Turk J Hematol 2007; 24:69-74

RESEARCH ARTICLE

Lupus anticoagulant and anticardiolipin antibodies in SLE with secondary Antiphospholipid Antibody Syndrome

Shveta Garg, Annamma Kurien

Department of Pathology Kasturba Medical College, Manipal, India
✉ *shvetatanmaya@gmail.com*

Received: *Jun 12, 2007* • ***Accepted:*** *May 02, 2007*

ABSTRACT

Thirty patients with systemic lupus erythematosus (SLE) and suspected secondary antiphospholipid antibody syndrome (APLAS) were evaluated in the study based on their clinical manifestations. The aim was to study the prevalence of various antiphospholipid antibodies, compare the tests used for their detection and to find a correlation between clinical and laboratory parameters. Coagulation tests used were activated partial thromboplastin time, dilute Russell viper venom time and kaolin clotting time and the results were analyzed statistically.

In our study, arteriovenous thrombosis was more common than recurrent abortions and other clinical manifestations. Twelve percent of patients had positive lupus anticoagulant and 78% had elevated anticardiolipin antibody titers. We concluded that the prevalence of lupus anticoagulant and anticardiolipin antibodies in SLE patients with secondary APLAS was 12% and 17%, respectively. We also proved that dilute Russell viper venom time and kaolin clotting time proved to be much more specific tests and the anticardiolipin antibody a much more sensitive test.

Key Words: SLE, Lupus anticoagulant, anticardiolipin antibodies, kaolin test, dilute russel viper venom test

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by the presence of a wide spectrum of autoantibodies reactive against subcellular structures.

Hence, it is often considered to be the prototype of systemic autoimmune diseases.

Lupus anticoagulant (LA) and anticardiolipin (aCL) antibodies are acquired antiphospholipid antibodies usually found in SLE patients. They are strongly associated with a diverse set of clinical manifestations including arterial and venous thrombosis, neuropsychiatric disorders, thrombocytopenia and recurrent fetal wastage. These together comprise "antiphospholipid antibody syndrome" (APLAS) [1,2]. The severity of thrombosis and missed abortions coupled with their high frequency in the SLE population makes it essential to be able to predict which subset of patients are likely to develop thrombotic events. This would facilitate improved targeting of prevention efforts, as well as provide insight into the etiology and pathophysiology of these manifestations of SLE.

The present study is an attempt to evaluate the incidence of antiphospholipid antibodies in SLE patients. From the scrutiny of previous and contemporary literature, there are a myriad of laboratory tests to detect antiphospholipid antibodies. An attempt has been made to determine the usefulness of activated partial thromboplastin time (APTT), dilute Russell viper venom time (dRVVT) and kaolin clotting time (KCT) in the detection of APLAS in SLE. We have chosen these tests because of their cost effectiveness and availability and because they are the basic minimum tests required to detect APLAS.

MATERIALS and METHODS

Thirty patients with Systemic Lupus Eryrthematosus (SLE) and suspected secondary APLAS, as per the diagnostic criteria for antiphospholipid syndrome [1,2] were included in the study. The study was carried out after taking the patient's informed consent and after obtaining clearance from the ethical committee of our institute. These patients were diagnosed during their visit to hospital or during regular follow up over four years. All these patients fulfilled the 1982 American Rheumatism Association diagnostic criteria [3] for SLE and were suspected to have secondary APLAS due to SLE on the basis of one of the following clinical and/or laboratory parameters:

Clinical Parameters:

1. History of arterial/venous thrombosis.
2. History of recurrent miscarriages.

Associated clinical findings:

a. Seizures.
b. Peripheral neuropathy.
c. History of vascular headache/migraine.
d. Pulmonary hypertension.

Laboratory Parameters:

1. Prolonged APTT.
2. APTT not corrected by addition of normal plasma (prolonged Uncorr APTT).
3. Prolonged KCT.
4. Prolonged dRVVT.
5. Elevated levels of aCL antibodies (> 150/ml).

Associated laboratory findings:

a. Hemolytic picture on peripheral smear examination and positive Coombs test.
b. Thrombocytopenia.

Laboratory tests like Coombs test, Anticardiolipin antibody titers and the clotting assays like prolonged activated partial thromboplastin time (prolonged Uncorr APTT), dilute Russel viper venom time (dRVVT) and kaolin clotting time (KCT) were performed in those SLE patients with suspicion of APLAS.

Clotting tests:

The samples for these tests were collected in 3.8% trisodium citrate in the ratio of 1:9 of anticoagulant and blood. These samples were centrifuged at 2500 rpm for 10 minutes and the plasma was separated. The prolonged Uncorr APTT test was done within 2 hours of collection of the sample. The plasma samples were put in aliquots and frozen at –70°C for KCT and dRVVT tests. These tests were done within 1 month, in batches. The platelet count was checked in the separated plasma samples before freezing to ensure that it was less than 10,000/cu mm. This precaution was taken to avoid platelet interference with the KCT and dRVVT tests. The standard precautions of sample collection, separation, preservation and processing were also followed to ensure reliable results.

1) Activated partial thromboplastin time [4]**:**

The APTT was done by an automated method using Biopool APTT-EA reagent and was considered prolonged if it was at least 8 seconds more than that of the control APTT. If APTT was prolonged, it was repeated by adding equal volume of normal pooled plasma for corrected APTT to rule out any factor deficiencies and confirm inhibitors. APTT is sensitive to heparin, so special consideration was given to ensure the patient was off heparin during the time assays were done.

2) Kaolin clotting time [4]**:**

The KCT was done by Exner KCT screening test and the test was considered suitable only if control plasma had a KCT of >60 seconds. The test was carried out as normal pooled plasma, the patient's plasma and a 4:1 normal:patient plasma mixture. The results were expressed as a ratio of KCT of 4:1 Mix KCT of normal plasma. A ratio equal to or greater than 1.2 was considered positive for lupus anticoagulant (LA).

3) Dilute russell viper venom time [4]**:**

The dRVVT was done by Tulip's LA Detection System that uses LA screen reagent and LA confirm reagent. The clotting time was first calculated with LA screen reagent (screen time). Only if screen time was more than 45 seconds was the test repeated with LA confirm reagent to obtain confirm time (confirm time). Ratio (R) of screen time to confirm time was calculated. If the result was borderline, mixing studies were further done on 50:50 mixtures of test plasma and normal plasma. LA was confirmed if the screen time was not corrected by mixing with normal plasma. The lupus anticoagulant was considered positive if R=1.5.

4) Anticardiolipin antibody assay [4]**:**

Anticardiolipin antibody was done by enzyme linked immunosorbent assay using a NOVAMED kit.

The clinical parameters and the laboratory data of the 30 SLE patients with suspected Antiphospholipid antibody syndrome were subsequently tabulated and analyzed. The statistical methods like chi-square and Z test were applied wherever applicable.

Table 1. Clinical manifestation and hematological parameters

	No. of patients n=30	*Percentage %*
A/V Thrombosis	12	40
H/o Recurrent abortions	05	17
Seizures	05	17
Pulmonary hypertension	05	17
Anaemia	20/30	67
Normocytic normochronic	18/20	60
Hemolytic anaemia	09/30	30
Leukopenia	08/30	27
Thrombocytopenia	11/30	37

Table 2. Coagulation tests

Tests	*Number of patients*	*Percentage %*
Prolonged Uncorr APTT	9/23	39
KCT	2/16	12
dRVVT	2/16	12

RESULTS

A total of 30 patients with suspected secondary APLAS due to systemic lupus eryrthematosus (SLE) were evaluated in this study. The tests were done after obtaining patients' informed consent and approval from the local ethical committee of our institute. The minimum age observed was 15 years and maximum was 52 years. The majority of patients (74%) in the study were either in second or third decade of life and were female (87%). The duration of SLE in the majority of patients was less than five years. History of arterial/venous thrombosis (40% patients) was more common than history of recurrent abortions (17% patients) (Table 1). Hemolytic anemia was seen in 30% of patients (9 of 30) and was confirmed by positive Coombs test and peripheral smear findings in six patients. In the other three patients, peripheral smear findings were suggestive of hemolytic anemia. The Coombs test was not done in two of these and was negative in one patient. Of the nine patients with hemolytic anemia, five patients had additional thrombocytopenia suggestive of Evan's syndrome. Thrombocytopenia was seen in 37%

Table 3. Frequency of antiphospholipid antibodies in SLE patients

Patients	*Number*	*Lupus Anticoagulant by KCT, dRVVT*	*Anticardolipin Antibody*
SLE patients with suspicion of 2º APLA **GROUP (I)**	30	2/16 (12%)	18/23 (78%)
SLE patients without suspicion of 2º APLA **GROUP (II)**	42	0/0	3/11(27%)

Table 4. Antiphospholipid antibodies as predictors of presumed thrombotic events

Tests	*Elevated Anticardiolipin antibody (n=18)*	*Prolonged KCT/dRVVT (n=2)*	*Prolonged Uncorr APTT (n=9)*
Prolonged Uncorr APTT	4/13	2/2	-
Prolonged KCT/dRVVT	1/7	-	2/7
Anticardiolipin antibody	-	1/2	4/7
Arteriovenous thrombosis	8/18	2/2	5/9
Recurrent abortions	2/18	0	2/9
Pulmonary hypertension	1/18	0	-
Peripheral neuropathy	1/18	0	-
Seizures	1/18	0	1/9

of patients and remaining patients had normal platelet counts (Table 1).

APTT was prolonged in 39% of patients and in all these patients was not corrected by the addition of normal plasma, suggesting the presence of an anticoagulant or an inhibitor (Prolonged Uncorr APTT). Among the SLE patients with suspected APLAS, dRVVT and KCT were done in 16 patients and were positive in two patients (12%). Both tests were positive in the same patients (Table 2). Among the patients of SLE with suspected APLA, dRVVT and KCT were done in 16 patients and were positive in 2 patients (12%). Anticardiolipin assay was done in 23 patients and was elevated in 18 patients (78%).. On applying chi-square test, we obtained x^2=13.806 and p=0.0002, which was highly significant (Table 3). On comparing the anticardiolipin antibody assay done in Group I (SLE patients with suspicion of 2º APLAS) and Group II (SLE patients without suspicion of 2º APLAS) patients, we determined x^2= 6.175 and p=0.013, which was again significant. Comparison of LA tests could not be done in the two groups as LA tests were not done in Group II. None of the Group II patients who were positive for anticardiolipin antibody (27%) had a history of arterial/venous thrombosis or recurrent abortions (Table 3). Of nine patients with prolonged Uncorr APTT, eight patients (89%) had a current or past history of presumed thrombotic event, recurrent abortions and seizures. Two patients with prolonged dRVVT had prolonged KCT and history of past thrombotic event. Out of the 18 patients with elevated aCL antibody levels, only 13 (72%) had a presumed thrombotic event, recurrent abortions, pulmonary hypertension, peripheral neuropathy and seizures (Table 4).

The specificity and sensitivity of the prolonged Uncorr APTT, dRVVT/KCT, and anticardiolipin antibodylevels for presumed thrombotic events, recurrent abortions, pulmonary hypertension, peripheral neuropathy and seizures were calculated (Table 5). The prolonged dRVVT/KCT proved to be more specific than the elevated aCL antibody levels (100% versus 84%, p<0.001 significant by Z test) and less sensitive (13% versus 59%, p<0.001 significant by Z test). Similarly, the prolonged Uncorr APTT test proved to be more specific than the elevated aCL antibody levels (93% versus 84%, p<0.05 significant by Z test), whereas prolonged Uncorr APTT and elevated aCL antibody levels were equally sensitive (6% versus 47%, p>0.05 not significant by Z test).

Table 5. Specificity and sensitivity of the prolonged tests for presumed thrombotic events

Tests	*Sensitivity*	*Specificity*
Uncorr APTT	35	50
dRVVT /KCT	13	100
Anticardiolipin antibody	59	16

Table 6. Comparison of antiphospholipid antibody positivity in various studies

Present study		*Petri M et al[5] (1987)*	*Love E et al[11] (1990)*	*Saxena et al[12] (1994)*
aCL antibody	78%	25%	44%	19%
LA	12%	6%	34%	16%

DISCUSSION

Systemic lupus eryrthematosus (SLE) is a disease of unknown etiology in which tissues and cells are damaged by pathogenic autoantibodies and immune complexes. A prevalence study in India found a point prevalence of 3 per 100,000 [4]. Among the various autoantibodies, APLA, though not very specific for SLE, identified patients at increased risk for venous and arterial thrombosis, and risk of fetal loss. This set of antibodies along with their clinical manifestations and their laboratory detection methodology were evaluated in this study.

In the present study, we detected 30 SLE patients with suspicion of APLAS by the clinical signs, symptoms and the laboratory parameters. The number of patients in this study is low because SLE is an uncommon disease and SLE patients with APLAS are even rarer, especially in the Indian scenario. Of the 30 patients, there were a total of 26 females (87%) and 4 males (13%). The female patients were in the second and third decades of life, similar to the sex and age prevalence noted in the study of Petri *et al.*[6]. The duration of disease (SLE) ranged from 3 months to 20 years, with a mean of 2.6 years as compared to 10 years in the study of Petri *et al.*[6]. Thus, most of the patients in present study did not have a prolonged history of illness and were diagnosed early because of improved methods of detection and regular follow up.

In the present study, 40% of patients presented with history of either arterial or venous thrombosis, as compared to only 18% in the study of Petri *et al.* [6]. However, Cervera *et al.* [7] reported 60% of patients with thrombotic episodes due to APLAS. History of recurrent abortions was present in 17% patients, which correlates with 10% in the study of Petri *et al.* [6].

Interestingly, Derksen *et al.* [8] reported 73% fetal loss in APLAS in those patients who were not treated. The main reason for low prevalence of recurrent abortions in the present study could be that most patients during their presentation had a mean duration of disease of three years or less and were on steroids, so conception was not advised. Among the rare manifestations of APLAS, the incidence of seizures, peripheral neuropathy and pulmonary hypertension was slightly more in our study, when compared to results of Petri *et al.* [6] and Cervera *et al.* [8], possibly due to the small study group.

Among the hematological manifestations, hemolytic anaemia (as confirmed by positive Coombs test and/or peripheral smear finding) was detected in 30% of patients. Levine *et al.* [9] also reported the prevalence of hemolytic anemia as 14 to 23%. Thrombocytopenia was found in 37% of our patients, which correlates with 20-40% prevalence as studied by Cuadrado *et al.* [10]. The concomitant prevalence of thrombocytopenia and autoimmune hemolytic anemia (Evan's syndrome) was 55%. Comparatively, Fong *et al.* [11] reported it as 64%.

We found elevated levels of aCL antibodies in 78% and positive LA in 12%, which was statistically highly significant (P = 0.0002). The present study correlated with other studies, in stating that the prevalence of LA is much less than of aCL antibody (Table 6).

We also found that the prevalence of aCL antibody was much more in those SLE patients with suspicion of APLA (78%) in contrast to those without suspected APLA (27%), and this was statistically significant. In the present study, 1 of 7 (14%) aCL antibody-positive patients with SLE had LA. This did not correlate with 45% association of aCL antibody positivity with LA as given by Love *et al.* [12] and 46% as given by Saxena *et al.* [4], possibly because of the small number of cases in whom LA was done. The treatment with steroids in most of these patients could also be a reason for the lower number of LA-positive cases.

There is a 100% association between LA and major manifestation of APLAS (history of abortion or arterial/venous thrombosis), whereas it was 72% with aCL antibody and 88% with prolonged Uncorr APTT. These results correlated with those of Petri *et al.* [6] (100% with LA and 53% with aCL antibody). Petri *et al.* [6] reported prolonged RVVT to be more specific (100%) than elevated aCL antibody levels (83%) as a risk factor for thrombosis and abortion. Similarly, in our study, dRVVT proved to be 100% specific as compared to aCL antibody. The aCL assay was found more sensitive (47%) as compared to dRVVT (25%), as given by Petri *et al.* [6]. Harris *et al.* [13] had also reported LA assay (dRVVT/KCT) to be more specific and aCL assays more sensitive for presumed thrombotic events and abortions. Our study correlates well with these.

Hence, we concluded that the prevalence of Lupus anticoagulant and anticardiolipin antibodies in SLE patients with secondary APLAS was 12% and 17%, respectively. All the patients who had LA had either arteriovenous thrombosis or recurrent abortions, as compared to 72% of patients with anticardiolipin antibodies. The dilute Russel viper venom time and kaolin clotting time proved to be much more specific tests and the anticardiolipin antibody more sensitive.

References

1. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch W, Rauch J. International consensus statement as preliminary classification criteria for definite antiphospholipid syndrome. Report of an international workshop. Arthritis Rheumatol 1999;42:1309-11.
2. Brandt JT, Tripett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulant: an update. On behalf of the Subcommittee on Lupus Anticoagulant/ Antiphospholipid Antibody of the Scientific and Standardization Committee of the ISTH. Thromb Hemostasis 1995;74:1185-90.
3. Hahn BH. Systemic lupus erythematosus. In: Kasper, ed. Harrison's Principles of Internal Medicine. 16th ed. New York: Mc Graw-Hill Medical Publishing Division, 2005:1960-67.
4. Saxena R, Savaya AK, Dhot PS, Singh Y, Malaviya AN. Lupus anticoagulant and anticardiolipin antibodies in systemic lupus erythematosus. Natl Med J India 1994;7:163-5.
5. Malaviya AN, Singh RR, Singh YN, Kapoor SK, Kumar A. Prevalence of systemic lupus erythematosus in India. Lupus 1993;2:115-8.
6. Petri M, Rheinschmidt M, Whiting-O'Keefe Q, Hellmann D, Corash L. The frequency of lupus anticoagulant in systemic lupus erythematosus. Ann Intern Med 1987;106:524-31.
7. Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, Jacobsen S, Lakos G, Tincani A, Kontopoulou-Griva I, Galeazzi M, Meroni PL, Derksen RH, de Groot PG, Gromnica-Ihle E, Baleva M, Mosca M, Bombardieri S, Houssiau F, Gris JC, Quere I, Hachulla E, Vasconcelos C, Roch B, Fernandez-Nebro A, Boffa MC, Hughes GR, Ingelmo M; Euro-Phospholipid Project Group. Clinical and immunological manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum 2002;46:1019-27.
8. Derksen RH. Lupus anticoagulant. Revival of our old phenomenon. Clin Exp Rheumatology 1985;3:349-57.
9. Levine JS, Branch W, Rauch J. The antiphospholipid syndrome. N Engl J Med 2002;346:752-63.
10. Cuadrado MJ, Mujie F, Munoz E, Khamashta MA, Hughes GR. Thrombocytopenia in antiphospholipid antibody syndrome. Ann Rheum Dis 1997;56:194-6.
11. Fong KY, Loizou S, Boey ML, Walport MJ. Anticardiolipin antibodies, hemolytic anaemia and thrombocytopenia in systemic lupus erythematosus. Br J Rheumatol 1992;31:453-5.
12. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in SLE and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990;112:682-98.
13. Harris EN, Gharavi AE, Patel SP, Hughes GR. Evaluation of the anticardiolipin antibody test: report of an international workshop held 4 April 1986. Clin Exp Immunol 1987;68:215-22.