ANTI-β_2-GLYCOPROTEIN I ANTIBODIES ARE HIGHLY PREVALENT IN A LARGE NUMBER OF BRAZILIAN LEPROSY PATIENTS

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Abstract

Objectives: To determine the prevalence of anticardiolipin (aCL) and anti-β_2-glycoprotein I (anti-β_2GPI) antibodies in leprosy patients, during and after specific multidrug therapy (MDT), and to evaluate a possible association between these antibodies and some clinical characteristics of leprosy, including clinical forms, reactional episodes and treatment.

Methods: The study included 158 leprosy patients, 129 gender-and-age matched healthy individuals, and 38 women with primary antiphospholipid syndrome (APS). Clinical and demographic characteristic of leprosy patients were collected, and serum samples, obtained from all participants, were kept frozen at - 20°C. Antibodies were measured either by an in house-developed ELISA (aCL) or by a commercial ELISA (anti-β_2GPI**).**

Results and Conclusions: Increased levels of aCL and anti-β_2GPI antibodies were found in leprosy patients and in the APS group, however, in contrast to APS, the predominant isotype in leprosy was IgM. The frequency of aCL and anti-β_2GPI antibodies was significantly higher in leprosy patients than in healthy individuals (15.8% vs. 3.1%; p<0.01; 46.2% vs. 9.4%, p<0.01**),** respectively. The lepromatous form predominated among aCL positive leprosy patients (p<0.01). There was no difference in aCL and anti-β_2GPI positivity between leprosy patients taking MDT and those completed MDT as cured. Furthermore the duration of discharged period (period between discharge from MDT and the realization of the study) had no effect on anti-β_2GPI positivity, and a slight increase in aCL positivity was observed in patients with longer follow up periods (p=0.04), suggesting that the presence of antiphospholipid antibodies (aPL) was not a transient phenomenon. Although aPL in leprosy were frequent and β_2GPI-dependent as those found in APS, IgM was the predominant isotype, and there was no association with thrombosis or other APS manifestations.

Keywords: Leprosy; Anti-β_2-glycoprotein I antibodies; Antiphospholipid antibodies; Anticardiolipin antibodies; Antiphospholipid syndrome.

Introduction

Antiphospholipid antibodies (aPL) are a heterogeneous group of autoantibodies, which have been reported in many autoimmune diseases, mainly in systemic lupus erythematosus (SLE) and primary antiphospholipid syndrome (APS), in association with vascular thrombosis, pregnancy morbidity, and a number of other less commonly found manifestations[1]. aPL have also been reported in the context of several infections such as leprosy, tuberculosis, malaria, syphilis, hepatitis C virus (HCV), human immunodeficiency virus (HIV), leptospirosis, and B19 parvovirus infections[2-5]. Interestingly, the presence of aPL in infectious diseases is not usually associated with the clinical complications attributed to APS[6], and they are often transient and may disappear after treatment[7].

Anticardiolipin (aCL) antibodies constitute the main group of aPL studied in primary and secondary APS and other autoimmune and infectious diseases. In the last two decades, several studies have indicated that β_2-glycoprotein I, a phospholipid binding protein, besides its role as cofactor for aCL antibodies detection[8-10], may be itself an antigen, eliciting pathogenic anti-β_2-glycoprotein I antibodies in APS[11].

Although the aCL antibodies detected in infectious disorders were initially reported to be main-

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ly β_2GPI-independent, β_2GPI-dependent aCL, and antibodies against the proteins β_2GPI and prothrombin have also been reported in some infections[12,13].

Leprosy is an infectious disease caused by *Mycobacterium leprae* affecting primarily the peripheral nervous system, and secondarily involving skin and other tissues. The clinical spectrum is characterized by two stable poles forms of disease. At one pole, tuberculoid leprosy, the host is able to keep the disease under control due to an efficient T-cell--mediated immune response; while at opposite pole, lepromatous leprosy, cell-mediated immunity is inefficient leading to excessive bacillary multiplication and dissemination. Between the two stable poles are unstable forms of leprosy, named borderline that may combine characteristics of the polar forms. A broad spectrum of clinical lesions and a high frequency of autoantibodies, especially aCL antibodies are seen in leprosy[14-16]. Despite the disease chronicity and the aCL persistence[16], aPL-related thromboembolic complications have rarely been reported in leprosy[17,18].

Although aCL have been reported in 20-98% of leprosy patients with different clinical forms, it seems to be more prevalent in the lepromatous form[3,4,15,16,19-24]. The prevalence of anti-β2-glycoprotein I antibodies (anti-β2GPI) is highly variable, ranging from 2.9 to 89%[3,16,21,23,24].

The objectives of this study were to determine the prevalence of aCL and anti-β_2GPI antibodies in a large number of Amazon leprosy patients taking multidrug therapy (MDT) and in completed MDT as cured, and to evaluate a possible association between these antibodies and clinical forms of disease, and reactional episodes.

Patients and Methods

The study group included 158 leprosy patients (113 males and 45 females, mean age of 39.9±15.2 years) followed up at the Tropical Dermatology and Venereal Clinic of Alfredo da Matta Foundation (Manaus, Amazon, Brazil) from July 2004 to October 2006. Leprosy diagnosis was established according to Ridley and Jopling classification criteria[25]. To determine the presence of antiphospholipid antibodies, 20 mL of peripheral venous blood were collected from all participants. The leprosy patients present with the following clinical and therapeutic characteristics: Fifty-six cases had lepromatous, 35 borderline lepromatous, 25 borderline, 32 tuberculoid borderline, 6 tuberculoid, and 4 indeterminate form. Reactional episodes as erythema nodosum lepromatosum (ENL), reversal reaction (RR) and neuritis were present in 58/158 (36.7%) of the patients, during the study. The criterion for a diagnosis of ENL was the presence of tender skin nodules. This could be accompanied by fever and other systemic symptoms such as joint pain, bone tenderness, neuritis, edema, malaise, anorexia, and/or lymphadenopathy; for the reversal reaction, acute inflammation, as pain, erithema, infiltration and edema of pre existing lesions sometimes accompanied of new lesions and neuritis. Sixty four patients were taking MDT and 94 had already completed MDT. In those patients who completed MDT(period cured), the period between discharge and the realization of the study ranged from 1 to 115 months (median of 31 months). All were submitted to complete physical exam and a questionnaire and none of them had any event suggestive of APS (vascular thrombosis, pregnancy morbidity or thrombocytopenia) or any concomitant rheumatic disease.

The control group was composed by 129 healthy individuals, gender-and-age matched to the leprosy group (mean age of 40.9±14.1 years), living in the same geographic region. The APS group was constituted by 38 women with primary APS (mean age of 40.2±13.8 years)[26] , who were recruited from a outpatient clinic of rheumatology, Universidade Federal de São Paulo (UNIFESP), Brazil, meeting the Sapporo criteria[27]. The most frequent manifestations of these patients were pregnancy morbidity (44.7%), lower limb venous thrombosis (39.4%) and stroke (28.9%). Many of them had more than one manifestation. The age distribution of the APS group did not differ from the leprosy and control groups (p=0.649).

Blood samples were collected from all participants and kept at -20°C, until analyzed at the Rheumatology Division of Universidade Federal de São Paulo. The study was approved by both Institutional Review Boards, and all participants signed the informed consent form.

Anticardiolipin antibodies (aCL)

IgG and IgM aCL antibodies were tested in all serum samples with an in house-developed ELISA. Normal ranges for aCL antibodies in Brazilian po-

pulation were previously determined in a sample of 200 healthy blood donors and the 95th percentile cutoff points, established with the use of international APL calibrators (LAP-GM-200 calibrators, Louisvulle APL Diagnostics, Inc, GA, USA) were 20 GPL and 10 MPL, respectively. One GPL or MPL unit is defined as the cardiolipin binding activity of 1 g/mL of an affinity purified IgG or IgM aCL preparation from a standard serum.

Anti-β_2-glycoprotein I antibodies

Serum IgG and IgM anti-β_2GPI antibodies were tested by a commercial ELISA (The Binding Site, Birmingham, UK) according to the manufacturer's instructions. The cutoff values according to the kit were 10 U/mL for IgM and 20 U/mL for IgG. Due to our limitations, only 106 control sera were included in this test.

Statistical analysis

Statistical analysis was performed with the SPSS 15.0.1 software (Chicago, USA). Kruskal-Wallis test was used for age distribution comparison between groups. Chi-square test was used to compare gender distribution, and aCL and anti-β_2GPI antibody frequencies between groups. Binomial test was used to compare the proportion of clinical forms among aCL and anti-β_2GPI antibodies positive leprosy patients. Student's t-test was used to compare duration of completed MDT (cured period) and aPL positivity between groups. P values < 0.05 were considered statistically significant.

Results

In the APS group aCL antibodies were positive in 34/38 (89.5%) patients, and GPL or MPL titers >40 U/mL were found in 26/34 (76.5%) of the positive sera. In the leprosy group, aCL antibodies were positive in 25/158 (15.8%) patients, and GPL or MPL titers >40 U/mL were found in 20/25 (80%) of the positive sera. There was no difference in aCL positivity between leprosy patients in completed MDT and those taking MDT (p=0.41). In the control group, aCL antibodies were found in only 4/129 (3.1%) individuals, none of them with GPL or MPL titers higher than 40 U/mL. Anticardiolipin antibodies were more frequent in leprosy patients than in healthy controls (p <0.01), but less frequent than in APS (p <0.01). Mean aCL titers in leprosy were 57 GPL U/mL and 56 MPL U/mL.

Among the 25 aCL positive leprosy sera, IgM isotype was more frequent than IgG (88% vs 16%; p<0.01). In contrast, in the APS group, IgG isotype was more frequent than IgM (91.2% vs 44.1%, respectively; p<0.01). All the four positive healthy controls had IgM aCL antibodies (Table I).

The clinical forms of disease were not equally distributed between aCL positive and negative leprosy patients (p<0.01,) as shown on Table 2. Among the aCL positive leprosy patients, the lepromatous form was found in a higher percentage (72%) than the other forms (p<0.01), while among aCL negative patients there was no predominant form. There was no association between the presence of reactional episodes and aCL antibodies (p=0.41). However, among the 58 leprosy patients with reactional episodes, the distribution of reaction type was different between aCL positive and negative patients (p=0.03). ENL was the most prevalent reaction type in both aCL positive and aCL negative patients with reactional episodes, however, all the aCL positive patients presented ENL type (100%) while in aCL negative, ENL was found in only 57.4% (p=0.02).

Anti-β_2GPI antibodies were found in 9/38 (23.7%) patients of the APS group, and titers >40

Table I. Frequency of aCL and anti-β2GPI antibodies according to isotype distribution in leprosy patients, healthy controls, and APS group

	Leprosy patients (n=158)		Healthy controls (n=129)	(n=106)	Primary APS patients (n=38)	
Isotype	**aCL**	**anti-β2GPI**	**aCL**	**anti-β2GPI**	**aCL**	**anti-β2GPI**
IgG alone	3	1	0	1	18	3
IgM alone	21	64	4	9	3	2
IgG + IgM	1	8	0	0	13	4
Total	25 (15.8%)	73 (46.2%)	4 (3.1%)	10 (9.4%)	34 (89.5%)	9 (23.6%)

Table II. Distribution of anticardiolipin antibody in leprosy patients accordingly to Ridley & Jopling classification

	Classification													
	I		TT		BT		BB		BL		LL		TOTAL	
aCL	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Negative	4	3,0	6	4,5	31	23,3	22	16,5	32	24,1	38	28,6	133	100
Positive	0	0	0	0	1	4	3	12	3	12	18	72	25	100
TOTAL	4	2,5	6	3,8	32	20,3	25	15,8	35	22,2	56	35,4	158	100

aCL=anticardiolipin antibodies; BB=borderline; BV=borderline lepromatous; BT=borderline tubercuoid; I=indeterminated; TT=tuberculoid; VV= lepromatous

U/mL were found in 66.7% of them. In the leprosy group, anti-β_2-GP1 antibodies were found in 73/158 (46.2%) patients, and titers >40 U/mL were found in 26 (35.6%) of them. There was no difference in anti-β_2GPI positivity between leprosy patients who completed MDT and those taking MDT (p=0.07). In the control group, anti-β_2-GPI antibodies were found in only 10/106 (9.4%) individuals, and none of them had titers >40 U/mL. Anti-β_2-GPI antibodies were more frequent in leprosy patients than in healthy controls (p<0.01) or APS (p=0.01). The mean titers of anti-β_2-GPI in leprosy patients were 112 U/mL for IgG and 94 U/mL for IgM antibodies.

Among the 73 anti-β_2GPI positive leprosy sera, IgM isotype was more frequent than IgG (97.3% vs. 12.3%, p<0.01). In the APS group, both isotypes were equally frequent. Among the anti-β_2GPI positive healthy controls, IgM isotype was also more frequent than IgG (90% vs. 10%, respectively; p <0.01) (Table I).

The distribution of the clinical forms of disease according the Ridley Jopling classification was similar in anti-β_2GPI positive and negative leprosy patients (p=0.09), Table III. Anti-β_2GPI positivity was also similar in patients with and without reactional episodes (51.7% vs. 43%, respectively, p=0.29), There was no difference in the proportions of the reaction types between anti-β_2GPI positive and negative patients (p=0.18).

Among the 158 leprosy patients, 77 (48.7%) were positive for at least one aPL antibody (Figure 1).

Interestingly, as depicted in Table II, among the

Table III. Distribution of anti-β_2GPI in leprosy patients accordingly to Ridley & Jopling classification

	Classification													
anti-	I		TT		BT		BB		BL		LL		TOTAL	
β_2GPI	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Negative	3	3,5	5	5,9	21	24,7	14	16,5	20	23,5	22	25,9	85	100
Positive	1	1,4	1	1,4	11	15,1	11	15,1	15	20,5	34	46,6	73	100
TOTAL	4	2,5	6	3,8	32	20,3	25	15,8	35	22,2	56	34,4	158	100

BB=borderline; BL=borderline lepromatous; BT=borderline tubercuoóid; I=indeterminated; TT=tuberculoid

Table IV. Prevalence of aCL and anti-ß2GPI antibodies among leprosy patients

		aCL		
		Positive	Negative	TOTAL
anti-β_2GPI	Positive	21	52	73
	Negative	4	81	85
TOTAL		25	133	158

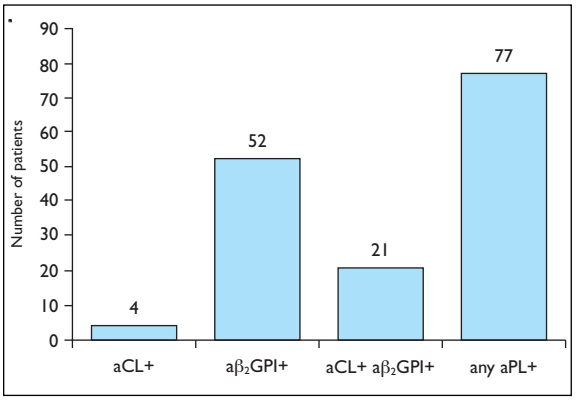

Figure 1. Frequency of the diverse aPL antibodies in leprosy patients

25 aCL positive leprosy patients, 21 (95%) were also positive for anti-β_2GP1 antibodies ($p<0.01$).

Among the leprosy population under study, 94 patients had completed MDT in the period of sera collection to test the antibodies aFL. This period ranged from 1 to 115 months (median 31.3 months) and had no influence in anti-β_2GPI positivity ($p=0.20$). A slight increase in aCL positivity was observed in patients with longer time completed MDT ($p=0.04$).

Discussion

Antiphospholipid antibodies have been widely studied in autoimmune diseases, and their association with thrombotic events as well as pregnancy morbidity is well known. However, aPL related to some viral or bacterial infections are not usually associated with the clinical manifestations attributed to APS. This lack of pathogenicity was first attributed to β_2GPI independency, either as a cofactor or as an autoantigen[7-10].

Nevertheless, the distinction between autoimmune (APS associated), and infectious (β_2GPI independent) aPL has been recently challenged, with some studies reporting the occurrence of antibodies to β_2GPI in patients with viral or bacterial infections[3,23,28].

In infectious diseases, aPL could be induced by disturbances of cellular and humoral immune regulation, during the immune response. Alternatively, this induction could be attributed to the exposure of phospholipid antigens by tissue damage in the course of infection. One current hypothesis is that infections may be a «trigger» for the generation of pathogenic aPL in genetically predisposed individuals[4,29,30]. In this case, bacterial or viral peptides with homology to the β_2GPI molecule would be presented to T lymphocytes, which will stimulate B lymphocytes to produce antibodies against the cross-reacting heterologous sequence[30].

Anticardiolipin and anti-β_2GPI antibodies have been reported in leprosy patients, mainly in association with lepromatous leprosy, and usually without APS-related clinical manifestations[3,4,15,16,19-24].

In our study, the positivity of cofactor-dependent aCL antibodies was lower than that reported in other studies, although there is a large variability in the literature[3,4,16,20-24]. This variability could be attributed to methodological differences including the cutoff value established for different populations, variation in the reagents used in home-based assays, calibration aspects, and other factors[31].

Our study selected a large cohort of leprosy patients representing the spectrum and clinical features of the disease. The frequency of aCL antibodies in our leprosy patients was higher in the lepromatous form, in agreement with several authors[3,15,16,20,22,23], and the presence of aCL antibodies, even in high titers, was not associated with reactional episodes. However, we observed that all patients with a reactional episode who were positive for aCL presented erythema nodosum leprosy. Several studies addressed the isotype prevalence of aPL in the sera from leprosy patients with varied results. We observed a predominance of the IgM isotype in aCL antibodies, corroborating the data of some authors[3], however, other studies found a higher prevalence of the IgG isotype[15,16,20], and one study, in black South African patients, found a higher prevalence of the IgA isotype[4].

Antibodies with specificity towards β_2GPI have been suggested to be a better marker of APS than aCL alone [32,34] and in 3-10% of APS patients, anti-β_2GPI antibodies may be the only positive test[35-37]. According to these evidences, recently, the presence of anti-β_2GPI antibodies regardless of isotype was included as part of the modified Sapporo classification criteria for APS[27].

According to the literature, anti-β_2GPI antibodies are less frequently found in infectious diseases than in APS[12,13,32,34]. Furthermore, most APS studies have shown a relationship between IgG isotype anti-β_2GPI antibodies and venous thrombosis and

lack of association with the IgM isotype[12,32,34,38,39]. However, associations between IgM anti-β_2GPI antibodies and arterial thrombosis, fetal loss and thrombocytopenia have also been reported in APS[39-43].

In the literature, the prevalence of anti-β_2GPI antibodies in leprosy ranges from 2.9% up to 89%[4,21]. We found anti-β_2GPI antibodies in 46.2% of leprosy patients, some of which displaying high titers. In our leprosy population, IgM was the most frequent isotype of anti-β_2GPI antibodies, in agreement with other studies[3,4]. However, another study, including 177 leprosy patients, showed no favorite isotype[23].

In our study, 20.5% of the anti-β_2GPI positive leprosy patients had antibody titers higher than 100 U/mL. According to the literature, high titers of anti-β_2GPI antibodies are associated with risk of thrombosis, but the definition of the boundaries for medium and high titers is a difficult task[27].

We found a higher prevalence of anti-β_2GPI (46.2%) than aCL (15.8%) antibodies in leprosy, corroborating the results of Loizou et al (2003)[4]. However, there are other studies reporting a higher prevalence of aCL than of anti-β_2GPI antibodies in leprosy[16,21].

Anticardiolipin and anti-β_2GPI antibodies were simultaneously found in 13.3% of our leprosy patients, and in 70% of those, antibody titers for both specificities were higher than 40 U/mL. However, in agreement with the literature, we found no association between the presence of aCL and/or anti-β_2GPI antibodies and thrombotic events[3,4,23,24].

In APS, thrombotic events are usually associated with the IgG isotype of aCL and anti-β_2GPI antibodies. In our study, IgM was the most prevalent isotype in leprosy whereas IgG was more frequent in primary APS. It has been suggested that IgG isotype may be an important factor in determining clinical complications of aCL.[44] There is also an interesting paper suggesting that IgG anti-β_2GPI antibodies specifically directed against a positively charged epitope on the first domain of β_2GPI correlate with thrombosis[45].

In addition, it has been reported that aPL cause endothelial cell activation and blood coagulation by binding to β_2GPI on the surface of endothelial cells[46,47]. Martinuzzo et al, demonstrated that sera from leprosy patients APL positive showed platelet and endothelial cell activation to the same extend that patients with APS, however they not show a procoagulant state as demonstred by normal levels of markers of blood coagulation[48].

We did not find association between treatment and positivity for aCL or anti-β_2GPI antibodies, and the increase in those who completed MDT as cured was not associated with a decrease in aPL positivity, suggesting that aPL may not be as transient as in other infections. Arvieux et al, 2002 also reported persistence of anti-β_2GPI antibodies in six leprosy patients followed for over two years.

There are a few case reports referring aPL and thrombotic phenomenon in patients with leprosy[17,18,49]. However, this may be just a coincidence since the pathological significance of these antibodies in leprosy is not very clear.

In conclusion, aCL and β_2GPI-dependent in leprosy are as frequent as those found in APS patients, however leprosy patients do not present clinical manifestations of APS. In leprosy-related aPL, IgM was the prevalent isotype whereas in APS, IgG was the main isotype. It is possible that the presence of aPL in leprosy may be simply another marker of autoimmunity. In contrast to previous suggestions, we found no evidence to support the presence of aPL as a transient phenomenon. It would be interesting to perform a longitudinal follow-up to evaluate the persistence of these antibodies, and to compare the fine specificity of APS and leprosy-associated anti-β_2GPI antibodies.

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2º Simpósio SPR – Artrite e Osso

Aveiro, Portugal
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