

Prevalence of Classic and Non-Classic Antiphospholipid Antibodies in Multiple Sclerosis

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ABSTRACT: **Background:** The association between antiphospholipid antibodies (aPL) and multiple sclerosis (MS) has been suggested previously, but prior studies provided contradicting findings. **Objectives:** To characterize the expression profile of eight classic and non-classic aPL in patients diagnosed with MS. **Methods:** Using the BioPlex™ 2200 immunoassay, we measured the levels of serum immunoglobulin (Ig)M and IgG isotypes of three classic aPL and five non-classic aPL in 98 subjects with MS and 237 healthy controls. **Results:** Three non-classic aPL were significantly more prevalent among MS patients in comparison to the control group. These antibodies included IgM and IgG against phosphatidylserine-β2GPI (PS-B2), IgG prothrombin complex (PT-PT) and IgM prothrombin (PT). The positive results according to Bonferroni correction are PS-B2 IgG and PT-PT IgG. The remaining aPL profiles did not differ significantly between the two groups. **Conclusions:** An association between certain non-classic aPL and MS has been established. The specific role of these autoantibodies in the pathogenesis of the condition remains uncertain.

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Multiple sclerosis (MS) is an immune mediated disease characterized by multifocal areas of demyelination, as well as by inflammation, axonal/neuronal loss and gliosis, in both the white and gray matter of the central nervous system (CNS). The pathophysiology of MS is multifactorial and is affected by genetic, environmental and immunological factors [1].

Antiphospholipid syndrome (APS), also known as Hughes' syndrome, is another systemic autoimmune disorder affecting

young adults. It is associated with thrombotic and non-thrombotic events and may manifest clinically as recurrent spontaneous abortions, arterial and/or venous thromboembolism, thrombocytopenia, livedo reticularis, pulmonary hypertension, or a neurological condition [2]. CNS involvement may present as migraine headache, cerebrovascular accident, transient ischemic attack, cognitive impairment, or chorea/ballismus, but it might also mimic MS, clinically as well as radiologically [2,3]. It is a potentially life-threatening condition, marked by the presence of circulating antiphospholipid antibodies (aPL). These antibodies are a heterogeneous group of autoantibodies that bind phospholipid (PL), protein-PL complexes, or PL-binding proteins [4]. aPL include the classic antibodies commonly used in the diagnosis of APS: lupus anticoagulant (LA), beta 2-glycoprotein I (β2GPI), and anticardiolipin (aCL) antibodies [5]. The non-classic aPL include phosphatidylserine-β2GPI (PS-B2), phosphatidylethanolamine (PE), prothrombin (PT), prothrombin complex (PT-PT), and phosphatidylserine-prothrombin (PS-PT) [6].

The diagnosis of MS is based on dissemination of signs in time and space, supported by findings of magnetic resonance imaging (MRI), evoked potentials and cerebrospinal fluid (CSF), and the exclusion of other diagnoses. However, definite diagnosis and differentiation of MS from other autoimmune conditions such as APS remain a challenge [3].

The prevalence of aPL in MS patients differs among various studies, with several researchers suggesting that the pathophysiology of MS in patients with positive aPL sera titers differs from that of seronegative patients [7]. In the present study, we characterized the expression profile of the three classic aPL, as well as five non-classic aPL, in subjects with MS and compared the results with those in healthy controls.

PATIENTS AND METHODS

This cross-sectional study included 98 subjects with a diagnosis of MS and 237 healthy controls. MS patients were recruited at the Asociación para la Lucha contra la Esclerosis Multiple

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(ALEM) in Medellín, Colombia, while the healthy controls were recruited both in Columbia and Italy. All subjects in the study met the Poser criteria for the diagnosis of MS [8].

The study was approved by the Institutional Review Board or Ethics Committee at the participating institutions and adhered to the Declaration of Helsinki and to Resolution 008430 of 1993 of the Ministry of Health of Colombia.

We assessed and quantified 16 different autoantibodies in the sera of 335 patients (98 MS, 237 controls). Due to technical factors, two MS patients were excluded from the IgM isotype analyses, hence 333 samples were analyzed.

The levels of serum IgG and IgM isotypes of the three classic and five non-classic aPL were measured using the BioRad BioPlex™ 2200 system (USA). The classic aPL included β 2GPI, aCL and cardiolipin- β 2GPI (CL-B2). The non-classic aPL included PS-B2, PE, PT, PT-PT and PS-PT.

The BioPlex™ 2200 immunoassay uses sets of magnetic 8 μ m beads that are infused with varying ratios of fluorescent dyes, creating unique bead identifiers corresponding to a specific antibody. Flow cytometry-based technology allows for simultaneous detection and quantification of the 10 isotypes tested from a single sample. Each subject aliquot specimen was added to a reaction well containing bead reagent and sample diluent. Following an incubation period (37°C) and washing procedures a phycoerythrin-conjugated antibody was added. After a second incubation and further washing, the beads were read by a flow-based detector which quantified each analyte and compared it to a pre-established calibration curve. The proteomic data were initially calculated as relative fluorescence intensity and then converted to a fluorescence ratio (FR) using an internal standard bead included in every bead set to normalize the detector signal. Finally, The FR was compared to an assay-specific calibration curve to determine the reading as antibody concentration units (antibody index, AI). For quality assurance two additional control beads were included in all incubations and a serum verification bead and a blank bead were added to verify the addition of serum to the reaction vessel and the absence of significant non-specific binding, respectively. The cutoff values to determine a positive result were calculated based on the control cohort by applying the following formula: mean antibody index + 2 standard deviations.

Statistical analysis was conducted using two-tailed Fisher's exact test by GraphPad prism 5 software (GraphPad Software Inc, San Diego, CA, USA). A *P* value < 0.05 was considered statistically significant.

RESULTS

The study population included 23 males and 75 females with MS; their mean age was 45.1 ± 10.3 years and mean duration of disease 12.4 ± 8 years. As previously mentioned, 237 sub-

jects served as the control group. The results are summarized in Table 1, and Figures 1 and 2.

A review of the titer analyses of IgM and IgG isotypes of classic aPL did not reveal a higher prevalence of these antibodies among MS patients when compared to control subjects. Moreover, both CL-B2 IgG and β 2GPI IgG serum profiles were significantly more widespread among healthy controls. While 19 of the 237 controls (8%) were found to be positive for β 2GPI IgG and 12 of the 237 controls (5.1%) were found positive for CL-B2 IgG, none of the MS patients had either β 2GPI IgG or CL-B2 IgG antibodies (*P* < 0.001 and *P* < 0.014, respectively).

An analyses of IgG and IgM isotypes of non-classic aPL revealed a higher incidence of both IgM and IgG PS-B2 antibodies among MS patients when compared to healthy controls (*P* < 0.011 and *P* < 0.0001, respectively). In addition, 45 of 96 MS patients (46.9%) had high titers of PT IgM, as compared to 83 of 237 controls (35%) (*P* < 0.03). However, the PT IgG occurrence was significantly lower among MS patients (3.1% among MS patients and 12.7% among controls, *P* < 0.004). Another interesting finding was the higher prevalence of PT-PT IgG antibodies among MS patients: 71.4% (70 of 98 MS patients) compared to 9.7% (23 of 237 controls) (*P* < 0.0001).

Table 1. Antiphospholipid antibodies profile in MS patients and healthy controls

Antiphospholipid antibody	Positive MS patients (n) %	Positive control subjects (n) %	<i>P</i> value*
aCL IgM	(5/96) 5.2	(15/237) 6.3	0.459
aCL IgG	(3/98) 3.1	(13/237) 5.5	0.261
β 2GPI IgM	(3/96) 3.1	(13/237) 5.5	0.273
β 2GPI IgG	(0/98) 0.0	(19/237) 8.0	0.001
CL-B2 IgM	(7/96) 7.3	(20/237) 8.4	0.461
CL-B2 IgG	(0/98) 0.0	(12/237) 5.1	0.014
PS-B2 IgM	(14/96) 14.6	(14/237) 5.9	0.011
PS-B2 IgG	(22/98) 22.4	(13/237) 5.5	0.0001
PS-PT IgM	(3/96) 3.1	(13/237) 5.5	0.273
PS-PT IgG	(4/98) 4.1	(24/237) 10.1	0.049
PT-PT IgM	(6/96) 6.3	(35/237) 14.8	0.021
PT-PT IgG	(70/98) 71.4	(23/237) 9.7	0.0001
PT IgM	(45/96) 46.9	(83/237) 35.0	0.03
PT IgG	(3/98) 3.1	(30/237) 12.7	0.004
PE IgM	(3/96) 3.1	(13/237) 5.5	0.273
PE IgG	(5/98) 5.1	(13/237) 5.5	0.563

*Two-tailed Fisher's exact test

aCL = anticardiolipin, β 2GPI = beta 2-glycoprotein I, CL-B2 = cardiolipin- β 2GPI, IgG = immunoglobulin G, IgM = immunoglobulin M, MS = multiple sclerosis, PE = phosphatidylethanolamine, PS-B2 = phosphatidylserine- β 2GPI, PS-PT = phosphatidylserine-prothrombin, PT = prothrombin, PT-PT = prothrombin complex

However, PT-PT IgM was significantly less frequent among MS patients compared to controls (6.3% vs. 14.8%, $P < 0.021$). The prevalence of both IgG PS-PT and IgM was lower among MS patients in comparison to the controls (3.1% vs. 5.5%, and 4.1% vs. 10.1%, respectively), yet only the former was statistically significant ($P < 0.05$). The statistically significant results according to Bonferroni correction were found for PS-B2 IgG and PT-PT IgG. Other aPL profiles that were measured in the two groups did not show any significant or meaningful differences.

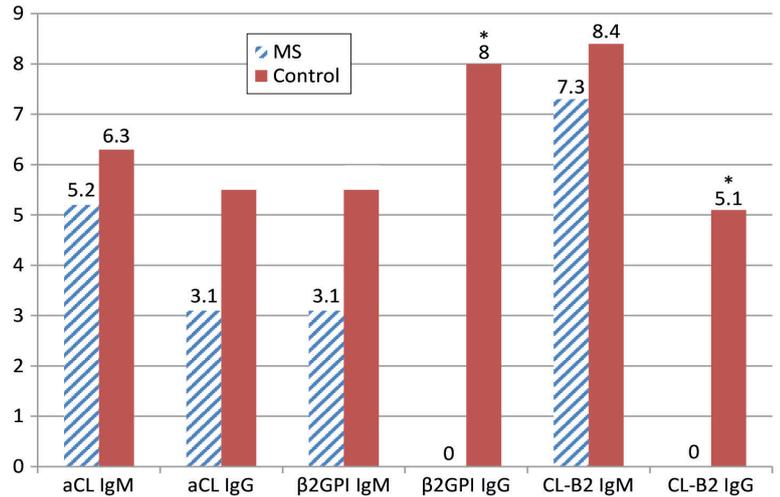
DISCUSSION

Non-thrombotic neurological manifestations in APS present a diagnostic challenge and on occasion can mimic classic presentation of MS [9]. In this study, we demonstrated that three non-classic aPL – IgM and IgG PS-B2, PT-PT IgG, and PT IgM – were significantly more prevalent among MS patients.

As mentioned, both the prevalence of aPL in MS patients and its clinical significance are highly debated. The prevalence of aPL in MS, without clinical manifestations of autoimmune disease, is mostly reported to be lower than 10%, yet several studies reach as high as 80% [10-17]. Although the prevalence of aPL reported by Chapman [11] was 32%, none of his subjects had β 2GPI, a main APS antibody. A Polish study showed increased prevalence of β 2GPI IgM (20% vs. 3.3%), but not aCL, among MS patients. These were associated with secondary progressive MS (SPMS), but not with the predominant site of neurological involvement [12]. Nearly half of 33 Brazilian MS patients were found to have aCL IgG in their CSF samples [13]. Eighty percent of 24 relapsing-remitting MS (RRMS) patients were found to have elevated aPL IgM, but no IgG, during disease exacerbation (aCL, β 2GPI, PC, PS, and PE) with correlation to MRI-enhancing lesions associated with PC and PS. In another study, β 2GPI was positive in 80% of acute MS patients [14]. The authors suspected that aPL may be involved in the pathogenesis of MS, although this might have been epiphenomenal [15]. A Dutch study showed that aPL occurred in 55% of MS patients, namely IgM aCL and PE, and were more common in SPMS when compared to RRMS [10]. Estonian researchers showed a higher prevalence of aCL in patients with absent CSF oligoclonal bands, but without clinical or laboratory significance [16]. Unique brain abnormalities were evident in RRMS and SPMS patients with aPL in comparison to seronegative patients [17]. Five of 17 British MS patients (29%) were found to have aCL IgM [18]. A French study found a prevalence of 32.6% of IgG and IgM aCL and/or β 2GPI among 89 MS patients [19].

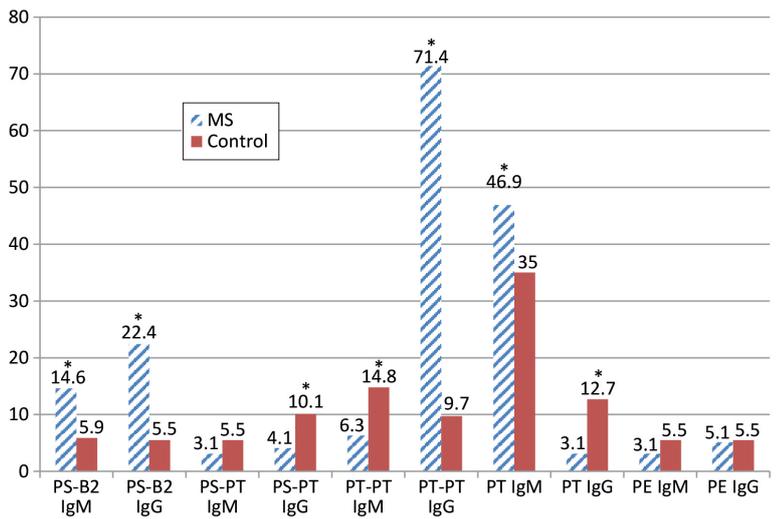
In contrast to the above, a Dutch study showed no increase in aCL in MS patients [20], and 12 Mexican MS patients, mainly females, were found to have no VDRL or CL- β 2GPI complex in their sera or CSF [21]. aCL or β 2GPI were found in only 5 of 62 French MS patients (8%) [22], while aPL in French MS patients were reported to be 6.2% [23]. Presence of aPL was not found to

Figure 1. Classic antiphospholipid antibodies in MS patients and healthy controls. The percentage of classic aPL isotypes among MS patients is compared to healthy controls



* P value < 0.05 . aCL = anti-cardiolipin, β 2GPI = beta 2-glycoprotein I, CL-B2 = cardiolipin- β 2GPI, IgG = immunoglobulin G, IgM = immunoglobulin M, MS = multiple sclerosis

Figure 2. Non-classic antiphospholipid antibodies in MS patients and healthy controls. The percentage of non-classic aPL isotypes among MS patients is compared to healthy controls



P value < 0.05

IgG = immunoglobulin G, IgM = immunoglobulin M, MS = multiple sclerosis, PE = phosphatidylethanolamine, PS-B2 = phosphatidylserine- β 2GPI, PS-PT = phosphatidylserine-prothrombin, PT = prothrombin, PT-PT = prothrombin complex

be associated with age, gender, disease duration, symptomatology, type of MS, disease course, or MRI findings [19].

The controversy is evident in Asia as well, with a Japanese study on 32 MS patients showing rates of aCL and PS to be as high as 44%, mainly IgM, yet with no clinical differences between seropositive and seronegative patients [24].

Our study demonstrated a significant association between certain aPL and MS. The specific role of these autoantibodies in the pathogenesis of MS remains uncertain and requires additional research. Measurement of aPL should be routinely performed in patients with neurologic disorders, especially those with atypical presentations, along with the utilization of visual evoked potential, which was found to be abnormal more frequently among MS patients in comparison to APS [25].

Further collaborative studies should focus on the clinical presentation of the recruited MS patients and explore an association between the prevalence of a specific aPL and the clinical manifestation, type and course of the disease. Emphasis should also be given to the ethnicity of the subjects, with the goal of recruiting a diverse population from multiple centers worldwide. Another factor that needs to be considered is the effect of MS therapy, since up to a quarter of MS patients who are treated with interferon-beta (IFN β) develop antibodies against it. The presence of aPL is reported to be associated with concurrent anti-IFN β antibodies. Future studies should also take into account autoimmunity. Our patient population did not include data regarding diagnosis of APS, whether as a primary or secondary condition.

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