myelin and axolemma, especially at the node of Ranvier.[58,59] Moreover, glycolipid composition is particular of every glial cell subset, GD2 is characteristic of mature oligodendrocytes, and GD1and GD3 are present on their precursors.[60]

Consequently, numerous researchers have been working to develop new diagnosis techniques and to detect antibodies to lipids in MS.

Enzyme-linked immunosorbent *assay*s (ELISAs) demonstrated the high prevalence of antibodies to lipids in serum and CSF samples from MS patients (Table 1). They recognize a broad spectrum of gangliosides[61] (aGM1,[62] GM1,[37,58,62–66] GM2[37] GM3,[37,63,64] GM4,[37] GD1a,[58,62–64,67] GD1b,[37,58,63] GD2,[37,67] GD3,[37,63,64,67] GT1a,[37] and GTB[37]), sulfatide,[37,58,64,68,69] cerebrosides,[61,68,70] phosphatidylinositol,[68] cardiolipin,[71,72] and cholesterol.[68]

Table 1. Analysis of the reactivity to lipids in CSF and serum samples from MS patients.

Author	Antigen and positive patients (%)	Lipid positive MS patients (%)			
Acarin *et al.*[62] (S)	aGM1 (24% IgG/IgM); GM1 (38% IgG/IgM); GD1a 33% (IgG/IgM)	48% of Total 36% RR IgG/IgM	33% SP IgG/IgM	100% PP IgG/IgM	
Sadatipour *et al.*[63] (S)	GM1; GM3; GD1a, GD1b; GD3	2.9% RR IgG/IgM/IgA/IgD	42.9% SP	56% PP	
Mata *et al.*[58] (S)	GM1 (10% IgG); GD1a (23% IgG; 10% IgM); GD1b (13% IgG; 6% IgM); Sulfatides (3% IgG; 7% IgM); Cardiolipin (3% IgG)	50% MALIGNANT IgG	6% BENIGN IgG		
(CSF)	GM1 (7% IgM); GD1a (13%, IgG); Sulfatides (3% IgG, 16% IgM); Cardiolipin (20% IgM)	26% MALIGNANT IgG	6% BENIGN IgG		
Giovannoni *et al.*[64] (S)	GM1; GM3; GD1a; GD3; GT1; GQ1; Sulfatides				
Marconi *et al.*[67] (S)	GD1a; GD2; GD3	30% of Total (IgM) 24.2% RR GD2+	50% SP GD2+	26.7% GD2+	
Menge *et al.*[70] (S)	Galactocerebroside	<10% CIS	40% RR IgG	26.7% SP IgG	<10% PP IgG
Jurewicz *et al.*[68] (S)	Sulfatides; Galactocerebroside; Phosphatidylinositol; cholesterol (IgG/IgM)	RR			
Ilyas *et al.*[69] (CSF)	Sulfatides	19.74% of Total IgG/IgM 15% RR	30% SP	14% PP	
Ivanova *et al.*[37] (S)	GM1 (10% IgG/IgM); GM2; GM3; GM4; GD1a; GD1b; GD2; GD3; GT1a; GT1b; GQ1b; Sulfatides (33.3% IgG/IgM)	42.3% Ig/IgM 38.1% RR	51.4% SP		
Colaço *et al.*[71] (S)	Cardiolipin (29.4% IgM)	29.4% IgM			
Lolli *et al.*[72] (S)	Cardiolipin	2% IgG; 7% IgM			
(CSF)		5% IgG; 7% IgA, 9% IgM			
Mathiesen *et al.*[65] (CSF)	GM1 (16.7% IgG)	16.7% IgG			
Marchiori *et al.*[61] (CSF)	Gangliosides (19.2% IgG; 7.7% IgM); Cardiolipin (46.2% IgG; 6.0% IgM); Galactocerebrosides (17.7% IgG; 11.8% IgM)				
Bech *et al.*[66] (S)	GM1 IgM				
Sádaba *et al.*[73] (S)	Phosphatidylcholine (IgM)	88.2% CIS; 88.7% RR	58.0% SP	59.5% PP	11.1% BENIGN

aGM1, asialoganglioside; CIS, Clinically isolated syndrome; CSF, cerebrospinal fluid; GD, ganglioside D; GM, ganglioside M; GT, ganglioside T; GQ, ganglioside M; MS, Multiple sclerosis; PP, Primary progressive; RR, Relapsing-remitting; S, serum; SP, Secondary progressive.

Interestingly, new microarray techniques showed the presence in the CSF of antibodies to lipids up to 60% of MS patients, being sulfatides the antigens recognized in most cases.[74–76]

Nevertheless, the incidence of the antibodies to this lipids is lower compared with that of the OIgGBs,[14,17,18,77] and these techniques are not currently used in the diagnosis of MS.[73]

Serum antibodies to PC are a diagnostic marker in MS

We developed a high sensitive ELISA to detect antibodies to lipids. We overcame the difficulties regarding the antigen solubility, accessibility of reactive groups, and the requirement of auxiliary lipids.

This assay demonstrated the upregulated concentration of IgMPC in serum samples from MS patients. More interesting, almost 90% of MS patients in the first stages of the disease, clinical isolated syndrome (CIS) or relapsing remitting MS, had serum IgMPC. However, a minimum percentage of benign patients and control group showed serum IgMPC[73] (Figure 1).

The sensitivity of our new assay is similar to the best obtained using the detection of OIgGBs, the gold standard for the diagnostic of MS at the moment. In other words, the detection of IgMPC in serum samples is a diagnostic marker in this disease and has numerous advantages compared with the detection of OIgGBs.[73,78] It is an easily reproducible technique; it can be automatable, and it does not use CSF.

Antibodies to lipids are a prognosis marker

Different reports demonstrated the higher prevalence of antibodies to gangliosides in the progressive forms than in the first stages of the disease.[62,63] In this line, it was also published that a significant percentage of MS patients with malignant course have IgG to gangliosides, sulfatides, and cardiolipin in serum and CSF.[58] However, other authors did not find this correlation, and the role of the antibodies to these antigens as prognosis biomarker remains unclear.

Intrathecal IgM synthesis correlates with a severe disease course of the disease.[79–82]

The most sensitive assay to detect intrathecal IgM synthesis[83] demonstrated the presence of oligoclonal IgM bands (OIgMBs) in CSF in approximately 45% of the MS patients.[84] Patients with OgIgMBs predict the conversion to clinically defined MS and a rapid progression. We observed

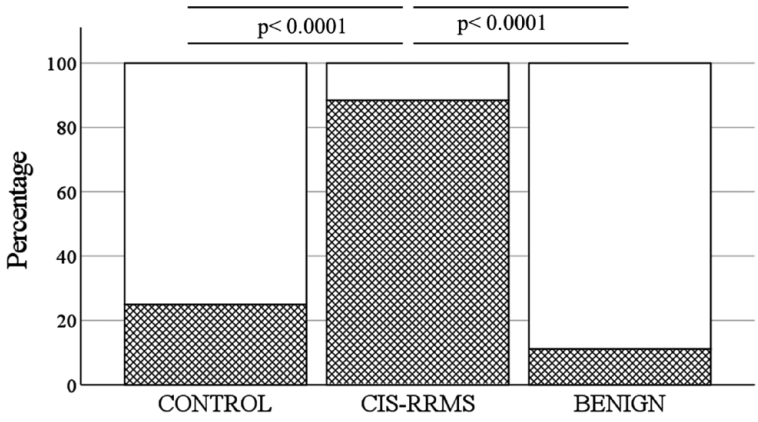

Figure 1. Percentage of positives (white bars) or negatives (squared bars) for IgMPC in MS patients in the first stages of the disease (CIS or RR) or benign form and control group. The percentage of positives for IgMPC was higher in patients in the first stages of the disease (88.5%) than in patients with the benign form (11.1%) and control group (25%)
Modified from Sádaba *et al.*[73]
CIS, clinical isolated syndrome; IgMPC, IgM to phosphatidylcholine; MS, multiple sclerosis; RR, relapsing remitting.

that these immunoglobulins recognized phospholipids and glycolipids, being PC the antigen recognized in most cases.[85] Patients with OIgMBs to lipids have a poor prognosis, with higher rate of relapses, faster increase of disability, remarkable brain atrophy and lesion load, and early development of the secondary progressive phase.[85–90] Currently, the detection of OIgMBs to lipids is the most sensitive biomarker for the prognosis of MS.[91,92]

Nevertheless, the sensitivity to detected IgM in CSF varies among laboratories,[92] as described for the detection of OIgGBs, and some authors did not observe relation between the presence of OIgMBs and the progression of the disease.[93–95]

However, using our reproducible and high sensitive ELISA, we observed that most of the benign MS patients did not have serum IgMPC.[77]

Antibodies to lipids are pathogenic

The increased concentration of IgM in CSF is related with the MRI lesion load,[79] and the presence of antibodies to lipids in CSF[87] and serum[45] correlates with brain atrophy.

The presence of OIgMBs in MS patients correlate with increased percentages of B1-lymphocytes in CSF and blood,[85,88,96] the B cells subset producer of antibodies to lipids. Moreover, the increased number of B1-lymphocytes in peripheral blood predicts the conversion to MS in those patients with an unclear diagnosis,[41] demonstrating the pathogenic role of this cells.

The histological studies also demonstrated the pathological role of antibodies to lipids and B cells. We observed in brain samples from MS patients, IgM deposits colocalizing with complement cascade factors, which in turn could mediate the lysis of oligodendrocytes and axons, and the phagocytosis of debris by activated macrophages.[97] In fact, IgM deposits colocalize with oligodendrocyte and axonal damage.[98] In this line, other authors also demonstrated that antibodies are the main characteristic of new forming lesions.[99] Corroborating the data obtained in CSF and serum, we also observed that the activity of the lesions is related with the number of B cells and plasma cells in the perivascular space and meninges.[98]

Other authors demonstrated that IgM anti-sulfatide or anti-galactocerebroside from MS patients recognize oligodendrocytes and myelin, causing demyelination 'in vitro' and 'in vivo'.[100]

Moreover, immunization of experimental animals with lipids or antibodies to lipids causes the development of experimental autoimmune encephalomyelitis. These models demonstrated that antibodies are necessary to induce similar pathology to that observed in humans.[74,101–106]

Antibodies to lipids predict the response to disease modifying treatments

Treatments do not cure the disease but delay the progression in most cases. Unfortunately, a considerable percentage of patients do not respond to the first-line therapies or suffer from important side effects.[107–109] Currently, the response or not to treatment is identified based on the occurrence of new relapses or the increase of disability. This is problematic because as the disease progress, patients do not recover completely the neurological function after the relapse. Thus, numerous researches aimed to characterize biomarkers of response to treatment, and thus, to develop personalized therapies, improving their effectiveness while minimizing adverse events.

Recently, we observed that the rapid decrease of serum IgMPC is a biomarker of response to interferon-β (Figure 2(a)). Most of responders to Rebif® (Merck) (83.3%) or Betaferon® (Bayer) (85.7%) showed a diminution of serum IgMPC after 6 months of treatment [Figure 2(b)].

Some patients who did not respond to interferon-β were treated with natalizumab. We demonstrated that those with the highest levels of IgMPC responded subsequently to natalizumab.[78] This was expected because these patients have an aggressive course, with more relapses and faster increase of the disability, and they do not respond to first-line therapies.[78]

Natalizumab prevents the migration of lymphocytes through the blood–brain barrier, but, as we and other groups demonstrated, there are resident immune cells in the central nervous system.[98,110,111] Regarding this, natalizumab does not eliminate completely the presence of OIgMBs

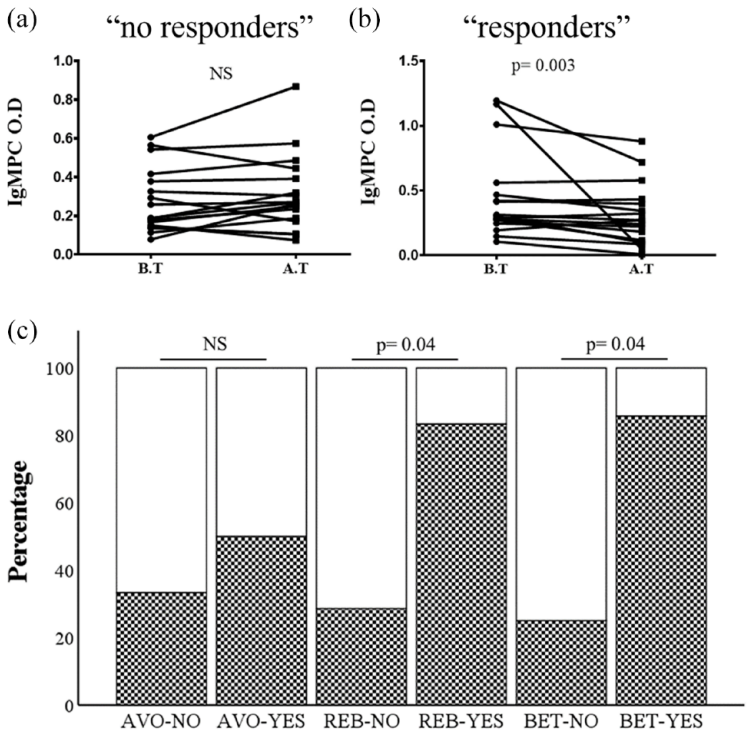

Figure 2. Levels of IgMPC before (B.T) and after (A.T) treatment in patients no responders (a) and responders (b) to interferon-β. (c) Percentage of patients showing a decrease of the levels of IgMPC after 6 months of treatment.
Source: Modified from Muñoz *et al.*[78]
AVO-NO, Patients who did not respond to Avonex® (Biogen); AVO-YES, Patients who respond to Avonex®; REB-NO, Non-responders to Rebif®; REB-YES, Responders to Rebif®; BET-NO, Non-responders to Betaferon®; BET-YES, Responders to Betaferon®; IgMPC, IgM to phosphatidylcholine; Squared bars, Decreased IgMPC levels; White bars, Non-decrease of IgMPC levels.

in CSF,[112] that, as described above, could play a main role in the tissue damage.

To predict the response to first-line therapies and natalizumab is of great interest to initiate the treatment of natalizumab as soon as possible, and thus to avoid the migration to the central nervous system of lymphocytes and the progression of the disease.

Conclusions

The detection of serum IgMPC is of great utility for the rapid diagnosis of MS and thus to treat the patients earlier. The later avoids the progression of the disease, but a considerable percentage of patients do not respond to first or second-line therapies. The levels of serum IgMPC predict the response to interferon-β and natalizumab. Thus, the quantification of these immunoglobulins is of great interest to decide when to change to a second-line treatment and then, the best therapeutic option.

Declarations

Ethics approval and consent to participate

Not applicable for Review articles.

Consent for publication

Not applicable for Review articles.

Author contributions

Isabel Sánchez-Vera: Conceptualization; Formal analysis; Writing – original draft; Writing – review & editing.

Esther Escudero: Conceptualization; Formal analysis; Writing – original draft; Writing – review & editing.

Úrsula Muñoz: Conceptualization; Formal analysis; Funding acquisition; Writing – original draft.

María C. Sádaba: Formal analysis; Funding acquisition; Methodology; Writing – original draft; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The corresponding authors will provide anonymized data of this study on reasonable request from any qualified investigator, following relevant data protection regulations.

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