

# Prevalence of Antiphospholipid Antibodies in Syrian Patients with Thrombosis

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## ABSTRACT

**Background:** Antiphospholipid antibodies (aPL) are a heterogeneous family of antibodies associated with thrombosis and other complications. **Objective:** To study the prevalence of aPL in patients with thrombosis at Aleppo University Hospitals, Syria. **Methods:** One hundred and fifty-seven patients with venous and arterial thrombosis and 63 healthy controls were studied. Anticardiolipin antibodies (aCL) and Lupus anticoagulant (LA) were determined. **Results:** Thirty-four out of 157 (21.7%) patients with thrombosis had some type of aPL. aPL was also found in four healthy subjects (4/63=6.3%). Eighteen patients (11.5%) were positive for LA, 20 (12.7%) for aCL antibodies and 4 (2.6%) were positive for more than one aPL. Patients without risk factors for thrombosis and having positive aPL were 23/34 (67.7%). Fourteen out of 78 (17.9%) patients with arterial thrombosis, and 20/79 (25.3%) with venous thrombosis were positive for at least one aPL. **Conclusion:** Our study showed a significant prevalence of aPL in patients with thrombosis. It seems that aPL is a risk factor for venous and arterial thrombosis, especially in patients with no conventional risk factors.

**Keywords:** Antiphospholipid Antibodies, Anticardiolipin Antibodies, SLE

## INTRODUCTION

Antiphospholipid antibodies (aPL) are a heterogeneous group of antibodies detected either by their binding to cardiolipin in the presence of B2 glycoprotein I (B2GPI) by ELISA or by their ability to prolong clotting time in vitro (lupus anticoagulant, LA). Anticardiolipin antibodies (aCL) have been described in autoimmune diseases such as systemic lupus erythematosus (SLE) (1,2) but may also be found in other clinical conditions, including infectious diseases (3,4), malignancies (5), and drug-induced diseases (6). Their persistent presence can be associated with venous and/or arterial thrombotic events (7,8). The main purpose of this study was to determine the prevalence of different aPL in Syrian patients with venous and arterial thrombosis.

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## PATIENTS AND METHODS

One hundred and fifty-seven patients with thrombosis from the Regional Hospitals of Aleppo, Syria, were studied between March 2007 and January 2009. The inclusion criterion was at least one episode of venous and/or arterial thrombosis. Mean age was 35 years; 37.6% were males and 62.4% were females. Seventy-eight (49.7%) patients had arterial thrombosis (23.6% ischemic stroke, IS; and 26.1% acute myocardial infarction, AMI) and 79 (50.3%) patients had venous thrombosis (43.3% deep venous thrombosis, DVT; and 7% retinal vein thrombosis, RVT). Thrombosis was confirmed by CT brain scan, electrocardiogram, cardiac enzymes and Doppler, depending on the case. Exclusion criteria were: age over 50 years, hepatic and cardiac insufficiencies, nephropathy, cancer, transplantation, seropositive cases of hepatitis B and C, human immunodeficiency virus, syphilis, and presence of several risk factors for thrombosis. Patients' data included the following clinical parameters: i) thrombosis type (arterial or venous), site, previous thrombosis history including sites, number of events and date of initial event; ii) clinical risk factors for thrombosis: including arterial hypertension (history or blood pressure > 140/90 mm Hg in at least two recordings), diabetes (history or fasting blood glucose > 120 mg/dl), hyperlipidemia (fasting triglycerides > 170mg/dl and/or fasting total cholesterol >230 mg/dl), smoking more than 15 cigarettes/day, obesity (BMI>30). The normal control group included 63 healthy blood donors who referred to the Blood Bank of the Regional Hospitals of Aleppo, Syria (mean age, 34 years; males, 34.9% and females, 65.1%). This control group was chosen according to the following inclusion criteria: age<50 years, absence of a previous history of thrombosis or any of the above-mentioned exclusion criteria. One blood sample was collected from each patient. In the case of AMI patients, samples were collected within the first 24 hours after infarction. Sera were separated and stored at -70°C until analysed.

**ELISA.** An ELISA assay for the determination of aCL antibodies (IgG, IgM) was used as previously described by Loizou et al. (9) with some modifications (9, 10). Briefly, non-irradiated microtiter plates (NUNC, Maxisorp, Denmark) were coated with 50 µl of CL (Sigma-Aldrich, USA) at a concentration of 50 µg/ml in 70% ethanol. The plates were air-dried overnight at 4°C. After washing with a 0.01 M phosphate-buffered saline (PBS) pH 7.4, the plates were blocked with 10% fetal bovine serum (FBS) (GIBCO, Scotland) in PBS for 1 h at room temperature. After washing with PBS, 50 µl of serum samples (diluted 1:50 in FBS-PBS), or the negative and the positive controls were added to the plates in duplicate. aCL ELISA standards (Louisville APL Diagnostics Inc, USA) were included to construct a calibration curve and to express the results in GPL and MPL units. After 1 hour incubation at room temperature, plates were washed with PBS, and 50 µl of alkaline phosphatase-conjugated anti-human IgG and IgM (Sigma-Aldrich, USA) were added to the plates. After 1 h incubation at room temperature and washing with PBS, 100 µl of p-nitrophenyl-phosphate substrate (Merck) in diethanolamine buffer (pH 9.8) was added. When the optical densities (OD) of the highest concentration point of the standard curve reached 1.0, the plates were read at 405 nm in a Stat-Fax-2100 microplate reader (Awareness Technology Inc). The positive samples were tested twice to confirm the results. aCL activity was expressed in GPL and MPL. For the two isotypes the cut-off value was 15 units in all cases.

Laboratory diagnosis of lupus anticoagulants was performed according to the revised criteria proposed by the subcommittee for standardization of Lupus Anticoagulant of the

International Society on Thrombosis and Hemostasis (11) and included mixing studies, screening and confirmation tests.

**Statistical Analysis.** Statistical analysis was performed using the SigmaStat software (SPSS, version 12.0).

In order to compare the qualitative data between patients and controls, Chi<sup>2</sup>-test or, if appropriate, Fisher's exact test was performed. Statistical significance was considered at  $p < 0.05$ .

## RESULTS

Main demographic and biologic characteristics of patients and controls are listed in Table 1. Mean and sex ratio showing a female predominance were similar between both groups (59M/98F and 22M/41F) for patients and controls, respectively. There was a relationship between sex and the type of thrombotic event, 65.3% thrombosis in women and 25.4% in men and 74.6% arterial thrombosis in men and 34.7% in women. The clinical risks for thrombosis were also included in the characteristics of the thrombosis population and were compared to those of the controls (Table 1). The prevalence of the parameters was similar in both populations.

**Table 1. Main demographic data and risks for thrombosis in patients and controls.**

Variable	Patients(n=157)	Controls(n=63)	P
Age, years [mean(range)]	35 (16-50)	34 (19-50)	NS <sup>1</sup>
Sex ratio, M/F	0.6	0.53	NS
Thromboembolic events(%)			
Venous	50.3%	-	
Arterial	49.7%	-	
Clinical risks for thrombosis(%)			
Arterial hypertension	7%	6.3%	NS
Tobacco <sup>2</sup>	28%	22.2%	NS
Diabetes	3.2%	1.6%	NS
Hyperlipidemia	8.3%	7.9%	NS
Obesity <sup>3</sup>	15.9%	11.1%	NS

<sup>1</sup>NS, not significant. <sup>2</sup>Cigarettes/day: N>15. <sup>3</sup>BMI>30.

Thirty-four out of 157 (21.7%) patients with thrombosis had some type of aPL. Twenty out of 157 patients (12.7%) and 2/63 controls (3.2%) were positive for at least one isotype of aCL antibodies. The difference between the two groups was statistically significant ( $p < 0.04$ ) (Table 2). Sixteen were positive for IgG and 8 for IgM aCL antibodies (Table 2). Isolated isotypes were observed more frequently (IgG=10.2%, IgM=5.1%) than combinations of isotypes (2.6%). The mean of activity of different isotypes of aCL antibodies are shown in Table 2. Ten out of 20 (50%) aCL-positive cases were >40 GPL or MPL. The positive cases among the control group were low positive <19 GPL, MPL. Eighteen out of 157 (11.5%) patients and 2/63 (3.2%) controls were positive for LA, and this was statistically significant ( $p < 0.03$ ) (Table 2).

Eighteen out of 157 (11.5%) patients showed only aCL, and 16 (10.2%) demonstrated only LA. In four patients (2.6%) more than one aPL was detected.

**Table 2. Prevalence of different antiphospholipid antibodies in patients and controls.**

Antibodies <sup>1</sup>	Patients(n=157)		Controls(n=63)		P
	n	%	n	%	
aPL	30	21.7	4	6.3	0.007
LA	18	11.5	2	3.2	<0.03
aCL	20	12.7	2	3.2	<0.01
-IgG	16	10.2	1	1.6	0.021
-IgM	8	5.1	1	1.6	0.216

<sup>1</sup> aPL, antiphospholipid antibodies aCL, anticardiolipin antibodies; LA, lupus anticoagulant

The prevalence of aCL and LA antibodies in different types of thrombosis is shown in Table 3. 12/79 (15.2%) patients with venous thrombosis, 8/78 (10.3%) patients with arterial thrombosis, and 10/65 (15.4%) patients with DVT were positive for aCL antibodies compared to 2/63 (3.2%) in the control group. Eight out of 78 (10.3%) patients with arterial thrombosis and 4/37 (10.8%) patients with IS were positive for LA compared to 2/63 (3.2%) in the control group.

**Table 3. Antiphospholipid antibodies in patients with different types of venous and arterial Thrombosis**

Thrombosis	aCL		LA	
	n	%	n	%
Venous thrombosis (n=79)	12	15.2	10	12.7
Deep venous thrombosis (n=65)	10	15.4	9	13.9
Retinal venous thrombosis (n=14)	2	14.3	1	7.1
Arterial thrombosis (n=78)	8	10.3	8	10.3
Acute myocardial infarction (n=41)	4	9.8	4	9.8
Ischemic stroke (n=37)	4	10.8	4	10.8

Twenty-three out of 157 (14.7%) patients with thrombosis (AMI=4 and DVT=12 and IS=5 and RVT=2) showed aPL as the only identified thrombosis risk factor. Fifty-eight arterial thrombosis patients presented the classic risk factors of arterial thrombosis; of these, 5 (8.6%) patients (IS=2, AMI=3) presented both the aPL and the classic risk factors. On the other hand, 50 venous thrombosis patients presented the classic risk factors of venous thrombosis; of these, 6 (12%) patients (DVT=5 and RVT=1) presented both the aPL and the classic risk factors. Twenty-six of 157 (16.6%) patients did not present aPL or the classic risk factors (IS=5, AMI=7, DVT=11, and RVT=3).

## DISCUSSION

The main objective of this study was to determine the prevalence of different aPL in Syrian patients with venous or arterial thrombosis. Our results show that 34/157 (21.7%) patients who presented thrombotic events were positive for some type of aPL. In this study some type of aPL was found in 17/65 (26.2%) patients with DVT. Ginsberg et al. (12), while looking at the association between the presence of aPL in both initial and recurrent episodes of DVT, found that there was a strong association between LA and DVT but no association with aCL antibodies. On the other hand, Eschwege et

al. (13) found LA in 15% and anti-B2GPI antibodies in 8% of unselected patients with venous thrombosis. Lee et al. (14) found that venous thrombosis was associated with LA, IgG aCL, and IgA anti-B2GPI antibodies in SLE patients. We found that 3/14 (21.4%) patients with RVT were positive for some type of aPL. Many studies have found aPL in 19%–24% of patients with RVT (15-19). With regard to venous thrombosis, we found an association between the whole group and DVT with aCL antibodies. The lack of association between RVT and aCL antibodies may be explained by the small sample size used in this study. Seven out of 37 (18.9%) patients with IS were positive for some type of aPL. Cerebral ischemia is the most common arterial thrombotic manifestation associated with aPL (20). The importance of aPL as a cardiovascular disease risk factor is controversial. Many studies have found that aPL are associated with an increased risk for incident (21,22) and recurrent (22,23) episodes of cerebral ischemia, but some have not (25). Fiallo et al. (25) found that 24% of unselected patients with IS and 12% patients with non-ischemic neurological disorders presented these antibodies, respectively. We found that 7/41 (17.1%) patients with AMI were positive for some type of aPL. A variety of cardiac manifestations have been found in association with aPL. They mainly include valvular and coronary artery diseases, as well as intracardiac thrombus and cardiomyopathy (26). However, the pathogenic role of the aCL antibodies in the development of AMI is controversial (27,28). For some authors, the presence of aCL antibodies in the context of an AMI would suppose a secondary immunologic reaction and not a triggering factor (29, 30). The differences observed in the prevalence of different aPL, when compared with other studies, may be related to differences in patient populations or protocols used for testing.

In summary, this study shows that 21.7% of patients with arterial or venous thrombosis had some type of aPL. Our results showed that aCL and LA antibodies seem to be risk factors for venous and arterial thrombosis, therefore screening for aPL in patients with thrombosis seems to be warranted.

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