The Presence of Multiple Prothrombotic Risk Factors Is Associated with a Higher Risk of Thrombosis in Individuals with Anticardiolipin Antibodies

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ABSTRACT. ***Objective.*** To explore the effect of multiple prothrombotic risk factors in individuals with anticardiolipin antibodies (aCL), we evaluated immunologic, coagulation, and genetic prothrombotic abnormalities in a cohort of individuals with different aCL titers.

Methods. We recruited 87 individuals into 4 categories (normal, low, intermediate, or high) based on their baseline IgG aCL (aCL-IgG) titers. We measured at followup: repeat aCL-IgG, IgM aCL (aCL-IgM), antibodies to ß2-glycoprotein I (anti-$ß_2$-GPI), lupus anticoagulant (LAC) antibodies, protein C, protein S, activated protein C resistance, factor V^{506} Leiden mutation, methyl tetrahydrofolate reductase (MTHFR) C677T genotype, and prothrombin 20210A gene mutation. Thrombotic events were confirmed.

Results. At recruitment, 20 individuals were negative for aCL-IgG and 67 were positive (22 low, 20 intermediate, and 25 high titer). Twenty of the 87 participants had experienced a previous thrombotic event: 4 in the aCL-IgG negative group and 16 in the aCL-IgG positive group. Among the 87 individuals, the number of those with concomitant prothrombotic risk factors was as follows: 5 had no other prothrombotic risk factors, 32 had 1 risk factor, 24 had 2 risk factors, 10 had 3 risk factors, 10 had 4 risk factors, and 6 had 5 risk factors. Thrombotic events were observed in 20%, 13%, 33%, 10%, 30%, and 50% of these groups, respectively, and the odds ratio associated with a previous thrombotic event was 1.46 per each additional prothrombotic risk factor (95% confidence interval: 1.003-2.134).

Conclusion. In individuals with positive aCL-IgG, we observed an association between the number of prothrombotic risk factors and history of thrombotic events. (J Rheumatol 2003;30:2385–91)

Key Indexing Terms:
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The antiphospholipid syndrome (APS) is currently described as the presence of a persistent antiphospholipid antibody (aPL) in association with vascular thrombosis or pregnancy morbidity[1,2]. The aPL can be either moderate to high level IgG or IgM anticardiolipin antibody (aCL-IgG or aCL-IgM) or lupus anticoagulant (LAC). Among subjects with one or more aPL, up to 22% of people without systemic lupus erythematosus (SLE)[3] and 70% of those with SLE[4] develop thrombosis. Since the original description of APS, the fundamental question of why some patients with aPL develop thrombosis while others do not remains unanswered. One possibility, referred to as the double hit theory, is predicated on the idea that an individual's risk of thrombosis increases in the presence of more than one coagulation abnormality[5-8]. Based on this theory, aPL in the presence of other thrombophilic abnormalities would result in an increased risk of thrombosis.

aPL are a heterogeneous group of antibodies that includes LAC and aCL. LAC antibodies are immunoglobulins that inhibit phospholipid-dependent coagulation reactions *in vitro* and are detected by prolongation of functional

coagulation assays[9]. In contrast, aCL are immunoglobulins that react with cardiolipin, a phospholipid, and are detected by solid phase immunoassays[10]. The binding of autoimmune aCL to phospholipids usually requires ß$_2$-glycoprotein I (ß$_2$-GPI), a plasma phospholipid-binding protein of unknown physiological function[11-14]. Similarly, many LAC antibodies recognize phospholipid-bound ß$_2$-GPI or prothrombin[15-19]. Antibodies to ß$_2$-GPI itself can also be detected in solid phase immunoassays and appear to be clinically important in APS, although they are not part of the currently proposed APS criteria[1]. Recent studies suggest that some categories of aPL, or combinations of aPL, may carry a higher risk for thrombosis[20].

A number of coagulation abnormalities that increase an individual's thrombotic risk have been identified. This so-called thrombophilia includes defects in the anticoagulant regulatory pathway, namely protein C and protein S deficiencies; a point mutation in coagulation factor V (factor V^{506} Leiden) that results in resistance to activated protein C[21-24]; the C677T polymorphism of the methyl tetrahydrofolate reductase (MTHFR) gene, which is associated with elevated homocysteinemia[25-28]; and the recently described 20210A prothrombin gene mutation, a G to A nucleotide transition at position 20210 of the 3'-UTR (untranslated region) of the prothrombin gene[29].

We assessed immunologic and coagulation abnormalities in a group of individuals known to have aCL-IgG. We hypothesized that individuals with aCL-IgG and coexisting immunologic and thrombophilic abnormalities would be more likely to have experienced a previous thrombotic event.

MATERIALS AND METHODS

Patients. All individuals tested for aCL in the Clinical Immunology Laboratory of the Montreal General Hospital between September 1992 and December 1994 were identified. The initial cohort consisted of 1564 persons. They were divided into 4 groups according to their initial aCL-IgG titer: normal (< 23 GPL), low (23–29.9 GPL), intermediate (30–49.9 GPL), and high (≥ 50 GPL). No information was available on why the aCL test was ordered. Seventy individuals were randomly selected from each group to represent a spectrum of aCL titers. Of these 280 individuals, 52 were not available: 9 had died, 7 were minors, and 36 were lost to followup. Two hundred and twenty-eight individuals were thus contacted to complete a mailed survey. One hundred and thirty-eight individuals responded to the survey. The individuals who answered the survey were then invited to undergo a clinical evaluation and blood tests. Eighty-seven individuals agreed to visit our clinic and to donate blood. Of these, 67 had been found to have elevated aCL-IgG, while 20 had normal levels. Our institutional Ethics Committee approved the study and all patients provided written, informed consent.

Study design. This was a descriptive, cross-sectional study. All study participants underwent a clinical evaluation and selected immunologic, hematologic, and genetic tests. Ascertainment of the reported thrombotic events was done retrospectively and all reported events were confirmed through review of available medical records.

Clinical evaluation. The clinical evaluation consisted of a questionnaire and a physical examination. The questionnaire elicited information on the following: demographics (age, gender, race, education, family income, cigarette smoking, alcohol intake); general health including medications (e.g., aspirin, warfarin, or oral contraceptives); comorbid conditions [e.g., hypertension, diabetes mellitus, SLE, rheumatoid arthritis (RA), thyroid gland diseases, and chronic infections]; history of arterial and/or venous thrombosis [including coronary artery disease, cerebrovascular accidents (CVA), transient ischemic attacks, deep venous thrombosis (DVT), pulmonary embolus (PE)]; family history of thrombosis in a first degree relative; and history of obstetrical events.

The arterial and venous events reported by the study participants were verified by individual chart review, including both hospital and outpatient charts. We sought to confirm the following reported events: transient ischemic attack, cerebrovascular accident, retinal artery thrombosis, coronary artery disease, myocardial infarction (MI), DVT, PE, retinal vein thrombosis, and portal vein thrombosis. We relied on the diagnosis made by the treating physician and confirmed by our chart review.

Blood samples and laboratory tests. Serum, citrated plasma, and cells were obtained, aliquoted, and stored at -70ºC until used. Laboratory tests, including complete blood counts, antinuclear antibodies (ANA), extractable nuclear antigens (ENA), rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), liver and renal function tests, and urinalyses were performed.

Serology. aCL assays were performed both in the Clinical Immunology Laboratory, using Kallestad anticardiolipin microplate EIA (normal for aCL-IgG < 23 GPL units and for aCL-IgM < 11 MPL units) (Sanofi-Pasteur, Inc., Chaska, MN, USA), and in the Rheumatology Research Laboratory, using an anticardiolipin ELISA that has been described[30]. Anti-ß$_2$-GPI antibody was also measured by ELISA[30] (normal < 0.6 OD_{405} units). LAC antibodies were detected by activated partial thromboplastin time (APTT, Thrombosil I, Ortho Diagnostics Systems, Raritan, NJ, USA) and dilute APTT assay (Automated APTT, Organon Teknika, Scarborough, ON, Canada) in which plasma was diluted 1:1 with normal plasma. Confirmation of LAC activity was performed by neutralization with hexagonal phase phosphatidylethanolamine[19].

Hematological and genetic studies. The following coagulation variables were assessed: functional and immunological protein C (protein C amydolytic assay, Berichrom, Behring Diagnostic, Montreal, QC, Canada) (normal ≥ 0.7 mU/l); functional protein S (protein S amydolytic assay, Liatest, Diagnostica Stago, Wellmark, Mississauga, ON, Canada (normal ≥ 0.63 mU/l); and activated protein C resistance (APCR) (Chromogenix, Helena Laboratories, Mississauga). The following genetic studies were done: factor V^{506} Leiden mutation (amplification of genomic DNA with the polymerase chain reaction and digestion of fragments with *Mnl*I[23]); C677T MTHFR gene polymorphism (genomic DNA amplification using PCR and digestion of fragments with *hiwf*I[25]); and 20210A prothrombin gene mutation (genomic DNA amplification with PCR and digestion of fragment with *hind*III[29]).

Data analysis. All data were entered into Medlog (Medlog System, 1995) and verified. Descriptive univariate analyses were performed for demographic and health variables (Pearson's correlations). For those with elevated aCL-IgG titers at baseline, multivariate regression analysis was performed using thrombotic events as the outcome variable, the number of additional prothrombotic risk factors as the predictor variable, and age and gender as covariates. The additional prothrombotic risk factors considered were presence of aCL-IgG on repeat testing, aCL-IgM, LAC, anti-ß$_2$-GPI antibodies, protein C deficiency, protein S deficiency, factor V^{506} Leiden mutation, APCR phenotype (in the absence of factor V^{506} Leiden mutation), C677T MTHFR polymorphism (heterozygous or homozygous), and 20210A prothrombin gene mutation. Analysis was done using SAS[31].

RESULTS

Demographics of the cohort. Characteristics of the 87 individuals who agreed to participate in this study are shown in Table 1. Individuals are grouped according to their initial aCL-IgG titers: normal (< 23 GPL), low (23–29.9 GPL),

Table 1. Population characteristics.

Variable	aCL Category* Normal n = 20	Low n = 22	Intermediate n = 20	High n = 25	Total n = 87
Mean age, yrs (SD)	48.9 (13.9)	48.9 (13.9)	46.7 (12.7)	45.1 (15.5)	47.6 (14.2)
% Female	80	91	95	76	85
Ethnic origin, n (%)					
Caucasian	17 (85)	19 (86)	18 (90)	20 (80)	74 (85)
Black	2 (10)	0	0	2 (8)	4 (5)
Hispanic	0	0	1 (5)	0	1 (1)
Asian	1 (5)	2 (9)	0	2 (8)	5 (6)
Other	0	1 (5)	1 (5)	0	2 (2)
Smoking (ever), n (%)	4 (20)	5 (23)	5 (25)	3 (12)	17 (20)
Comorbidities, n (%)					
Hypertension	4 (20)	6 (27)	1 (5)	5 (20)	16 (18)
Diabetes mellitus	0	3 (14)	0	0	3 (4)
Thyroid disease	5 (25)	6 (27)	2 (10)	2 (8)	15 (17)
RA	3 (15)	2 (9)	3 (15)	2 (8)	10 (12)
SLE	4 (20)	6 (27)	6 (30)	10 (40)	26 (30)
Chronic infection	1 (5)	0	1 (5)	0	2 (2)
Thrombosis, n (%)					
Arterial	1 (5)	3 (14)	3 (15)	6 (24)	13 (15)
Venous	3 (15)	3 (14)	2 (10)	2 (8)	10 (12)
Arterial or venous	4 (20)	5 (23)	4 (20)	7 (28)	20 (23)

* aCL categories were defined using aCL-IgG test results at entry: normal < 23 GPL; low 23.0–29.9 GPL; intermediate 30–49.9 GPL; High ≥ 50 GPL. SD: standard deviation; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus.

intermediate (30–49.9 GPL), and high (≥ 50 GPL). Prior to entry into the study, 67 individuals had elevated titers of aCL-IgG: 25 were high, 20 were intermediate, and 22 were low. Twenty individuals were negative for aCL-IgG. Our sample consisted mostly of women (85%) of Caucasian race (85%). The mean age of the cohort was 48 years. Twenty-six individuals (29.9%) had SLE, 18% had hypertension, 4% diabetes mellitus, 17% thyroid disease, 13% RA, and 2% chronic infection.

Thrombotic events. Thirteen (15%) participants had experienced one or more (≥ 1) arterial events and 10 (12%) had experienced one or more (≥ 1) venous events. Three individuals had both arterial and venous events, and they were all aCL-IgG positive. Hence, 16 of the 67 aCL-IgG positive participants (24%) and 4 of the 20 aCL-IgG negative participants (20%) had thrombotic events.

Clinical evaluation. No association was found between the presence of aCL-IgG and specific diagnosis (such as CVA, MI, or DVT), comorbid diseases (such as hypertension, diabetes mellitus, SLE, RA, thyroid gland disease, or chronic infections), or physical findings (such as Raynaud's, livedo reticularis, or decreased pulses) (data not shown). In univariate analysis, no association could be identified for arterial events, venous events, or miscarriages, when gender, smoking, hypertension, and repeat aCL-IgG titers were considered as predictors (data not shown). This may be due to the relatively small number of individuals with events within each category.

Repeat aCL-IgG testing. Of those individuals initially identified with elevated aCL-IgG titers (n = 67), only 40 (60%) had elevated aCL-IgG titers on repeat testing. Twenty-six (26) participants had normal aCL-IgG status on repeat testing and the result of one participant was not available. Conversely, 2 individuals with normal aCL-IgG titers at entry into the study had elevated aCL titers (one low and one intermediate) on repeat testing. Fourteen (21%) of the 67 participants with elevated aCL-IgG at entry had elevated aCL-IgM on repeat testing, 19 (28%) had LAC antibodies, and 18 (27%) had anti-$ß_2$-GPI antibodies. In contrast, of the 20 participants negative for aCL at entry into the study, 1 (5%) had aCL-IgM, 1 (5%) had LAC antibodies, and 4 (20%) had anti-$ß_2$-GPI antibodies (data not shown).

Prothrombotic risk factors. The distribution of prothrombotic risk factors in the cohort of 87 participants is presented in Table 2 for the 67 individuals without thrombosis and the 20 individuals with thrombosis. Among those individuals with thrombosis, the following had prothrombotic risk factors: 12 (60%) had aCL-IgG on repeat testing, 3 (15%) had aCL-IgM, 9 (45%) had LAC, 7 (35%) had anti-$ß_2$-GPI, 2 (10%) had protein C deficiency, 1 (5%) had factor V^{506}, and 1 (5%) had acquired activated protein C resistance (APCR). There were 12 (60%) MTHFR heterozygotes and 2 (10%) MTHFR homozygotes, and 1 (5%) individual had a prothrombin gene mutation. No one in the thrombosis group had protein S deficiency.

Of the individuals without thrombosis (n = 67), the

Table 2. Distribution of prothrombotic risk factors in individuals without and with thrombosis.

Risk factor, n (%)	Without Thrombosis aCL Category*					With Thrombosis aCL Category*				
	Normal n = 16	Low n = 17	Intermediate n = 16	High n = 18	Total n = 67	Normal n = 4	Low n = 5	Intermediate n = 4	High n = 7	Total n = 20
aCL-IgG	2 (13)	7 (41)	7 (44)	14 (78)	30 (45)	0	2 (40)	4 (100)	6 (86)	12 (60)
aCL-IgM	1 (6)	4 (24)	2 (13)	5 (28)	12 (18)	0	1 (20)	0	2 (29)	3 (15)
LAC	1 (6)	2 (12)	3 (19)	5 (28)	11 (16)	0	1 (20)	1 (25)	7 (100)	9 (45)
aß$_2$GPI	2 (13)	2 (12)	5 (31)	6 (33)	15 (22)	2 (50)	0	0	5 (71)	7 (35)
Protein C deficiency	0	2 (12)	4 (25)	0	6 (9)	1 (25)	0	0	1 (14)	2 (10)
Protein S deficiency	0	0	1 (6)	1 (6)	2 (3)	0	0	0	0	0
APCR**	2 (13)	0	2 (13)	1 (6)	7 (7)	0	0	0	1 (14)	1 (5)
Factor V^{506}	1 (6)	0	2 (13)	0	3 (4)	1 (25)	0	0	0	1 (5)
Acquired	1 (6)	0	0	1 (6)	2 (3)	0	0	0	1 (14)	1 (5)
MTHFR										
Heterozygous	9 (56)	7 (41)	7 (44)	8 (44)	31 (46)	3 (75)	1 (20)	3 (75)	5 (71)	12 (60)
Homozygous	3 (19)	6 (35)	2 (13)	3 (17)	14 (21)	0	1 (20)	1 (25)	0	2 (10)
Prothrombin gene mutation***	n/a	0	1 (6)	0	1 (1)	n/a	0	1 (25)	0	1 (5)

* aCL categories were defined using aCL-IgG test results at entry: normal < 23 GPL; low 23.0–29.9 GPL; intermediate 30–49.9 GPL; high ≥ 50 GPL. ** One individual was heterozygotic for factor V Leiden and had a normal APCR. This individual was taking coumadin. *** Prothrombin gene mutation was not evaluated in the aCL normal group.

percentage of individuals with prothrombotic risk factors was generally lower than in those with thrombosis (n = 20). This applied to all risk factors, with the exception of aCL-IgM, protein S deficiency, APCR, and homozygous MTHFR. Interestingly, the 3 individuals in the cohort with protein S deficiency were all in the group without a previous thrombosis.

Relationship between number of risk factors and thrombosis. The relationship between the number of prothrombotic risk factors and a history of thrombosis is shown in Table 3 and Figure 1. Table 3 shows the aCL-IgG category of the study participants, as well as their thrombotic status. It is noteworthy that the percentage of individuals in each aCL category was similar (20% to 27%), whether the individuals had experienced a thrombotic event or not, with the exception of thrombotic individuals with high titer aCL-IgG (35%) (Table 3). In contrast, subgrouping the study participants according to their number of prothrombotic risk factors showed differences between the individuals with and without thrombosis (Figure 1). As the number of prothrombotic risk factors increases, there is a tendency towards an increased history of thrombosis. For example, 6/20 (30%) of individuals with thrombosis had 4 or more prothrombotic risk factors, compared to 10/67 (14.9%) of individuals without thrombosis (Table 3). This is particularly clear when the percentage of individuals with thrombosis is calculated for each prothrombotic risk factor category (Figure 1). In this figure, we observe a trend towards a higher proportion of individuals with thrombosis in the groups with a higher number of prothrombotic risk factors.

Multivariate regression analysis was performed to measure the association between risk of thrombosis and the

Table 3. Number of prothrombotic risk factors in individuals without and with thrombosis.

	Without Thrombosis aCL Category*					With Thrombosis aCL Category*				
	Normal	Low	Intermediate	High	Total	Normal	Low	Intermediate	High	Total
Prothrombotic risk factors										
0	2	1	0	1	4	0	1	0	0	1
1	9	8	5	6	28	2	2	0	0	4
2	4	4	6	2	16	1	2	3	2	8
3	0	2	2	5	9	1	0	0	0	1
4	1	2	2	2	7	0	0	1	2	3
5	0	0	1	2	3	0	0	0	3	3
Total**	16	17	16	18	67	4	5	4	7	20

* aCL categories were defined using aCL-IgG test results at entry: normal < 23 GPL; Low 23.0–29.9 GPL; intermediate 30–49.9 GPL; high ≥ 50 GPL. ** The percentage (%) of individuals in each aCL category was: 23.9, 25.4, 23.9, and 26.9 for individuals without thrombosis and 20.0, 25.0, 20.0, and 35.0 for individuals with thrombosis.

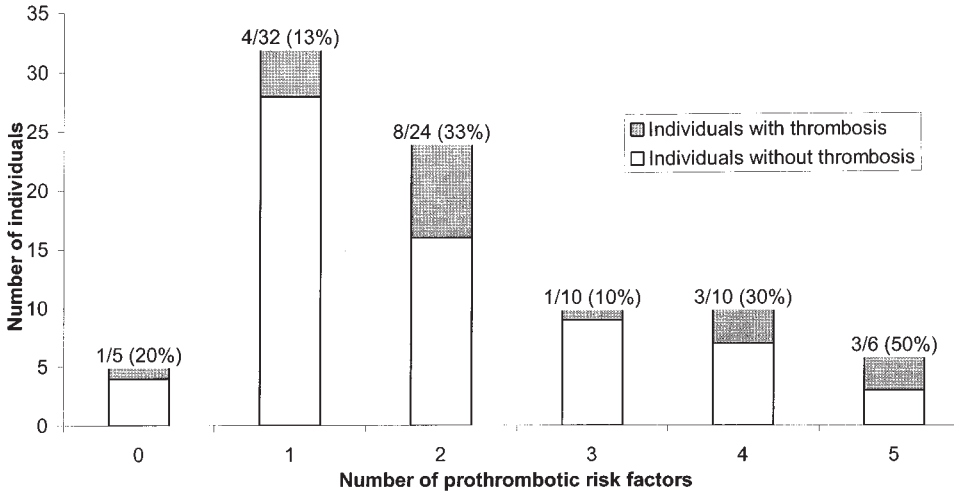

Figure 1. Association of number of prothrombotic risk factors with thrombosis. The number of individuals in the study and proportion of those with thrombosis are plotted for each number of prothrombotic risk factors. The proportion of individuals with thrombosis tends to increase with the number of prothrombotic risk factors.

number of risk factors, controlling for age and gender. Risk factors in the analysis using the single variable of number of risk factors included: aCL-IgG on repeat testing, aCL-IgM, protein C and S deficiencies, LAC antibodies, anti-ß$_2$-GPI antibodies, factor V^{506} Leiden mutation, APCR in the absence of factor V^{506} Leiden mutation, C677T MTHFR gene polymorphism (heterozygous or homozygous), and 20210A prothrombin gene mutation. In those with a positive aCL-IgG at study entry, the odds ratio (OR) of having had a thrombotic event prior to entry into the study was 1.46 for each additional prothrombotic risk factor documented [95% confidence interval (CI): 1.003–2.134, p = 0.048].

DISCUSSION

We have evaluated a group of individuals who had been sent for aCL testing by their physician and who were recalled by us for further testing one to 4 years later. No information was available on why the test was ordered, and a selection bias towards oversampling of persons with higher thrombophilic risk factors may have occurred. Consequently, we cannot use this sample to estimate the magnitude of the thrombophilic risk of multiple factors with certainty, and the results of our study are not sufficient to recommend a change in current clinical practice. Our results can, however, generate important hypotheses to be tested in future studies, and act as proof of principle justifying more research on this topic.

Twenty individuals were negative and 67 were positive for aCL upon entry into the study. We found a high prevalence of concomitant immunologic and coagulation abnormalities in this group of individuals. Sixteen (16) of the 67 subjects (24%) who initially tested positive for aCL-IgG were found to have suffered thrombotic events previously. Multivariate regression analysis was performed to study the association between previous thrombosis and the number of prothrombotic risk factors. When controlling for age and gender, the odds of a previous thrombotic event were increased by 46% (OR = 1.46, 95% CI: 1.003–2.134, p = 0.048) with each additional prothrombotic risk factor.

The risk of thrombosis in persons with aPL increases with increasing titers of antibodies[32-35], particularly those of the IgG isotype[36,37]. The coexistence of other thrombogenic conditions in the presence of aPL may be important in the development of thrombotic events. Reports have suggested an increased frequency of acquired protein S deficiency in patients with aPL[38,39]. The significance of this observation remains unknown. Factor V^{506} Leiden mutation is the most common inherited condition in Caucasians leading to a hypercoagulable state[6,8]. There is conflicting data concerning the role of factor V^{506} Leiden in patients with aPL. In one study of 30 women with APS, 10 of whom had a history of thrombosis, none of the women were found to be heterozygous or homozygous for factor V^{506} Leiden mutation[40]. Chopra reported an increase in the prevalence of Factor V^{506} Leiden in a population of persons with autoimmune aPL and thrombosis, but there was no significant association of this mutation with thrombosis, after adjustment for other clinical risk factors[41]. In another study of 60 patients with aPL, 26 of whom had venous thrombosis, 4 patients were heterozygous and one was homozygous for the factor V^{506} Leiden mutation[42]. Furthermore, there are reports of an increased frequency of an acquired APCR phenotype in patients with aPL without a factor V^{506} Leiden abnormality[43,44]. In contrast, there has been a lack of association between thrombosis in APS and the 20210A prothrombin gene mutation[45,46]. Finally, the mutation Ala677 to Val in the MTHFR gene, which is associated with elevated homocysteinemia, has been shown to be a risk factor for thrombosis[26].

Our study has some limitations. The number of study participants (n = 87) was small, as was the number of individuals with confirmed thrombotic events (n = 20). Also, fluctuations in aCL-IgG titers were apparent, as has been previously described[47,48]. Of the 67 patients with elevated aCL-IgG titers at study entry, only 40 (60%) had elevated titers on repeat testing. Given the tendency for aCL-IgG titers to fluctuate, isolated results are not conclusive and associations are more difficult to detect. A more recent definition of APS proposes to incorporate a certain level of positivity over a period of time[1]. Finally, we found a high prevalence of immunologic and coagulation abnormalities in participants without aCL-IgG. We also found that 4 of these individuals (20%) had experienced thrombotic events. This could be explained by the fact that we chose these participants from a group of individuals who had undergone aCL testing on at least one occasion. Hence, there may have been a selection bias in favour of patients more likely to have a coagulation abnormality. Therefore, our results may not be applicable to the general population, but rather to individuals with a thrombophilic predisposition. In addition, although our study participants were randomly chosen from a cohort of individuals who had undergone aCL testing, women were over-represented in both the aCL positive (87%) and the aCL negative (80%) groups. This may reflect a selection bias for testing women for aCL, due to the higher risk of thrombosis generally reported in women[49], or it may reflect a true preponderance of aPL in women.

The increased risk of thrombosis associated with increasing numbers of potential prothrombotic risk factors seems modest (OR = 1.46, 95% CI: 1.003-2.134, p = 0.048). Nevertheless, it is consistent with our hypothesis that the concomitance of aCL-IgG and other immunologic and thrombophilic abnormalities may increase the risk of thrombosis. Larger, prospective studies are needed to confirm this observation and to assess the relative importance of each prothrombotic risk factor.

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