# Thromboembolic risk in patients with high titre anticardiolipin and multiple antiphospholipid antibodies

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**Summary**

Asymptomatic antiphospholipid antibody (aPL) carriers with high risk for thrombosis may benefit from preventive anticoagulation.

It was our objective to test whether the risk of thrombosis increases with: 1) increasing titres of anticardiolipin antibodies (aCL) after adjustment for other cardiovascular risk factors and 2) the number of aPL detected.

In a cross-sectional study, blood was collected from clinics in two teaching hospitals.

The study included 208 individuals suspected of having an aPL and 208 age- and sex-matched controls having blood drawn for a complete blood count.

Clinical variables included history of previous arterial (ATE) or venous (VTE) thrombotic events, traditional risk factors for cardiovascular disease, and systemic lupus erythematosus (SLE). Laboratory variables included IgG/IgM aCL, lupus anticoagulant, and IgG/IgM anti-β2-glycoprotein I.

Mean age was 46.5 years and 83% were female. Seventy-five of the 416 participants had ≥ 1 aPL, and 69 had confirmed ≥ 1 ATE or VTE. Family history was positive in 48% of participants, smoking in 28%, hypertension in 16%, diabetes in 6%, and SLE in 20%. A 10-unit increase in aCL IgG titre was associated with an odds ratio (OR) [95% CI] of 1.07 [1.01-1.13] for ATE and 1.06 [1.02 - 1.11] for VTE. The odds of a previous thrombosis increased with each additional aPL detected: 1.5 [0.93-2.3] for ATE and 1.7 [1.1-2.5] for VTE.

These results indicate that increased titres of aCL and multiple aPL were associated with an increased risk of a previous thrombotic event.

**Keywords**

Antiphospholipid syndrome, antiphospholipid antibodies, anticardiolipin antibody, thrombosis

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## Introduction

New criteria for the diagnosis of antiphospholipid antibody syndrome (APS) have recently been proposed (1) and validated (2). These criteria require the presence of one clinical event (either thrombotic or obstetrical), accompanied by the persistence of an anticardiolipin antibody (aCL) in moderate to high titres or of a lupus anticoagulant antibody (LAC). The presence of antiphospholipid antibodies (aPL) has been associated with thrombotic events. More specifically, anticardiolipin antibodies (aCL),

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lupus anticoagulant antibodies (LAC), and anti-β2-glycoprotein I (aβ2GPI) have been implicated in arterial and venous thrombosis (3). Although aPL are associated with an increased risk of thrombosis, it remains unclear whether they are actively involved in the genesis of the blood clot itself or are indirect markers for another thrombophilic process. Furthermore, thrombosis develops in some, but not all, aPL positive individuals, suggesting the involvement of other thrombophilic factors in the development of aPL-related thrombotic events. Thus, at present, routine screening tests for aPL to identify those at higher risk for thrombosis are not recommended in the general population.

Since the treatment of thrombosis in APS implies lifelong oral anticoagulation, with a 1% to 5% risk of a major bleed (4, 5), asymptomatic aPL carriers are not usually treated preventively unless their risk for thrombosis is deemed higher than their risk of major bleed. Rheumatologists and hematologists are confronted daily with the difficult decision of how to treat asymptomatic aPL carriers. Awaiting the development of a thrombosis before treating is sub-optimal, since the first event may be fatal or cause significant morbidity. Therefore, a means of separating asymptomatic aPL carriers into high versus low risk groups for thrombosis would greatly benefit this patient population, allowing the clinician to intervene before a devastating thrombotic event (TE) occurs.

It remains unclear how to best characterize the risk for thrombosis associated with aPL. The presence of aCL, LAC or aβ2GPI may each carry a different risk and so, the presence of each antibody can be considered as an independent exposure. Other types of exposures include the titres of the quantifiable aPL, the number of aPL detected and persistence of aPL presence over time. In this paper, we focus on aPL positivity, aCL titres, and the number and combinations of aPL as independent measures of exposure to aPL. We look at the association of these exposures with thrombosis in a cross-sectional analysis of an ongoing prospective cohort. This cohort will be followed prospectively for the development of incident thrombotic events.

# Study population and methods

## Population

We selected a group of individuals with a high index of suspicion for the presence of an aPL and a second group with average suspicion for aPL. Specifically, two groups were identified: 1) individuals whose treating physician had requested testing for either aCL or LAC (aPL-request) and 2) age-, gender-, and site-matched individuals whose treating physicians had requested a routine complete blood count (CBC), but no aPL test (CBC-request). Participants were recruited from The McGill University Health Centre (MUHC) and Hôpital Maisonneuve-Rosemont (HMR), both university hospital test centres. All English- or French-speaking persons over the age of 18 years, who were identified in either of the two groups, were approached and asked to participate in the study. Participants completed a baseline evaluation questionnaire, provided a blood sample, and had their blood pressure measured. They also agreed to be contacted by phone semi-annually and to return to the clinic annually for blood samples and questionnaire completion. The study was reviewed and approved by Research Ethics Committees of MUHC and HMR.

## Clinical data at baseline

The baseline questionnaire included the following items: demographic parameters (age, gender, race, education, income); medication usage; a list of comorbidities; history of venous, arterial and obstetrical events; family history of cerebrovascular accident (CVA), transient ischemic attack (TIA), myocardial infarction (MI) or angina in first-degree relatives (FMH); smoking; diabetes mellitus (DM); systemic lupus erythematosus (SLE); and hypertension (HBP). The primary outcome was defined as any previously documented arterial or venous thrombotic event. Arterial thrombotic events (ATE) were classified as CVA, TIA, MI, angina, or other sites of arterial thrombosis. Venous events (VTE) were classified as deep vein thrombosis (DVT), pulmonary embolism (PE), or other sites of venous thrombosis. Combined ATE and VTE will be referred to hereafter as TE. At baseline, all events reported to have occurred prior to entry into the study were reviewed by a physician blinded to aPL status. A TE was confirmed if there was 1) a positive diagnostic test, or 2) a clinical diagnosis by the treating physician. Only confirmed events were used in the analyses.

## Laboratory tests at baseline

Participants were tested for IgG and IgM aCL, LAC, and IgG and IgM aβ2GPI. Plasma and serum were aliquotted and stored frozen at –70 degrees C. The laboratory technician performing the tests was blinded to the identity and the study group of the test samples. aCL was tested in the clinical laboratories of MUHC and HMR, using the Louisville assay (Louisville APL Diagnostics, Inc., Louisville, Kentucky, USA). LAC and aβ2GPI assays were performed in the Rheumatology Research Laboratory at the MUHC. LAC was detected using a dilute APTT assay (Automated APTT, Organon Teknika, Scarborough, Ontario, Canada) in which the plasma tested was diluted 1:1 with normal plasma. Confirmation of LAC activity was performed by neutralization with hexagonal phase phosphatidylethanolamine (6). The aβ2GPI was measured by ELISA as described previously (7), and positive results were confirmed by repeat testing. aPL positivity was defined as aCL (IgG or IgM) > 15, LAC ≥ 0.6 seconds, and aβ2GPI (IgG or IgM) ≥ 0.7.

## Data analysis

All data were double-entered into Medlog (Medlog Systems, Incline Village, NV, USA) and discordant entries were verified.

Analyses were performed using SAS (8). Means, for continuous variables, and proportions, for dichotomous variables, were calculated for each study group and the differences in means and proportions obtained, as well as the 95% confidence intervals (CIs) for these differences. Point estimates and 95% CIs were calculated for the difference between the aPL- and CBC-request groups in the proportions with previous confirmed thrombotic events and reported obstetric events. Within each request group, point estimates and 95% CIs were calculated for the difference in proportions of thrombotic and obstetric events in those who actually tested positive or negative for aPLs. Finally, the difference in proportions of these events in those testing positive or negative for aPLs, and the corresponding 95% CIs, were obtained for the entire group.

For the logistic regression models described below, we performed the analysis for each group (aPL-request and CBC-request) as well as for the overall population. In each model, the variable of interest was presence of aPL. This was defined in the following ways: aPL isotype titre, as a continuous or dichotomous variable; number of different types of aPL present, regardless of isotype; and combinations of aPL present. We adjusted for study group and for traditional cardiovascular disease (CVD) risk factors if they either had a confounding effect on the main variable or if they showed an important association with the outcome variable. The models with TE as outcome included as covariates study group, family history of CVD, gender, and hypertension, while the models with ATE as outcome included these variables as well as age. However, the models with VTE as outcome were adjusted only for study group, as none of the other variables acted as confounders. For the analyses performed within groups, study group was not included as a covariate.

The number of antibodies was defined as the total number of different aPL antibodies found in the blood sample. If two isotypes of an aPL were present, this was counted as only one aPL. For example, a value of 2 for this variable would mean that a patient had either aCL (IgG and/or IgM) with LAC, aCL (IgG and/or IgM) with aβ2GPI (IgG and/or IgM), or LAC with aβ2GPI (IgG and/or IgM).

In order to identify antibody profiles that were associated with a higher risk of having confirmed thrombosis prior to baseline, we performed logistic regression using ATE, VTE, and TE as the dependent variables in separate models, and specific combinations of aPL, represented by indicator variables, as covariates. The antibody combinations included were: aCL (IgG and/or IgM) only, LAC only, aCL plus LAC, aCL plus aβ2GPI, and all three antibodies. The reference category was the absence of all three antibodies. It was not possible to accurately estimate the regression parameters for the aβ2GPI-only or the LAC/aβ2GPI subgroups in any of these models, as none of the 6 subjects with aβ2GPI alone experienced any thrombotic events and there were no subjects in the LAC/aβ2GPI subgroup. Similarly, it was not possible to accurately estimate these parameters for the aCL/aβ2GPI subgroup in the models with VTE as the outcome. Subjects in these subgroups were omitted from this portion of the analyses to avoid biasing the regression parameter estimates for the other antibody combinations.

# Results

Of a total of 576 persons approached, 92 declined and 14 were excluded due to their inability to speak either English or French. Four hundred and seventy (470) persons provided informed consent. Of these, 208 aCL/LAC-tested individuals were age-, gender- and referral center-matched to 208 CBC-tested individuals. Forty-eight CBC-tested and six aCL/LAC-tested individuals could not be matched and were not studied further.

## Description of confirmed events at baseline

One hundred twenty-eight TE were confirmed in 69 participants: 13 with CVA, 5 with TIA, 15 with MI, 9 with angina, 32 with DVT, 14 with PE, 2 with amaurosis fugax and 6 with other thrombotic events. The number of events per person were as follows: CVA (nine with one, three with two, one with four); TIA (one each); DVT (22 with one, five with two, three with three, and two with four); PE (twelve with one, two with two); amaurosis fugax (one each); and other thrombotic events (two with a lacunar infarct, two with renal thrombosis, one with three femoral arterial occlusions, and one with Budd-Chiari and a ureteral artery infarct).

## Characteristics of the cohort

Characteristics of the cohort of 416 participants are shown in Table 1. The mean age was 46.5 years and 83% were female. No clinically important differences in education or ethnic group were seen between the aPL-request and the CBC-request groups. Smoking was reported in 28% of the cohort, HBP in 16%, DM in 6%, SLE in 20%, and FMH in 48%. As expected, prednisone, hydroxychloroquine, aspirin, and warfarin use were higher in the aPL-request group. There was also a clinically relevant difference in the number of people in each group with confirmed thrombotic events, multiple thrombotic events, and reported obstetrical events, with the percent risk being higher for the aPL-request group and the 95% CI for the percent risk difference excluding zero.

Table 2 shows the number and percentage of individuals who tested positive for aPL. As expected, the percentage of individuals who tested positive for aPL was higher in the aPL-request group for all aPL except aβ2GPI IgM. The frequency of aCL IgG and LA were highest among all of the aPL and similar within each group (~15-16% in the aPL-request group and ~8-10% in the CBC-request group). The percentage of individuals with aβ2GPI IgG was low (4.3% in the aPL-request group and 2.7% in the CBC-request group). Seventy-five (18%) of all

| | aPL request | CBC request | Total |
|---|---|---|---|
| | N= 208 | N=208 | |
| Age in years, mean (SD) | 46.3 (14.2) | 46.6 (14.2) | 46.5 (14.2) |
| Education in years, mean (SD)* | 12.7 (2.5) | 13.0 (2.8) | 12.9 (2.7) |
| Gender, % female | 173 (83.2) | 173 (83.2) | 346 (83.2) |
| Race, % Caucasian | 178 (85.6) | 172 (82.7) | 350 (84.1) |
| Smoking (%) | 59 (28.4) | 56 (26.9) | 115 (27.6) |
| HBP (%)† | 34 (16.4) | 33 (15.9) | 67 (16.1) |
| DM (%)† | 9 (4.3) | 14 (6.7) | 23 (5.5) |
| SLE (%)† | 82 (39.4) | 3 (1.4) | 85 (20.4) |
| FMH (%)† | 109 (52.4) | 92 (44.2) | 201 (48.3) |
| Prednisone (%) | 35 (16.8) | 1 (0.5) | 36 (8.7) |
| Hydroxychloroquine (%) | 52 (25.0) | 2 (1.0) | 54 (13.0 |
| Acetylsalicylic acid (%) | 46 (28.9) | 15 (13.5) | 61 (21.2) |
| Warfarin (%) | 28 (13.5) | 4 (1.9) | 32 (7.7) |
| Estrogen (%)* | 25 (14.5) | 37 (21.4) | 62 (17.9) |
| Confirmed thrombotic events (%)§ | 57 (27.4) | 12 (5.8) | 69 (16.6) |
| Thrombotic events >1 (%)§ | 25 (12.0) | 2 (1.0) | 27 (6.5) |
| Reported miscarriages (%)* | 0 (0.0) | 1 (0.5) | 1 (0.2) |
| Reported fetal loss (%)*§ | 30 (17.3) | 12 (6.9) | 42 (12.1) |
| Reported premature births (%)* | 13 (7.5) | 9 (5.2) | 22 (6.4) |

* For education, N = 201 and 206 for the aPL request and CBC request groups, respectively; for estrogen and obstetrical events, N = 173 each for the aPL request and CBC request females, respectively.

† HBP = high blood pressure; DM = diabetes mellitus; SLE = systemic lupus erythematosus; FMH = family history for CVD.

§ Risk difference (95% CI) = 21.6% (14.8% to 28.5%) for confirmed thrombotic events; 11.1% (6.4% to 15.7 %) for thrombotic events; and 10.4% (3.6% to 17.2%) for reported fetal loss.

**Table 1:** Demographic characteristics, cardiovascular risk factors, medications, and previous thrombotic and obstetrical events at baseline.

| aPL measured | aPL request | CBC request | Total |
|---|---|---|---|
| | N= 208 | N= 207* | N= 415 |
| aCL (IgG or IgM)† | 43 (20.7)§ | 6 (2.9) | 49 (11.8) |
| aCL IgG† | 32 (15.4) | 2 (1.0) | 34 (8.2) |
| aCL IgM† | 21 (10.1) | 5 (2.4) | 26 (6.3) |
| LAC† | 33 (15.9) | 7 (3.4) | 40 (9.6) |
| aß2GPI (IgG or IgM)† | 12 (5.8) | 5 (2.4) | 17 (4.1) |
| aß2GPI (IgG)† | 9 (4.3) | 2 (0.9) | 11 (2.7) |
| aß2GPI (IgM)† | 3 (1.4) | 4 (1.9) | 7 (1.7) |

*aß2GPI status was not available for one person in the CBC-request group.

†aPL positivity were defined as follows: aCL (IgG or IgM) > 15, LAC ≥ 0.6 seconds, and aß2GPI (IgG or IgM) ≥ 0.7.

§Number (%) of individuals positive in the group

**Table 2:** Number and percentage of individuals testing positive for aPL.

participants had at least one aPL. Of these, 26 had aCL only, 20 had LA only, 6 had aβ2GPI only, 3 had both aCL and aβ2GPI, 12 had both LA and aCL, and 8 were positive for all three aPL (data not shown). None of the subjects were positive for LA and aβ2GPI, and aβ2GPI status was unknown for one subject who was otherwise aPL-negative. When aPL-positive and aPL-negative study participants were compared, regardless of original request group, a higher proportion of aPL-positive participants were found to have HBP, SLE, and FMH (results not shown).

Table 3 shows the proportions of confirmed TE and reported obstetrical events by study group and, within each study group, by aPL antibody status. The percent risk difference [95% CI] for TE in the aPL-request group was 23.3 [9.0 to 37.5], well into the range of clinically relevant difference. In contrast, the percent risk difference was shifted towards a less clinically relevant range for the CBC-request group (7.3 [-9.3 to 23.8]). For the pooled population, the percent risk difference was 25.3 [13.8 to 36.7], and the entire 95% CI was in the clinically relevant range. Similarly, more obstetric events were reported in aPL-positive subjects in the aPL-request group (percent risk difference [95% CI] = 15.8 [0.1 to 30.9]), with the difference reaching clinical relevance. A smaller difference was observed in the CBC-request group (percent risk difference [95% CI] = 2.9 [-16.1 to 21.9]).

## Association of aCL with thrombosis

Table 4 shows the adjusted odds ratios (OR) and 95% CI for the associations between aCL IgG or IgM and ATE, VTE, or TE, for all 416 subjects. When the associations were modeled with the aCL IgG titre as a dichotomous variable, the ORs and their 95% CIs were in a clinically relevant range for VTE and TE. Similarly, the associations between aCL IgG titre, as a continuous variable, and ATE, VTE, or TE were all clinically relevant. For aCL IgM, the ORs were closer to 1 than those for aCL IgG,

**Table 3:** Percentage risk differences in individuals with thrombotic and obstetric events.

| | aPL request (N = 208) | | | CBC request (N = 207)* | | | Pooled Population (N = 415) |
|---|---|---|---|---|---|---|---|
| aPL status | Positive (N = 59) | Negative (N = 149) | Risk difference (95% CI)† | Positive (N = 16) | Negative (N = 191) | Risk difference (95% CI)† | Risk difference (95% CI) † |
| Thrombotic events (%)‡ | 44.1 | 20.8 | 23.3 (9.0 to 37.5) | 12.5 | 5.2 | 7.3 (-9.3 to 23.8) | 25.3 (13.8 to 36.7) |
| Obstetric events (%)‡§ | 34.0 | 18.3 | 15.8 (0.1 to 30.9) | 14.3 | 11.4 | 2.9 (-16.1 to 21.9) | 15.1 (2.9 to 27.2) |

*aß2GPI status was not available for one person in the CBC-request group.

†Risk difference = risk in aPL-positive subjects – risk in aPL-negative subjects

‡Thrombotic events were confirmed events and obstetric events were reported events.

§ For obstetric events, risk differences were calculated for females only: N (aPL-positive) = 47 and N (aPL-negative) = 126 in the aPL request group; N (aPL-positive) = 14 and N (aPL-negative) = 158 in the CBC request group.

**Table 4:** Association of aCL IgG or IgM and thrombosis in the pooled population (N = 416).

| | OR (95% CI) | | |
|---|---|---|---|
| | ATE*† | VTE*† | TE*† |
| aCL IgG titre > 15 ‡ | 2.38 (0.90 to 6.33) | 5.72 (2.54 to 12.88) | 5.20 (2.29 to 11.78) |
| aCL IgG titre (per 10-unit difference) | 1.07 (1.01 to 1.13) | 1.06 (1.02 to 1.11) | 1.08 (1.03 to 1.13) |
| aCL IgM titre > 15 ‡ | 1.95 (0.62 to 6.10) | 1.87 (0.68 to 5.13) | 2.25 (0.88 to 5.73) |
| aCL IgM titre (per 10-unit difference) | 1.05 (0.92 to 1.21) | 1.05 (0.94 to 1.18) | 1.60 (0.95 to 1.18) |
| aCL IgG or IgM positive§ | 1.81 (0.74 to 4.43) | 3.18 (1.50 to 6.77) | 2.98 (1.45 to 6.10) |

*ATE = arterial thrombotic events; VTE = venous thrombotic events; TE = arterial or venous thrombotic events.

†ATE models are adjusted for study group, family history, sex, hypertension, and age; VTE models are adjusted for study group; and TE models are adjusted for study group, family history, sex, and hypertension.

‡For aCL IgG or IgM titre (dichotomous), the reference category is ≤15.

§ For aCL IgG or IgM (dichotomous), the reference category is aCL negative individuals.

when aCL IgM presence was modelled as a dichotomous variable, and were similar to those for aCL IgG, when aCL IgM presence was modelled as a continuous variable. However, the confidence intervals were wider and included values that were outside the range of clinical relevance when either the dichotomous or continuous variable was used. Presence of aCL, regardless of isotype, was associated with both VTE and TE. Similar results were found when the analysis was restricted to those in the aPL-request group (results not shown).

### Association of the number of different aPL present, and aPL profiles, with thrombosis

Table 5 shows the estimated adjusted ORs and 95% CI for the associations of ATE, VTE, or TE with the presence of each additional aPL, and with specific aPL combinations, for the pooled population. One subject in the CBC-request group was excluded from this analysis because his or her aβ2GPI status was not available. The ORs for a 1-antibody increase were 1.5, 1.7, and 1.7 for the models with ATE, VTE, and TE as the outcome, respectively. As the 95% CI excluded 1.0 in the models with either VTE or TE as the outcome, these values can be considered to be within the clinically relevant range. In these regression models, the type of aPL antibody involved was not taken into account.

In the regression models with aPL combinations as the main variables, ORs and 95% CIs could not be accurately estimated for the subgroup with aβ2GPI alone (n = 6), due to the lack of thromboses. For the same reason, ORs and 95% CIs could not be estimated for the subgroup with aCL and aβ2GPI in models with VTE as the outcome (n = 3), and the OR estimates for this subgroup lacked precision in the other models. aCL with LAC was the combination with the highest OR for TE. Comparatively equivalent OR were found for aCL only, LAC only, aCL and aβ2GPI, and all three aPL. Similar results were found when the analysis was restricted to the aPL-request group (data not shown).

## Discussion

We have described an association between thrombosis and the presence of aCL (IgG or IgM), after adjusting for traditional cardiovascular risk factors. The association of aCL with thrombosis increased with increasing titres of aCL (IgG or IgM). We have also found that the odds of having a confirmed thrombosis increased with each additional aPL detected and that LAC, in combination with aCL, was most strongly associated with previous TE (in particular, VTE).

Our cohort presents some limitations. The presence of aPL is a relatively rare condition, with a prevalence of 1.2-5.6% for aCL, 1.0-4.2% for aCL IgG, 1.0-5.0% for aCL IgM and 3.6% for LAC among healthy controls (9-14) and, furthermore, not all persons with aPL develop thrombosis. Therefore, an extremely large sample would be required to observe, prospectively, the development of TE in asymptomatic aPL carriers. A longitudinal study of such a population would be costly, impractical and inefficient. For this reason, we identified and followed prospectively two groups of individuals based on the level of suspicion (average or high) for the presence of aPL. This might have

**Table 5:** Association of the number of different aPL present, and aPL profiles, with thrombosis for the pooled population (N = 415).

| | OR (95% CI) | | |
|---|---|---|---|
| | ATE*† | VTE*† | TE*† |
| Number of aPL antibodies present (per 1-antibody difference) | 1.46 (0.93 to 2.27) | 1.69 (1.14 to 2.50) | 1.70 (1.16 to 2.51) |
| Profile‡ | | | |
| aCL only | 1.89 (0.55 to 6.46) | 3.02 (1.11 to 8.23) | 3.13 (1.24 to 7.90) |
| LAC only | 2.43 (0.68 to 8.67) | 1.63 (0.43 to 6.20) | 1.99 (0.67 to 5.92) |
| aCL + LAC | 1.05 (0.21 to 5.35) | 6.32 (1.86 to 21.45) | 3.33 (0.90 to 12.25) |
| aCL + aß2GPI | 8.20 (0.66 to 102.60) | *NI* | 2.61 (0.22 to 30.49) |
| aCL + LAC + aß2GPI | 3.20 (0.60 to 17.18) | 2.47 (0.45 to 13.40) | 3.11 (0.65 to 15.04) |

* ATE = arterial thrombotic events; VTE = venous thrombotic events; TE = arterial or venous thrombotic events. N = 409, 406, and 409 for the models with ATE, VTE, and TE as the outcomes, respectively.

† ATE models are adjusted for study group, family history, sex, hypertension, and age; VTE models are adjusted for study group; and TE models are adjusted for study group, family history, sex, and hypertension.

‡ The reference category was the absence of all three antibodies. NI = not included in the model. The categories "aß2GPI only" and "LAC + aß2GPI" were not included in any of the regression models, as none of the subjects with only aß2GPI had any thrombotic events, and no one had the LAC + aß2GPI antibody combination. The category "aCL + aß2GPI" was not included in the model with VTE as the outcome due to a lack of thrombotic events.

created a channeling bias that could cause confounding between group status and risk for thrombosis, resulting in overestimation of the association between aPL presence and thrombotic events. Therefore, we analyzed our data on the basis of the true aPL status within each group and for the pooled population, and adjusted for study group when analyzing the pooled population. In addition, the precision of our estimates, especially in our analysis of aPL combinations, was limited by the low number of subjects in some of the aPL categories. In particular, the small number of patients with aβ2GPI affected the OR estimates and 95% CIs for aPL profiles that included this antibody. Thus, although our study design allows for the generation of interesting hypotheses and the description of potentially important associations, it does not allow us to comment on causality between aPL status and thrombosis. Furthermore, it is impossible to determine if there is a cause-and-effect or a temporal relationship between the presence of aPL and thrombosis.

Our study demonstrates that aCL IgG in higher titres was associated with an increased risk of both ATE and VTE, even after adjustment for cardiovascular risk factors. This concurs with findings of previous studies that adjusted for cardiovascular risks (15-20).

We also show that the risk of thrombosis increases with the number of aPL present, such that the risk for TE nearly doubles with each additional antibody. Other studies have commented on the role of multiple thrombophilic markers in increasing the risk of thrombosis, but were limited either by the number of patients (21) or analyses restricted to genetic thrombophilic risk factors (22-24). We, then, explored which aPL or combination of aPL was associated with higher odds of thrombosis and found that LAC, in combination with aCL, was associated with the highest odds for TE and VTE, but not ATE. This concurs with previous studies (25, 26). Similarly, higher odds of ATE or VTE were associated with LAC alone (27, 28) or aCL alone (19, 29). A very high odds for ATE was associated with the combination of aCL and aβ2GPI, but the confidence intervals were very wide, making it unclear whether this finding is clinically relevant. Some studies (30-35) have found an association with aβ2GPI alone and thrombosis, while others (36-39) only showed an association for VTE when aβ2GPI was found with concomitant aCL. The range of aβ2GPI titres reported in these studies may explain this discrepancy. Cuadrado (40) found aβ2GPI to be associated with recurrent thrombosis only in the highest titres.

In conclusion, we have established a cohort of individuals selected for high or average suspicion of having an aPL, in order to prospectively monitor thrombotic events. Here, we report on aPL status and previous thrombotic events at entry into the cohort. Our findings demonstrate that, after adjustment for cardiovascular risk factors, the presence of aCL is associated with thrombosis. Furthermore, higher titres of aCL increase the odds for thrombosis. Importantly, we have shown that the risk for thrombosis increases with each additional aPL. Finally, we demonstrate that different combinations of aPL may be associated with varying odds of thrombosis. Classification of patients with aPL at high or low risk for thrombosis is an essential step towards achieving the goal of preventing the occurrence of thrombosis in asymptomatic aPL carriers.

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