Evaluation of antiphospholipid antibodies testing for the diagnosis of antiphospholipid syndrome

Paula Gonçalves Perches[1], Daniela Pezzutti Domingues[1], Andréia Latanza Gomes[2], Augusta Morgado Ribeiro[2], Felipe Moraes Toledo Pereira[2], Ivan Elias Rassi[3], Alexandre Wagner Silva de Souza[4]

ABSTRACT

Objective: To evaluate the prevalence of lupus anticoagulant (LAC) and isotypes of anticardiolipin (ACL) antibodies and its possible clinical associations. **Patients and methods:** A retrospective study analyzed clinical and laboratorial manifestations in individuals who showed positive antiphospholipid antibodies followed-up at Hospital Edmundo Vasconcelos from March 2005 to June 2006. **Results:** 106 participants (mean age of 42.2 ± 14.1 years at inclusion and female gender in 84% of patients) were included in the study. The prevalence of thrombosis was 17.9% (19/106 patients) and pregnancy morbidity was 12.3% (13/106 patients). The antiphospholipid syndrome (APS) was confirmed in 23.6% (25/106 patients), and it was primary in 68% (17/25 patients) and secondary in 32% (8/25 patients). The ACL antibodies were found in 97.1% (103/106) and LAC in 11.4% (5/44 of the serum samples tested). IgM, IgG and IgA ACL isotypes were respectively found in 100%, 23.3% and in 4.9% of these ACL positive sera. For APS diagnosis the sensitivity of IgM ACL was 92% and its specificity was 1.2%, while IgG ACL had a sensitivity of 40% and specificity of 82.5%. The absence of IgG ACL had a high negative predictive value for APS diagnosis (81.4%).The analysis of the Receiver Operating Characteristic (ROC) curve showed larger area under the curve for ACL IgG and LAC. **Conclusion:** In a random sample of individuals with positive antiphospholipid antibodies, IgG ACL and LAC showed a larger specificity for APS diagnosis which had been characterized by a higher prevalence of thrombosis.

Keywords: antiphospholipid syndrome, anticardiolipin antibodies, lupus anticoagulant, thrombosis, fetal loss.

INTRODUCTION

The antiphospholipid syndrome (APS) is characterized by the presence of antiphospholipid antibodies associated with arterial thrombosis, venous and/or microangiopathic, recurrent gestational morbidity and/or thrombocytopenia. It can be primary or secondary to other autoimmune diseases, mainly to systemic lupus erythematosus (SLE), to chronic infectious diseases or to neoplasias.[1,2]

Antiphospholipid antibodies are autoantibodies whose targets are cellular membrane phospholipids and/or proteins combined with phospholipids, like beta-2 glycoprotein I (β_2-GPI) and prothrombin. The main antiphospholipid antibodies are the lupus anticoagulant (LAC), and anticardiolipin (ACL), which can be IgG, IgM or IgA.[2] The presence of LAC or high titers of ACL is an important independent risk factor for thrombosis.[3] Furthermore, these autoantibodies also help in the diagnosis of APS, composing the classification criteria for this entity.[4,5]

In this study we attempted to evaluate the prevalence of clinical manifestations associated with the presence of antiphospholipid antibodies, to compare the prevalence of these manifestations and different antiphospholipid antibodies

Received on 08/07/2008. Approved on 01/30/2009. We declare no conflict of interest, except for the author Alexandre Wagner Silva Souza, who informed the following conflicts of interest: Received honoraria to minister the class "The immunologic system" and financing to participate in the ACR 2008 – Roche Brasil.
1. Medical Practice Service of the Hospital Professor Edmundo Vasconcellos. 2. Rheumatology Division at UNIFESP

1. Medical practice resident physician at the Hospital Professor Edmundo Vasconcellos
2. Medical Student, UNIFESP
3. Chief of the Laboratory at the Hospital Professor Edmundo Vasconcellos
4. Assistant physician of Rheumatology at UNIFESP
Correspondence to: Alexandre Wagner Silva de Souza. Rua Botucatu, 740, 3º andar – Disciplina de Reumatologia – Vila Clementino. São Paulo, SP - Brasil - Zip Code: 04023-900. E-mail: alexandre_wagner@uol.com.br

in patients with and without APS and to verify the performance of different tests to confirm the syndrome.

PATIENTS AND METHODS

A retrospective study was performed through active search of positive tests results for ACL and LAC in the clinical analyses laboratory at Hospital Professor Edmundo Vasconcelos, from March 2005 to June 2006. Later, the medical records of these individuals were analyzed. A hundred and six patients were included according to the following criterion: presence of ACL superior to 10 MPL, APL and/or GPL and/or positivity of LAC. The patients with antiphospholipid antibodies were divided into two groups: those who fulfilled the Sapporo Classification Criteria for APS[4] and those who did not fulfill these criteria. The groups were compared regarding clinical manifestations, and the contribution of each antiphospholipid antibody to the diagnosis of APS was statistically evaluated.

The search of ACL was performed by enzyme immunoassay (ELISA) with the participant's serum using INOVA Diagnostics' kits. The results were issued in MPL, APL and GPL units for IgM, IgA and IgG, respectively. The LAC test was performed with the subjects' plasma with the following tests: activated partial thromboplastin time (APTT) and dilute Russell's viper venom time (dRVVT), according to international guidelines.[6]

The study was approved by the Hospital Professor Edmundo Vasconcelos ethics commission.

STATISTICAL ANALYSIS

The chi square or Fisher tests were employed to analyze categorical variables, while the *Student's t* or Mann-Whitney's tests were used to compare the numeric variables. The kurtosis and skewness coefficients were calculated to verify if the numeric variables presented normal distribution. Values of P inferior to 0.05 were considered significant.

The correlation among the ACL IgM, IgA and IgG levels and with the presence of LAC was performed by the Spearman's rank correlation coefficient. For each diagnosis test, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated, besides the construction of the ROC (Receiver Operating Characteristic) curve with the area below the curve and confidence interval of 95% (CI 95%). The gold standard used for the diagnosis of APS was the Sapporo Classification Criteria.

The risk of thrombosis associated with each test diagnosis of the study was done by logistic regression, stepwise. The results were expressed in odds ratio with confidence interval (CI) of 95%.

RESULTS

Positive tests for antiphospholipid antibodies were found in 106 individuals. The participants of the study had mean age of 42.2 years with standard deviation (SD) of 14.1 years in the age range between 10 and 80 years, and most were female (84%).

Episodes of thrombosis were diagnosed in 17.9% (19/106) of the patients. Venous thrombosis was present in 73.7% (14/19) and arterial thrombosis in 31.6% (6/19). Gestational morbidity was observed in 12.3% (13/106) of the patients. The Sapporo Classification Criteria for APS were fulfilled in 23.6% of the cases (25/106 patients). Among them 68% presented the primary form and 32%, the secondary form of the disease – six of them with SLE diagnosis, one with chronic hepatitis C, and one with chronic infection by HIV.

ACL prevalence was 97.1% (103/106 patients) and LAC was 11.4% (5/44 of the patients evaluated). ACL IgM, IgG and IgA were respectively found in 100%, 23.3% and 4.9% of the ACL positive sera (Table 1). ACL IgM, IgG and IgA had mean titers of 35.5 MPL, 32.9 GPL and 19.1 APL, respectively.

Patients with and without APS presented similar data regarding age (45.2 ± 13.1 *versus* 41.2 ± 14.3 years, P = 0.198) for the female gender (84% *versus* 83.9%, P = 0.995) and for ACL IgM (92% *versus* 98.7%, P = 0.075) and ACL IgA (4% *versus* 4.9%, P = 0.889) frequencies. Nevertheless, the prevalence (40% *versus* 17.2%, P = 0.019) and mean levels of ACL IgG (38.8 ± 21.8 GPL *versus* 28.7 ± 28.7 GPL, P = 0.038) were higher in patients with APS compared with those without APS. A significant large number of individuals with mean levels of ACL IgG above 40 GPL was also observed among patients with APS compared with those without APS (16% *versus* 2.5%, P = 0.001). As for ACL of IgM class, there was no significant difference between the groups with and without APS regarding the number of individuals with mean levels superior to 40 MPL (24% *versus* 13.6%, P = 0.215) (Table 1).

Of the 44 patients in the LAC search, five had a positive test (11.3%) – three with diagnosis of APS according to the Sapporo Classification Criteria, P = 0.336. Patients with APS presented a higher frequency of ACL IgM and IgG concomitant (40% *versus* 17.2%, P = 0.018) compared with those without APS. Patients with APS presented a greater prevalence of arterial

Table 1
Demographic, laboratorial and clinical data of antiphospholipid positive patients with and without APS

Variables	APS (n = 25)	Absence of APS (n = 81)	P
Age, years	45.2 (± 13.1)	41.2 (± 14.3)	0.198
Female	21 (84%)	68 (83.9%)	0.995
LAC§ (n = 44)	3/16 (18.8%)	2/28 (7.1%)	0.336
ACL IgM	23 (92%)	80 (98.7%)	0.075
ACL IgA	1 (4%)	4 (4.9%)	0.889
ACL IgG	10 (40%)	14 (17.2%)	0.019*
ACL IgM e IgG	10 (40%)	14 (17.2%)	0.018*
ACL IgM > 10MPL	43.6 (± 49.0)	33.1 (± 35.0)	0.382
ACL IgM > 40MPL	6 (24%)	11 (13.6%)	0.215
ACL IgA > 10 APL	14.3 (NA)	20.3 (± 7.5)	0.480
ACL IgG > 10GPL	38.8 (± 21.8)	28.7 (± 28.7)	0.038*
ACL IgG > 40GPL	4 (16%)	2 (2.5%)	0.010*
Thrombosis	15 (60%)	4 (4.9%)	< 0.001*
Venous	11 (44%)	3 (3.7%)	< 0.001*
Arterial	5 (20%)	1 (1.2%)	< 0.001*
Gestational morbidity	12 (48%)	1 (1.2%)	< 0.001*

NA = non appliable; ACL = anticardiolipin; LAC = lupus anticoagulant. § The LAC test was performed in 44 of the patients included in the study. *Significant statistic difference.

thrombosis (20% *versus* 1.2%, P < 0.001), venous thrombosis (44% *versus* 3.7%, P < 0.001) and gestational morbidity (48% *versus* 1.2%, P < 0.001) compared with individuals who presented antiphospholipid antibodies without fulfilling the classification criteria for APS (Table 1).

In a multivariated analysis, the antiphospholipid antibodies LAC and ACL (IgM, IgA, and IgG) did not seem significantly associated to a greater risk of thrombosis. ACL IgG presented a risk of 2.4 times for thrombosis, but the CI 95% from 0.4 to 13.7 was not significant (P = 0.315).

The sensitivity of ACL IgM was 92%, and its specificity was 1.2% for the diagnosis of APS. PPV was 22.3% and NPV was 33.3% for ACL IgM. The ROC curve demonstrated an area under the curve of 0.466 with CI 95% of 0.332-0.600 (Figure 1). The sensitivity of ACL IgG was 40%, and its specificity was 82.5% for the diagnosis of APS. The PPV for ACL IgG was 41.6% and NPV, 81.4%. The ROC curve demonstrated an area under the curve of 0.613 with CI 95% of 0.479-0.746 (Figure 2). The sensitivity of LAC was 12%, and its specificity was 97.5% for the diagnosis of APS. PPV was 60% and NPV was 78.2%. The ROC curve demonstrated an area under the curve of 0.558 with CI 95% of 0.376-0.740 (Figure 3). The very low prevalence of ACL IgA did not allow a similar analysis regarding the performance of this diagnostic test.

DISCUSSION

In this study, all ACL IgM, IgG and IgA tests were researched, besides LAC tests performed in the general hospital laboratory for 16 months. The obtained results of the antiphospholipid antibodies research were confronted with the clinical manifestations and personal antecedents presented by the participants of the study and with the Sapporo classification criteria for APS.[4] ACL of IgG class and LAC were the most specific tests for the diagnosis of APS, while ACL of IgM class was too sensitive and little specific. Patients with APS diagnosis presented higher titers of ACL class IgG. However, the retrospective design of the present study prevents causality conclusions.

The standardization of diagnostic tests for antiphospholipid antibodies research has been a challenge, since diverse studies demonstrated a great variability of the results obtained with different commercial kits and in-house techniques of ELISA among laboratories for the ACL research.[7-10] In this regard, it would be interesting to extend our findings using commercial kits for the detection of ACL of different origins. Also for the LAC search, heterogeneity of results is observed in different laboratories and used techniques.[11-14] International consensuses for standardization the antiphospholipid antibodies research have been performed to improve interlaboratorial agreement.

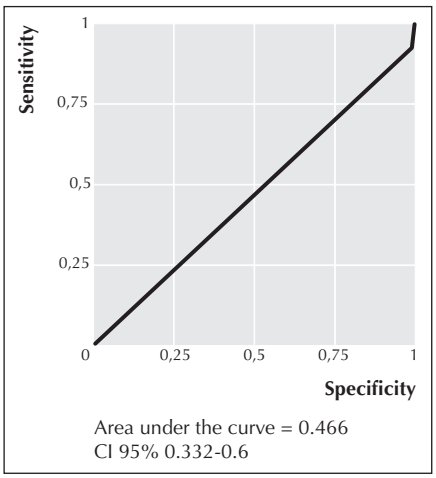

Figure 1. ROC curve for ACL IgM.

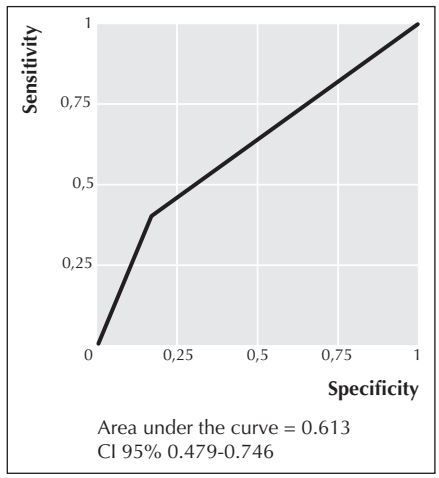

Figure 2. ROC curve for ACL IgG.

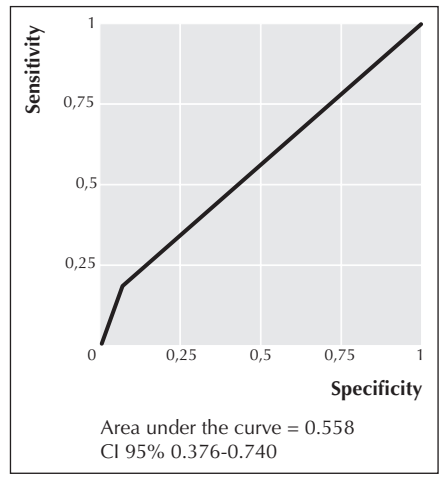

Figure 3. ROC curve for LAC.

The detection of ACL by a commercial kit in the present study showed high prevalence of ACL IgM in low titers in individuals without the diagnosis of APS, suggesting its high sensitivity for ACL IgM. However, the literature demonstrates that there is a tendency to the association of false positive results and low titers of ACL IgM, mainly in individuals with rheumatoid factor or cryoglobulins.[18-19]

The discovery of high specificity and a larger area under the ROC curve for the diagnosis of APS with LAC positivity in our study is in agreement with previously reported data.[3] The presence of LAC is the main risk factor for gestational morbidity in APS and for thrombotic events, despite the vascular bed or type of thrombosis, the presence of SLE and the method used to find it.[3] In this study, the finding of LAC positivity (12%) was inferior to the one observed in the literature (50-55%).[1,23-25] This finding can be underestimated since its research was performed in only 44 of the 106 participants. Maybe a minor knowledge about the importance of LAC as a risk factor for thrombosis and its importance for the diagnosis of APS has contributed for its minor solicitation.

In our study, the presence of ACL class IgG also presented a high specificity for the diagnosis of APS, in agreement with the literature.[3] The presence of moderate to high titers of ACL class IgG is a risk factor for thrombosis, but with less intensity in relation to LAC.[3] The great number of individuals without APS and with ACL in low titers observed in the present study is probably due to a low cut off for ACL positivity (10 units GPL, MPL, or APL) oriented by the kit's manufacturer. As it would be expected, most individuals without APS presented ACL IgM in low titers. Nevertheless, the difference between the groups was not significant regarding the mean of MPL units, because a few individuals without APS presented elevated titers of ACL class IgM. In the Sapporo classification criteria, the cut off used to define the ACL positivity was 20 GPL or MPL units.[4] Recently, with the objective of obtaining a higher specificity in the diagnosis of APS, the Sapporo criteria were updated and the cut off was raised to 40 GPL or MPL units, or levels above the 99 percentage.[5] The high prevalence of antiphospholipid antibodies in individuals without APS is also due to the fact that these tests were requested for patients with some clinical suspicion. The prevalence of antiphospholipid antibodies varies from 1.0 to 5.6% in the general population without suspicion of APS clinical scenario and tends to increase with aging, mainly in individuals with comorbidities.[20]

ACL class IgA was only detected in four individuals and among them only one patient had APS. Due to the low prevalence of this autoantibody, it was not possible to analyze it separately. This isotype does not seem to increase the sensitivity for the diagnosis of APS.[5] It just helps to identify subgroups of patients and it is highly prevalent in african-american patients with SLE and correlating with thrombocytopenia, cutaneous ulcers and vasculitis.[21,22]

The patients with diagnosis of APS evaluated in this study presented similar data to those in most studies in the literature regarding the frequency of the female gender and the prevalence of venous and arterial thrombosis, and of obstetrical morbidity.[1,23-25]

As for antiphospholipid antibodies in patients with the diagnosis of APS, the prevalence of ACL class IgM was 92%, superior than the 76.4 to 77% described in other studies[23,24] and far above the 39.4% described in a study performed by the same author using the in-house ELISA technique,[25] which confirms the high sensitivity of ACL IgM of the tested kit. Although ACL IgG was more specific for the diagnosis of APS in this study,

its prevalence of 40% was inferior to what was previously described in patients with APS (75.7-81.6%).[23-25]

In conclusion, our data reveal the importance of the positivity of LAC and ACL class IgG in the diagnosis of APS in individuals cared for in a general hospital. Besides that, the present study shows that ACL antibodies of the IgM isotype, despite its high prevalence, does not seem to contribute in helping the syndrome diagnosis, as well as the IgA isotype.

REFERÊNCIAS

REFERENCES

1. Levine JS, Ware Branch D, Rauch J.The antiphospholipid syndrome. N Engl J Med 2002;346(10):752-763.
2. Roubey RAS. Immunology of the antiphospholipid syndrome. Arthritis Rheum 1996;39(9):1444-1454.
3. Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. Blood 2003;101(5):1827-1832.
4. Wilson AW, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC e*t al*. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Report on an international workshop. Arthritis Rheum 1999;42(7):1309-1311.
5. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R e*t al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome. J Thromb Haemost 2005;4(2):295-306.
6. Brandt JT, Triplett DA, Alving B, Scharrer A. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid antibody of the Scientific Standardization Committee of the ISTH. Thromb Haemost 1995;74(4):1185-1190.
7. Pengo V, Biasiolo A, Bison E, Chantarangkul V, Tripodi A. Antiphospholipid antibody ELISAs: survey on the performance of clinical laboratories assessed by using lyophilized affinity-purified IgG with anticardiolipin and anti-beta2-Glycoprotein I activity. Thromb Res 2007;120(1):127-133.
8. Reber G, Arvieux J, Comby E, Degenne D, de Moerloose P, Sanmarco M e*t al*. Multicenter evaluation of nine commercial kits for the quantitation of anticardiolipin antibodies. Thromb Haemost 1995;73(3):444-452.
9. Tincani A, Allegri F, Sanmarco M, Cinquini M, Taglietti M, Balestrieri G e*t al*. Anticardiolipin antibody assay: a methodological analysis for a better consensus in routine determinations. A cooperative project of the European Antiphospholipid Forum. Thromb Haemost 2001;86(2):575-583.
10. Harris EN, Pierangelli S. Revisiting the anticardiolipin test and its standartization. Lupus 2002;11(5):269-275.
11. Exner T, Triplett DA, Taberner DA, Howard MA, Harris EN. Comparison of test methods for the lupus anticoagulant: international survey on lupus anticoagulants-I (ISLA-1). Thromb Haemost 1990;64(3):478-484.
12. Hemostasis Committee of the ''Societé Française de Biologie Clinique'': Laboratory heterogeneity of the lupus anticoagulant: a multicentre study using different clotting assays on a panel of 78 samples. Thromb Res 1992;66(4):349-364.
13. Goudemand J, Caron C, De Prost D, Derlon A, Borg JY, Sampol J e*t al*. Evaluation of sensitivity and specificity of a standardized procedure using different reagents for the detection of lupus anticoagulants. The Working Group on Hemostasis of the Societé Française de Biologie Clinique and for the Groupe d'Études sur I'Hemostase et la Thrombose. Thromb Haemost 1997;77(2):336–342.
14. Jennings I, Kitchen S, Woods TA, Preston FE, Greaves M. Potentially clinically important inaccuracies in testing for the lupus anticoagulant: an analysis of results from three surveys of the UK National External Quality Assessment Scheme (NEQAS) for Blood Coagulation. Thromb Haemost 1997;77(5):934–937.
15. Tincani A, Allegri F, Balestrieri G, Reber G, Sanmarco M, Meroni P e*t al*. Minimal requirements for antiphospholipid antibodies ELISAs proposed by the European Forum on antiphospholipid antibodies. Thromb Res 2004;114(5-6):553-558.
16. Pierangeli SS, Stewart M, Silva LK, Harris EN. Report of an anticardiolipin wet workshop during the VIIth International Symposium on antiphospholipid antibodies. J Rheumatol 1998;25(1):156-162.
17. Wong RCW on behalf of the Australasian aCL working party. Consensus guidelines for anticardiolipin antibody testing. Thromb Res 2004;114(5-6):559-571.
18. Bahar AM, Kwak JY, Beer AE, Kim JH, Nelson LA, Beaman KD *et al*. Antibodies to phospholipids and nuclear antigens in non-pregnant women with unexplained spontaneous recurrent abortions. J Reprod Immunol 1993;24(3):213-222.
19. Spadaro A, Riccieri V, Terracina S, Rinaldi T, Taccari E, Zoppini A. Class specific rheumatoid factors and antiphospholipid syndrome in systemic lupus erythematosus. Lupus 2000;9(1):56-60.
20. Petri M. Epidemiology of the antiphospholipid antibody syndrome. J Autoimmun 2000;15(2):145-151.
21. Tajima C, Suzuki Y, Mizushima Y, Ichikawa Y. Clinical significance of immunoglobulin A antiphospholipid antibodies: possible association with skin manifestations and small vessel vasculitis. J Rheumatol 1998;25(9):1730-1736.
22. Cucurull E, Gharavi AE, Diri E, Mendez E, Kapoor D, Espinoza LR. IgA anticardiolipin and anti-beta2-glycoprotein I are the most prevalent isotypes in African American patients with systemic lupus erythematosus. Am J Med Sci 1999;318(1):55-60.
23. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT *et al*. For the Euro-Phospholipid Project Group: Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum 2002;46(4):1019-1027.
24. Mejia-Romero R, Garcia-Carrasco M, Galarza-Maldonado C, Santos P, Mendonza-Pinto C, Escárcega RO e*t al*. Primary antiphospholipid syndrome in Latin American mestizo patients: clinical and immunologic characteristics and comparison with European patients. Clin Rheumatol 2008;27(7):891-897.
25. de Souza AWS, Silva NP, de Carvalho JF, D'Almeida V, Noguti MAE, Sato EI. Impact of hypertension and hyperhomocysteinemia on arterial thrombosis in primary antiphospholipid syndrome. Lupus 2007;16(10):782-787.