Lymphocytopenia is associated with anti-Beta-2 glycoprotein-1 in patients with systemic lupus erythematosus

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ABSTRACT

Background: Anti B2-Glicoprotein 1 (B2-GPI) is an antiphospholipid antibody that may be present in primary or secondary antiphospholipid syndrome (APS). Systemic Lupus erythematosus (SLE) is the main disease associated with secondary APS.
Objective: To study the prevalence of anti B2-GPI in SLE patients.
Methods: Anti B2-GPI (IgM/IgG) was studied by ELISA in 88 patients with SLE of both genders; 18.6% of which with secondary APS. Charts were reviewed for clinical and serological profile.
Results: Anti B2-GPI was present in 18.6% of the whole sample and in 29.4% of those with secondary APS. At univariated analysis, the presence of anti B2-GPI was more common in patients with serositis (p=0.04), lymphocytopenia (p=0.003) and anti cardiolipin (aCl) IgM antibodies (p=0.04). In a logistic regression study, only the associations with lymphocytopenia (OR=8.2; 95%CI=2.1-39.3) and aCl IgM (p=0.04; OR=3.4; 95%CI=1.05-11.1) remained significant.
Conclusion: There is a 18.6% prevalence of positive anti B2-GPI in SLE population that is associated with the presence of aCl IgM and lymphocytopenia.

Keywords: Antiphospholipid antibody syndrome; Anticardiolipin; Antiphospholipid antibodies; Systemic lupus erythematosus

INTRODUTION

Antiphospholipid antibodies are a family of autoantibodies that may be related to antiphospholipid antibody syndrome (APS) or that may appear without clinical significance[1]. Anti Beta-2 glycoprotein-I (B2-GPI) is one of these antiphospholipid antibodies that is used as laboratory Sapporo Classification Criteria for APS[2]. Other antibodies are anticardiolipin (aCl) IgG and IgM and lupus anticoagulant (LA)[2].

APS is a disease that causes gestational morbidity due to placental insufficiency, venous and arterial thrombosis of vessels of all sizes and some non thrombotic manifestations such as thrombocytopenia, *livedo reticularis*, skin ulcers, endocarditis, seizures and chorea[1]. The mechanism of action of these autoantibodies is not completely clear. It is believed that they may result from a variety of effects upon coagulation pathways, including the pro-coagulant actions on protein C, S, annexin V, platelets, serum proteases, tissue factor and impaired fibrinolysis[4-8]. Antiphospholipid antibodies may also increase vascular tone enhancing susceptibility to atherosclerosis[8].

B2-GPI is a naturally occurring inhibitor of coagulation and platelet aggregation[9]; the properties of this protein as a clotting inhibitor is one of the explanations why this neutralizing antibody promotes thrombosis[10].

APS occurs either as a primary condition or in the setting of an underlying disease, mainly systemic lupus erythematosus (SLE) and other connective tissue diseases[1]. Antibodies to B2-GPI are found in both primary or secondary disease[11] and usually appear associated with other antiphospholipid antibodies although they may be the only one present in up to 11% of patients[11].

A study done with 281 patients (111 with primary and secondary APS) showed that a total of 28% were positive for B2-GPI and that its presence was strongly associated with the presence of another antiphospholipid antibody[11]. In the same study, anti B2-GPI was present in 42% of APS patients and was more common in primary than in secondary disease[11]. Another study in a Serbian population[12] with 374 APS patients (260 with primary disease) positivity was found in 31.9%

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for IgG B2-GPI and 37.7% for IgM B2-GPI in primary APS and in 43% for IgG B2-GPI and 44.7% for IgM B2--GPI in the secondary disease. In Malaysian population IgG B2-GPI was found in 54.6% of 11 patients with primary APS, in 35% of 20 lupus patients with secondary APS and in 7.8% of 51 SLE patients[13].

In the present study, we aimed to investigate the prevalence of anti B2-GPI in a sample of Brazilian SLE patients with and without secondary APS seeking to understand the associations of this autoantibody with other clinical and serological manifestations of lupus.

METHODS

This is a transversal and observational study that was approved by the local Committee of Ethics in Research. All patients signed consent prior to participation.

A total of 88 SLE patients, with at least 4 American College of Rheumatology classification criteria for this disease[14], were included. This was a convenience sample from a single Outpatient Clinic chosen according to consultation order and willingness to participate in the study. This sample had 86 (97.7%) women and two men; and 16/88 (18.2%) were diagnosed with secondary APS syndrome according to the Sapporo classification criteria[2].

Venous blood samples were taken from all patients, aliquoted and preserved at -80°C until serological tests were performed. Serum levels of anti B2-GPI (IgG and IgM) were measured by ELISA (Orgentec®, Germany). Cut-off used was set to 40 U/ml according to manufacturer's instructions. Medical charts were reviewed for cumulative clinical data and autoantibody profile. Collected clinical data were considered as defined by the 1997 revised Classification Criteria of American College of Rheumatology for SLE[14]. Autoantibodies considered for analysis were: anti Ro/SS-A, anti La/SS-B, anti RNP, anti Sm, anti dsDNA, IgG anticardiolipin (aCl), IgM aCl, LA (lupus anticoagulant), direct Coombs and rheumatoid factor (RF). Anti Ro/SS-A, anti La/SS-B, anti RNP, anti Sm, IgG aCl, IgM aCl were performed by ELISA (Orgentec®, Germany); anti dsDNA by immunofluorescence (IFI) using *Crithidia luciliae* as substrate (Inova, USA). Lupus anticoagulant was examined through a screening test, the dRVVT (dilute Russell viper venom test) and confirmed by RVVT. Antiphospholipid antibodies were considered positive if present in, at least, two ocasions.

Data were collected in contingency and frequency tables. Distribution was analyzed by Kolmogorov Smirnov test. Central tendency was expressed in mean and standard deviation (SD) for parametric data and median and interquartile range (IQR) for nonparametric data. Fisher and chi-squared tests were used for association studies of nominal data and unpaired t test and Mann Whitney were used for numeric data. Variable with $p<0.05$ in the univariate analysis, were submitted to analysis through a model of logistic regression to determine the odds ratio (OR) and 95% confidence interval. Calculation was done with help of the software Graph Pad Prism version 4.0 and Medcalc version 12.1.3.0. The adopted significance was 5%.

RESULTS

Patients from the studied sample had a median age of 41.6 (IQR = 29-48) years and mean disease duration of 99.0±56.05 months. The clinical and serological profile is seen in Table 1.

Anti B2-GPI was present in 17/88 (19.3%) of the SLE patients. In the SLE population with APS, there was one patient positive for anti B2-GPI which was the only antiphospholipid antibody present. In 40/88 (45.4%) SLE patients there were at least one aCl (IgG and/or IgM) and/or LA positive. In this subgroup, 11/40 (27.5%) were also positive for anti B2-GPI. Considering all positive patients for anti B2-GPI (n=17), six of them (35.2%) had no other concomitant antiphospholipid antibody.

Comparing the SLE population positive for anti B2--GPI with those negative, we found data demonstrated in Table 2. It is evident that patients with positive anti B2-GPI had more serositis, lymphopenia and IgM-aCL. With a logistic regression study, where anti B2-GPI was considered the dependent variable, including all variables with $p \leq 0.05$ (serositis, lymphopenia and IgM-aCl) only lymphopenia (p=0.002; OR=9.2; 95% CI=2.1-39.3) and IgM aCl (p=0.04; OR=3.4; 95%CI=1.05-11.1) remained significant.

DISCUSSION

Our results showed a 19.3% prevalence of B2-GPI in lupus patients in a sample where almost 30% had APS syndrome. This result is very similar to those of a large European series of 574 SLE patients from 7 countries

TABLE I. CLINICAL AND SEROLOGICAL CUMULATIVE PROFILE OF 88 PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

	n	%
Arthritis	46/87	52.8
Discoid rash	10/86	11.62
Photosensitivity	60/85	70.5
Malar rash	39/88	44.3
Aphta	40/87	45.9
Psycosis	6/87	6.8
Seizures	11/87	12.6
Serositis	12/88	13.6
Nephritis	32/88	36.6
Leucopenia	25/88	28.4
Thrombocytopenia	23/88	26.1
Lymphocytopenia	11/82	13.4
Hemolysis	8/88	9.09
Anti RNP	19/81	23.4
Anti Sm	14/86	16.2
Anti Ro	29/87	33.3
Anti La	16/86	18.6
Anti dsDNA	25/88	28.45
Antiphospholipid syndrome (obstetrical criteria)	9/86	10.34
Antiphospholipid syndrome (vascular criteria)	8/86	9.3
Antiphospholipid syndrome (total)	16/86	18.6
IgG anticardiolipin	23/87	26.4
IgM anticardiolipin	22/88	25
Lupus anticoagulant	19/83	22.9
Positive coombs	4/78	5.1

where 20% positivity was found[15]. In one of the studied patients with SLE and secondary APS, B2-GPI was the only antiphospholipid antibody detected demonstrating the importance of its search when APS is suspected.

In our sample it was possible to note an association between B2-GPI presence and finding of positive aCl IgM and lymphopenia. The association of various antiphospholipid antibodies is well known and expected[1,11]. Although anticardiolipin antibodies react with cardiolipin, they may also react with other phospholipids such as phosphatidylserine, phosphatidylinositol, annexin V, phosphatidylglycerol, prothrombin and B2-GPI[16].

On the other hand, the association with lymphopenia was an unanticipated finding and the relationship found was striking with an OR of 9.2 (95% CI=2.1--39.3). To our knowledge this association has not been described before.

Lymphopenia (≤1.500 cells/mm^3 in the absence of medications that can explained it) is a classification criteria for SLE of the American College of Rheumatology[14] and has been linked to higher disease activity[17], more severe damage[17], higher prevalence of infections[18] and some clinical disease characteristics such as neurologic involvement[19,20]. A low lymphocyte count has been found to contribute to leucopenia, however, it occurs independently[20]. It involves specially suppressor T cells[17,21] and it is strongly associated with IgM, cold reactive, complement fixing and presumably cytotoxic anti-lymphocyte antibodies[22]. Another potential mechanism of lymphopenia in lupus is increased apoptosis as reflected by increased expression of Fas antigen on T cells[23]. Low lymphocyte count is also associated with accelerated atherogenesis as studied by carotid media-intima measurement in children with SLE[24].

The finding, in present study, of an association between lymphopenia and the presence of antiphospho-

TABLE II. COMPARISON OF ANTI B2-GPI POSITIVE AND ANTI B2-GPI NEGATIVE SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

	Anti β2-GPI positive N= 17/88 = 19.3%	Anti β2-GPI negative N=71/88 = 80.6 %	P
Female gender	17/17	69/71	1.00 (*)
Age (years)	21-49 Median 40.0; IQR = 32.0-46.5	17-64 Median 42.0; IQR = 29.0-49.0	0.65 (#)
Disease duration (months)	12-231 Median 87.0; IQR=57.0-139.5	9-264 Median 96.0; IQR = 60.0-132.0	0.90 (#)
Arthritis	12/17-70.5%	34/71-47.8%	0.11 (*)
Discoid rash	0/17-0	10/70-14.2%	0.19 (*)
Photossensitivity	12/17-70.5%	48/69-69.5%	1.00 (**)
Malar rash	8/17-47.0%	31/72-43.0%	0.76 (**)
Aphtae	9/17-52.9%	31/71-43.6%	0.49 (**)
Psycosis	2/17-11.7%	4/71-5.6%	0.32 (*)
Seizures	3/17-17.6	8/72-11.1%	0.43 (*)
Serositis	**5/17-29.4%**	**7/72-9.7%**	**0.04 (*)**
Nephritis	6/17-35.2%	26/69-37.6%	0.85 (**)
Leucopenia	5/17-29.4%	20/72-27.7%	1.00 (*)
Thrombocytopenia	5/17-29.4%	18/72-25%	0.76 (*)
Lymphocytopenia	**6/15-40%**	**5/68-7.3%**	**0.003 (*)**
Hemolysis	1/17-5.8%	7/72-9.7%	1.00 (*)
Anti RNP	5/16-31.25%	14/66-21.2%	0.50 (*)
Anti Sm	3/17-18.7%	11/70-15.7%	1.00 (*)
Anti Ro	6/17-35.2%	23/71-32.3%	1.00 (*)
Anti La	3/16-18.7%	13/71-18.3%	1.00 (*)
Anti dsDNA	6/17-35.2%	19/72-26.3%	0.46 (**)
APS (obstetrical criteria)	3/17-17.6%	6/71-8.4%	0.36 (**)
APS (arterial thrombosis)	1/17-5.8%	4/71-5.6%	1.00 (*)
APS (venous thrombosis)	2/17-11.6%	4/71-5.6%	0.32 (*)
APS-total	5/17-29.4%	11/70-15.7%	0.19 (*)
aCl IgG	7/17-41.4%	16/71-22.5%	0.11 (**)
aCl IgM	**8/17-47.0%**	**14/72-19.4%**	**0.04 (**)**
LA	5/16-31.2%	14/68-20.5%	0.34 (*)
Positive Coombs	0/17-0	4/63-6.3%	0.57 (*)

APS = antiphospholipid syndrome; aCl = anticardiolipin; LA = lupus anticoagulant; β2-GPI= Beta 2 glycoprotein I; (*)= Fisher test; (**) =Chi squared test; (#) =Mann Whitney test

lipid antibodies, brings to attention some similarities between these two situations. Both accelerate atherogenesis and both have been linked to neurological involvement.

Antiphospholipid antibodies permeabilize and depolarize brain synaptoneurosomes and this disrupts neuronal function by direct action in nerve terminals[20,26]. Anti B2-GPI has been found to bind itself to astrocytes and neurons of SLE patients[20,27,28]. Non thrombotic neurological involvement described in APS includes migraine, movement disorders such as hemiballismus and chorea, transverse myelopathy, sensorial hearing loss and seizures[3,28-30].

It has been described that secondary injection of lymphocytotoxic autoantibodies (also called antineuronal antibodies[20] in the ventricles of experimental animals caused a variety of neurological symptoms such as convulsions and impaired memory [20,31]. In humans, these lymphocytotoxic autoantibodies have been found to cross the blood brain barrier and to react with

brain tissue[20]. This has been associated with diffuse neurological manifestations[20].

Concerning accelerated atherogenesis, it has been shown that antiphospholipid antibodies cross react with oxidized low-density lipoprotein (oxLDL) and, particularly B2-GPI, accelaterates the influx of oxLDL into macrophages, a major step in the development of atheromatous plaques[32]. Macrophages, T cells and dendritic cells activation and CD40-CD40L interactions are pathogenic mechanisms seen in atherogenesis and APS[33, 34].

Accelerated atherogenesis in lupus lymphopenia has been attributed to high titers of anti-T and anti-CD4 antibodies with decreased peripheral count[24]. CD4+CD25+ regulatory T cells (Tregs) stabilize atherosclerotic plaques by inhibiting inflammatory cytokine secretion and matrix metalloproteinase (MMP) expression[25].

In conclusion, the present study shows that, in a Brazilian sample, the prevalence of anti B2-GPI in lupus is 19.3% and that this prevalence in SLE with secondary APS is 29.4%. Anti B2-GPI has been found more frequent in those with IgM-aCl and with lymphopenia. Although lymphopenia and anti B2-GPI both have been linked to neurological manifestations and accelerated atherosclerosis, the real meaning of this association remains to be established.

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