Antiphospholipid antibodies and thrombosis: association with acquired activated protein C resistance in venous thrombosis and with hyperhomocysteinemia in arterial thrombosis

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Summary

Although antiphospholipid antibodies (aPL) are associated with thrombosis, it is not known who with aPL is at higher risk for thrombosis. It was the aim of this cross-sectional study to investigate how thrombophilic factors contribute to venous or arterial thrombosis in aPL-positive individuals. In outpatient test centres at two tertiary care hospitals, two hundred and eight (208) persons requiring aPL testing were matched by age, gender and centre to 208 persons requiring a complete blood count. Persons were classified as aPL-positive (having anticardiolipin, lupus anticoagulant and/or anti- β_2 -glycoprotein I antibodies) or aPL-negative. Several thrombophilic factors were studied using logistic regression modelling. Results showed that the aPL-positive group had three-fold more events (37%) than the aPL-negative group (12%). In unadjusted analyses, clinically important associations were observed between factor V Leiden and venous thrombosis, hyperhomocysteinemia and arterial thrombosis, and activated protein C resistance (APCR) and venous thrombosis (OR, 95% CI = 4.00, 1.35–11.91; 4.79, 2.03–11.33; and 2.03, 1.03–3.97, respectively). After adjusting for recruitment group, persons with both APCR and aPL had a three-fold greater risk (OR, 95% CI = 3.31, 1.30–8.41) for venous thrombosis than those with neither APCR nor aPL. Similarly, after adjusting for hypertension, family history of cardiovascular disease, gender and recruitment group, persons with both hyperhomocysteinemia and aPL had a

five-fold increased risk (OR, 95% CI = 4.90, 1.37–17.37) for arterial thrombosis compared to those with neither risk factor. In conclusion, APCR phenotype and hyperhomocysteinemia are associated with a higher risk of venous and arterial thrombosis, respectively, in the presence of aPL.

Keywords

Antiphospholipid antibodies; antiphospholipid syndrome; thrombosis; activated protein C resistance; hyperhomocysteinemia

Introduction

The presence of antiphospholipid antibodies (aPL), in particular either a lupus anticoagulant antibody (LA) or an anticardiolipin antibody (aCL), has been associated with an increased risk for venous or arterial thrombosis (1). The criteria for the diagnosis of antiphospholipid syndrome (APS) require the persistence of either aCL, in moderate to high titres, or LA, as well as the presence of either a thrombotic or an obstetrical event (2, 3). The presence of an aPL, however, is not considered sufficient on its own in predicting the future development of thrombosis, and it remains unclear whether aPLs are actively involved in the prothrombotic process itself or are indirect markers for another thrombophilic process (1).

Since treatment of thrombosis in APS implies life-long oral anticoagulation with warfarin and a 1% to 5% risk for major bleed (4, 5), one does not want to submit persons with aPL and no thrombosis to anticoagulation prevention unless the thrombotic risk outweighs the risk of a major bleed. On the other hand, waiting for the development of a thrombosis before treating is sub-optimal, since the event may be fatal or cause significant morbidity. Therefore, a means of separating aPL carriers into high versus low risk groups for thrombosis would be of great benefit, allowing the clinician to intervene when indicated with a prophylactic measure.

There is growing interest in evaluating the contribution of concomitant thrombophilic risk factors to the presence of aPL for venous or arterial thrombotic events. For instance, initial reports looking at inherited thrombophilic conditions have demonstrated that persons with more than one inherited risk factor are at higher risk for thrombosis than those with only one (6, 7). These conditions include: deficiencies in physiologic coagulation inhibitors such as protein C, protein S, or antithrombin; and genetic mutations, such as factor V Leiden (leading to an activated protein C resistance [APCR] phenotype), the enzyme methyltetrahydrofolate reductase (MTHFR) gene C677T mutation genotype (implicated in the metabolism of homocysteine) and the prothrombin gene G20210A mutation.

It is possible that aPL combined with the above inherited or acquired thrombophilic factors may further increase the risk for either venous or arterial thrombosis. We are currently conducting a prospective cohort study of 416 individuals recruited based on a clinician's request for aPL testing. The aPL profiles of individuals at entry into this cohort have been described elsewhere (8). Here, we report on the prevalence of other thrombophilic risk factors in these individuals at baseline, as well as the associations of these risk factors with

both aPL and presence of previous thrombotic events. Our objective was to determine whether the presence of acquired or inherited thrombophilic risk factors contributes to the strength of the association between aPL and venous or arterial thrombosis.

Methods

Study population

We recruited study participants from consecutive persons attending the outpatient blood test centres at the McGill University Health Centre (MUHC, Montreal General Hospital Campus) and Hôpital Maisonneuve-Rosemont (HMR). We selected a group of individuals requiring aPL testing (aCL and/or LA) and thus at high suspicion for the presence of an aPL, and matched these by age, gender, and centre to a second group requiring a complete blood count (CBC) who were not suspected to have aPL. All persons identified in either of the two groups were approached and asked to participate in the study if they were between the ages of 18 and 80, able to write in either English or French, and not pregnant. 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 and/or LA-tested individuals were matched by age, gender, and referral centre to 208 CBC-tested individuals. One CBC-tested person was excluded because of missing information on their aPL status. Forty-eight CBC-tested and six aCL/LA-tested individuals could not be matched and were not studied further. The study was reviewed and approved by Research Ethics Committees of both institutions. At entry into the cohort, participants completed a questionnaire, provided a blood sample, and had their blood pressure measured. The participants were informed that DNA was being collected for evaluation of factor V Leiden, prothrombin G20210A and MTHFR mutations and would then be discarded. They also agreed to be contacted by phone and to return to the clinic at regular intervals for the collection of blood samples and completion of questionnaires.

Clinical data at baseline

The questionnaire completed at entry into the cohort included demographic parameters (age, gender, ethnicity, education, and income); medication usage; a list of other illnesses; history of venous, arterial and obstetrical events; family history of cardiovascular disease (FMH); smoking; diabetes mellitus (DM); systemic lupus erythematosus (SLE) and hypertension (HBP). The primary outcome was defined as any previously documented venous or arterial thrombotic event. Venous events (VE) were classified as thrombophlebitis, pulmonary embolism, or other sites of venous thrombosis. Arterial thrombotic events (AE) were classified as cerebral vascular accident, transient ischemic attack, myocardial infarction, angina or other sites of arterial thrombosis. Combined AE and VE will be referred to hereafter as thrombotic events (TE). All reported baseline events were reviewed by a physician blinded to aPL status. A TE was considered confirmed if there was either a positive diagnostic test or a clinical diagnosis by the treating physician. If neither of these criteria was present, the event was considered to be “not confirmed”. This is the only information that was validated by a physician’s review.

Obstetric and thrombotic events are listed at the bottom of Table 1. Obstetric events could not be validated and are shown for descriptive purposes only. We validated all reported TE in order to confirm or reject events that were or were not true thromboses. Eight of the reported thrombotic events in the aPL-positive group (n=75) could not be confirmed, while in the aPL-negative group (n=340), 35 TE were not confirmed. In total, 43 TE were not confirmed. A higher proportion of the reported TE in the aPL negative group (35/76=46%) could not be confirmed, compared to those in the aPL-positive group (8/36=22%) or the total cohort (43/112=38%).

Laboratory tests

The aPL status for this study was determined at entry into the cohort and is unknown prior to reported TE. Participants were tested for IgG and IgM aCL, IgG and IgM anti-beta-2-glycoprotein I (a β ₂GPI) antibodies, and LA. Plasma and serum were aliquoted and stored frozen at -70° C. The laboratory technician performing the tests was blinded to the identity of the test samples and the group to which the sample tested belonged. Presence of aCL was tested in the clinical laboratories of MUHC and HMR by a standard enzyme linked immunoassay (ELISA) using the Louisville assay (Louisville APL Diagnostics Inc., Louisville, Kentucky). The a β ₂GPI antibody and LA assays were performed in the Rheumatology Research Laboratory at the MUHC. LA was detected using a dilute APTT assay (Automated APTT, Organon Teknika, Scarborough, Ontario) in which the plasma tested was diluted 1:1 with normal plasma. Confirmation of LA activity was performed by neutralization with hexagonal phase phosphatidylethanolamine (9). Anti- β ₂GPI antibodies were measured by ELISA as described previously (9). APCR phenotype was determined using APTT reagent (Diagnostic Stago, Abbott Laboratories, Mississauga, Ontario) and APC/CaCl₂ (Diagnostic Stago, Abbott Laboratories, Mississauga, Ontario). Equal volumes of APTT reagent and patient plasma were incubated at 37° C for 5 minutes, to which an equal volume of either CaCl₂ or APC/CaCl₂ was added. The time required for clot formation was recorded with an ACL300 (Instrumentation Laboratory, Beckman Coulter, Mississauga, Ontario). Duplicate samples were assayed. APC resistance ratio was defined as the ratio of the clotting time in the presence of added APC/CaCl₂ to the clotting time in the absence of APC. Controls for the assay were obtained from pooled normal plasma from healthy volunteers. APCR ratio >2.0 were classified as a normal APC response. Homocysteine was measured in sera, using a quantitative assay on the IMx Instrument Axis patented enzymatic conversion method. The homocysteine in the sample sera was reduced and enzymatically converted to S-adenosyl-L-homocysteine (SAH), using an enzyme prepared by Axis Biochemicals. Subsequently, a competitive fluorescence polarization immunoassay (FPIA) utilizing an Abbott monoclonal antibody and a fluoresceinated tracer (Abbott Laboratories, Mississauga, Ontario) was performed. Functional antithrombin was measured by amidolytic assay based on Xa inhibition (Chromogenix, DiaPharma, Ohio) Functional protein C was measured by amidolytic assay (Berichrom Protein C assay, Behring Diagnostic, Montreal, Quebec). Functional protein S was measured by amidolytic assay (Protein S Liatest, Diagnostic Stago, Abbott Laboratories, Mississauga, Ontario).

The following genetic studies were done on banked DNA using standard procedures: factor V Leiden mutation (amplification of genomic DNA with the polymerase chain reaction and

digestion of fragments with *MnII*) (10); C677T MTHFR gene polymorphism (genomic DNA amplification using PCR and digestion of fragments with *hiwII*) (11); and prothrombin gene G20210A mutation (genomic DNA amplification with PCR and digestion of fragment with *HindIII*) (12).

Data management and analysis

All data were entered twice into Medlog (MedLog Systems, Incline Village, NY) and crosschecked. A verification program in Medlog was used to detect errors in data entry.

For the purpose of this analysis, we subdivided the cohort into two groups, aPL-positive and aPL-negative, regardless of their original recruitment group (aPL or CBC testing). We defined aPL-positive individuals as persons testing positive at entry for one of the following: aCL IgG or IgM $> 15 \times 103$ U/L, confirmed LA, and/or $\text{a}\beta_2\text{GPI} \geq 0.7$. The thrombophilic factors of interest included protein C, protein S and antithrombin deficiency; APCR phenotype; hyperhomocysteinemia; factor V Leiden, C677T MTHFR, and prothrombin gene G20210A mutations.

All statistical analyses were performed using SAS version 8.02 (13) and S-Plus version 6.0 (Seattle, Washington) (14). Only confirmed events were used in the analyses. Persons with non-confirmed thrombotic events were retained in the analysis but considered as non-affected. For all participants and the two aPL subgroups, we calculated descriptive statistics for age, gender, ethnicity, thrombotic events, cardiovascular risk factors, medication use, and thrombophilic factors. Crude odds ratios (OR) and 95% confidence intervals (CI) were calculated for the association between each thrombophilic factor and venous, arterial, or any thrombosis. To determine whether these associations varied with the presence of aPLs, we calculated ORs and 95% CIs separately in strata of aPL-positive and aPL-negative persons.

We used logistic regression models to estimate these associations in our cohort while adjusting for possible confounders. Our outcome variables were, in separate models, venous and arterial thrombosis, while the main variables of interest were aPL status and APCR (for venous thrombosis) or hyperhomocysteinemia (for arterial thrombosis). The covariates initially considered for each model included study recruitment group, SLE diagnosis, DM, hypertension, FMH, age, gender, and smoking status. Those covariates that had a possible confounding effect on the main variables were retained in the final models. Recruitment group was included in the analysis because it was likely that persons in the aPL-test group were suspected of having aPL because of previous thrombotic events. In addition, we investigated the possibility of interaction between aPL and the thrombophilic factor of interest for each outcome by including an interaction term in our initial models. The interaction term would allow us to determine whether the resulting odds ratio (OR) for concurrent presence of aPL and the thrombophilic factor differed from what would be expected in a model without interaction terms (i.e., determined simply by multiplying the OR for aPL presence with the OR for the thrombophilic factor). The OR for presence of both APCR and aPL (venous thrombosis model), or for both hyperhomocysteinemia and aPL (arterial thrombosis model), were calculated by multiplying the adjusted ORs for both variables (i.e., the relevant variables in each model), and the 95% CIs were obtained using the regression covariance matrices.

Results

Baseline characteristics

Baseline characteristics of the cohort are presented in Table 1. One person was excluded from the analysis because of missing aPL information. There were no differences in age, gender, ethnicity, smoking, hypertension, diabetes, and use of prednisone, hydroxychloroquine or estrogen between the aPL-positive and aPL-negative groups. The aPL-positive group had higher prevalence of family history of cardiovascular disease, SLE, and use of ASA and coumadin. The prevalence of deficiencies in physiologic coagulation-inhibitor factors, such as protein C, protein S, and antithrombin, was very low overall (3.6%). The prevalences of factor V Leiden, prothrombin gene G20210A mutation and MTHFR genotype were similar in both aPL groups (4.4%, 4.2%, and 56.5% in the entire cohort, respectively). The APCR phenotype was observed more frequently in the aPL-positive than in aPL-negative individuals (47.9% versus 24.9%). Similarly, an elevated serum homocysteine was found more frequently in aPL-positive individuals (12.3% versus 7.2%). There was a high rate of confirmed thrombotic events in both aPL groups, with more being observed in the aPL-positive individuals (37.3% versus 12.1% in aPL-negative individuals).

Thrombophilic risk factors, aPL, and thrombosis

Table 2 shows the number of confirmed VE, AE, and TE in each thrombophilic risk factor category (APCR phenotype, factor V Leiden, elevated serum homocysteine, C677T MTHFR genotype, and prothrombin gene G20210A mutation), as well as unadjusted and aPL-stratified ORs (with 95% CIs) for associations between thrombosis and these thrombophilic factors. Clinically important associations were observed between factor V Leiden and venous thrombosis, and between elevated serum homocysteinemia and arterial thrombosis in unadjusted analyses (OR, 95% CI = 4.00, 1.35–11.91 and 4.79, 2.03–11.33, respectively). A more modest association was also observed between APCR phenotype and venous thrombosis (OR, 95% CI = 2.03, 1.03–3.97). The confidence intervals in the stratified analyses were too wide for definite conclusions, with the exceptions of factor V Leiden, where the association with VE was greater and remained clinically relevant for the aPL-negative group (OR, 95% CI = 1.69, 0.14–19.84 for aPL-positive group compared to 6.06, 1.76–20.93 for aPL-negative group), and hyperhomocysteinemia, where the association with AE was greater and clinically relevant for the aPL-positive group (OR, 95% CI = 8.75, 1.93–39.57 for aPL-positive group compared to 3.04, 0.94–9.80 for the aPL-negative group). For prothrombin gene G20210A mutation, we found the expected association with VE in the aPL-negative group (OR, 95% CI = 4.32, 1.11–16.80). Because of the low prevalence of this mutation, it was not possible to obtain an OR for the association between its presence and arterial thrombosis.

Tables 3 and 4 show the final logistic regression models for venous and arterial thrombosis, respectively. Study recruitment group was included in the venous thrombosis model and hypertension, family history of cardiovascular disease, gender, and study recruitment group were included as covariates in the arterial thrombosis model. In analyses adjusting for study recruitment group, persons with both APCR and aPL had an increased risk (OR, 95% CI =

3.31, 1.30–8.41) for venous thrombosis compared to those with neither APCR nor aPL. Similarly, after adjusting for hypertension, family history of cardiovascular disease, gender and study recruitment group, those with both hyperhomocysteinemia and aPL had an increased risk (OR, 95% CI = 4.90, 1.37–17.37) for arterial thrombosis compared to those with neither factor.

The models used to obtain the results presented in Tables 3 and 4 assume that the association of aPL with previous thrombosis increases in a multiplicative fashion when the additional thrombophilic factor is present. We had initially included a variable in each model to account for possible interactions between aPL and the thrombophilic factor, but wide confidence intervals meant that no definitive conclusions could be derived from our analysis with interaction terms and those results have not been presented in any of the tables. A larger population would allow us to determine more precisely if such an interaction exists. Nevertheless, the salient findings from these analyses showed that including an interaction term for the presence of both aPL and APCR in the regression model with an outcome variable of VE resulted in ORs, 95% CIs of 1.52, 0.60–3.86 for APCR alone; 2.20, 0.80–6.04 for baseline aPL presence alone; and 3.31, 1.27–8.61 for presence of both. When hyperhomocysteinemia, aPL and an interaction term between them were present in the regression model for AE, the adjusted OR, 95% CI were 1.85, 0.50–6.93 for hyperhomocysteinemia alone; 1.29, 0.49–3.39 for baseline aPL presence alone; and 8.65, 1.81–41.7 for presence of both.

Discussion

Our cohort demonstrated a high prevalence of confirmed thrombotic events (17%). The APCR phenotype was the most common thrombophilic feature with a prevalence of 29%, followed by elevated serum homocysteine (8.2%), the heterozygous Factor V Leiden mutation (4.4%), and the heterozygous prothrombin gene G20210A mutation (4.2%). The prevalence of deficiencies in physiologic coagulation inhibitors (protein C, protein S, or antithrombin) was 4%, similar to that in the general population (15;16). Genotype heterozygosity and homozygosity for MTHFR were 43.2% and 13.3%, similar to what is reported for Canadian Caucasian subjects (11).

We found the APCR phenotype and factor V Leiden to be associated with venous thrombosis, with unadjusted ORs (95% CI) of 2.03 (1.03, 3.97) and 4.00 (1.35, 11.91), respectively. This finding concurs with reports in the literature (6). Serum homocysteine, but not MTHFR mutation, was found to be associated with arterial thrombosis, with unadjusted ORs (95% CI) of 4.8 (2.0, 11.3) and 1.3 (0.7, 2.7) respectively. The stratified OR for hyperhomocysteinemia was greater in the presence (8.8, 1.9–39.6) than in the absence (3.0, 0.9–9.8) of aPL, but this was not the case for APCR, which had a stratified OR of 1.5 (0.5–4.7) in the presence of aPL and 1.7 (0.7–4.1) in its absence. Although we found associations between APCR phenotype and venous thrombosis, and between hyperhomocysteinemia and arterial thrombosis, we could not draw conclusions from our results for factor V Leiden and MTHFR genotypes and thrombotic risk because of very large confidence intervals for the analyses of the genotypes. This suggests that functional APCR and/or elevated serum

homocysteinemia may be more clearly associated with thrombosis than their corresponding genetic abnormalities.

The development of thrombosis in individuals with an APCR phenotype is highly variable. It appears to be dependent on the coexistence of other genetic or acquired risk factors (17). There have been numerous reports linking interference between LA and protein C activation pathway in vitro (18, 19), lending some weight to the hypothesis that the presence of both LA and APCR phenotype would be associated with higher risk for venous thrombosis. Only one clinical report, however, suggested an interaction between APCR, LA and thrombosis in SLE (20). On the other hand, a retrospective study failed to show that a combination of aPL and factor V Leiden had an increased risk of recurrent thrombosis (21). We have reported similar results for factor V Leiden in another multicentre cohort (22).

Hyperhomocysteinemia may be due to an inherited genetic defect in homocysteine metabolism, such as the C677T MTHFR gene mutation; to acquired conditions, such as nutritional deficiencies in vitamin cofactors, end-stage renal failure, hypothyroidism, or carcinoma (breast, ovarian or pancreatic); or secondary to drugs, toxins (methotrexate, phenytoin) or cigarette smoking (11, 23, 24). Hyperhomocysteinemia contributes to a prothrombotic state by disrupting endothelial function at several levels (25–28). A nested case-control study from the large Physician's Health Study (N=14,916) has shown that the relative risk of myocardial infarction was increased three-fold for those with homocysteine concentrations that exceeded by 12 percent or more the upper limit of normal (29). However, a meta-analysis of prospective studies suggested at most a weak association between hyperhomocysteinemia and atherothrombotic disease (23, 30). Few prospective studies have examined the association between hyperhomocysteinemia and venous thrombosis, and these have produced inconsistent results. The association seems to be positive with recurrent rather than incident venous thrombosis (7, 31). There has been no association found in SLE patients between hyperhomocysteinemia and venous thrombosis (32).

The evaluation of thrombophilic risk factors in thromboembolic disease is important for making decisions regarding treatment and prophylaxis. There have been reports of the increased risk of thrombosis as a result of the additive or synergistic effect of concomitant thrombophilic defects occurring in the same patient (6, 7). We reported similar results in another cohort of persons with an IgG-aCL and found an odds ratio associated with a previous TE of 1.5 (95%CI 1.0–2.1) for each additional prothrombotic risk factor (33). In the present cross-sectional study, we demonstrate that APCR phenotype and hyperhomocysteinemia are associated with a higher risk of prior venous and arterial thrombosis, respectively, in the presence of aPL. This finding raises the question as to whether those individuals with both aPL and APCR, or hyperhomocysteinemia, should be given anticoagulation prophylaxis even if they have not yet suffered a TE, or prolonged, perhaps lifelong, anticoagulation treatment if they have already experienced a TE. Prospective study designs and analyses evaluating these and other prothrombotic factors are needed to define aPL subsets at higher risk for primary thrombosis.

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Table 1

Baseline characteristics of the cohort.

	aPL-positive N=75	aPL-negative N=340	Total N = 415*
Demographic			
Age in years, mean (SD)	47.7 (13.8)	46.2 (14.3)	46.5 (14.2)
Gender, n (%) female	61 (81.3)	284 (83.5)	345 (83.1)
Ethnicity, n (%) Caucasian	68 (90.7)	282 (82.9)	350 (84.3)
Cardiovascular risk factors			
FMH, n (%)	48 (64.0)	153 (45.0)	201 (48.4)
SLE, n (%) *	26 (34.7)	59 (17.4)	85 (20.5)
Smoking, n (%)	23 (30.7)	92 (27.1)	115 (27.7)
HBP (%)	17 (22.7)	50 (14.7)	67 (16.1)
DM (%)	5 (6.7)	18 (5.3)	23 (5.5)
Thyroid disorder	7 (9.3)	53 (15.7)	60 (14.5)
Medication			
Prednisone (%)	11 (14.7)	25 (7.4)	36 (8.7)
Hydroxychloroquine (%)	14 (18.7)	40 (11.8)	54 (13.0)
Acetylsalicylic acid, n (%)	20 (26.7)	41 (12.1)	61 (14.7)
Warfarin, n (%)	18 (24.0)	14 (4.1)	32 (7.7)
Estrogen, n (% of women) †	8 (13.1)	54 (19.0)	62 (18.0)
Coagulation profile			
Antithrombin, Protein C, and/or Protein S deficiencies	1 (1.3)	14 (4.1)	15 (3.6)
APCR phenotype ‡	34 (47.9)	83 (24.9)	117 (29.0)
Hyperhomocysteinemia (positive >15 U/L) §	9 (12.3)	24 (7.2)	33 (8.2)
Factor V Leiden //			
Heterozygous	3 (4.1)	15 (4.5)	18 (4.4)
Homozygous	0 (0.0)	0 (0.0)	0 (0.0)
Prothrombin gene G20210A mutation **			
Heterozygous	3 (4.1)	14 (4.2)	17 (4.2)
Homozygous	0 (0.0)	0 (0.0)	0 (0.0)
MTHFR C677T mutation **			
Heterozygous	31 (42.5)	145 (43.4)	176 (43.2)
Homozygous	7 (9.6)	47 (14.1)	54 (13.3)
Obstetric Events			
Miscarriages, n (% of women)	0 (0.0)	1 (0.4)	1 (0.3)
Spontaneous abortions, n (% of women)	14 (23.0)	28 (9.9)	42 (12.2)
Premature births, n (% of women)	5 (8.2)	17 (6.0)	22 (6.4)
Reported thrombotic events (arterial or venous), n (%)	36 (48.0)	76 (22.4)	112 (27.0)
Confirmed thrombotic events (arterial or venous), n (%)	28 (37.3)	41 (12.1)	69 (16.6)
Arterial events only, n (%)	10 (13.3)	18 (5.3)	28 (6.7)

	aPL-positive N=75	aPL-negative N=340	Total N = 415*
Venous events only, n (%)	13 (17.3)	17 (5.0)	30 (7.2)
Both arterial and venous events, n (%)	5 (6.7)	6 (1.8)	11 (2.6)
>1 thrombotic events, n (%)	11 (14.7)	16 (4.7)	27 (6.5)

* One person has been excluded from the analysis because of missing information on aPL status and thrombophilic factors.

[†]N = 61/284 aPL+/aPL- women.

[‡]N = 404. APCR phenotype is unknown for 4 aPL+ and 7 aPL- persons.

[§]N = 405. Homocysteine levels are unknown for 2 aPL+ and 8 aPL- persons.

^{||}N = 406. Factor V Leiden is unknown for 2 aPL+ and 7 aPL- persons.

^{**}N = 407. Prothrombin and MTHFR genotypes are unknown for 2 aPL+ and 6 aPL- persons.

^{††}N = 413. Thyroid function is unknown for 2 aPL- persons.

Table 2

Associations between thrombotic events (AE, VE, or TE) and thrombophilic features, presented as crude and aPL-stratified ORs and 95% CIs.

	VE	AE	TE
APCR phenotype (n = 404) *			
N (%) events in APCR-positive participants (n = 118)	17 (14.5)	14 (12.0)	26 (22.2)
N (%) events in APCR-negative participants (n = 287)	22 (7.7)	24 (8.4)	40 (13.9)
Crude OR (95% CI)	2.05 (1.04 to 4.01)	1.49 (0.74 to 2.99)	1.76 (1.02 to 3.06)
Stratified OR (95% CI)			
aPL+	1.54 (0.50 to 4.73)	3.44 (0.96 to 12.28)	1.65 (0.62 to 4.42)
aPL-	1.67 (0.68 to 4.10)	0.58 (0.19 to 1.76)	1.29 (0.62 to 2.66)
Factor V Leiden mutation (APCR genotype) (n = 406) †			
N (%) events in participants with mutation (n = 18)	5 (27.8)	1 (5.6)	5 (27.8)
N (%) events in participants without mutation (n = 388)	34 (8.8)	34 (8.8)	60 (15.5)
Crude OR (95% CI)	4.00 (1.35 to 11.91)	0.61 (0.08 to 4.74)	2.10 (0.72 to 6.11)
Stratified OR (95% CI)			
aPL+	1.69 (0.14 to 19.84)	2.42 (0.20 to 28.85)	0.90 (0.078 to 10.43)
aPL-	6.06 (1.76 to 20.93)	not available‡	2.94 (0.89 to 9.73)
Elevated serum homocysteine (n = 405) §			
N (%) events in participants with elevated serum homocysteine (n = 33)	6 (18.2)	9 (27.3)	12 (36.4)
N (%) events in participants with normal serum homocysteine (n = 372)	34 (9.1)	27 (7.3)	54 (14.5)
Crude OR (95% CI)	2.21 (0.85 to 5.73)	4.79 (2.03 to 11.33)	3.37 (1.56 to 7.24)
Stratified OR (95% CI)			
aPL+	1.79 (0.40 to 8.06)	8.75 (1.93 to 39.57)	4.40 (1.00 to 19.39)
aPL-	2.06 (0.57 to 7.49)	3.04 (0.94 to 9.80)	2.69 (1.00 to 7.23)
MTHFR C677T mutation (n = 407)			
N (%) events in participants with mutation (n = 230)	19 (8.3)	22 (9.6)	36 (15.7)
N (%) events in participants without mutation (n = 177)	20 (11.3)	13 (7.3)	29 (16.4)
Crude OR (95% CI)	0.71 (0.37 to 1.37)	1.33 (0.65 to 2.73)	0.95 (0.56 to 1.62)
Stratified OR (95% CI)			
aPL+	0.41 (0.13 to 1.26)	1.09 (0.33 to 3.63)	0.69 (0.26 to 1.81)
aPL-	1.07 (0.45 to 2.58)	1.63 (0.65 to 4.12)	1.21 (0.61 to 2.40)
Prothrombin gene G20210A mutation (n = 407) //			
N (%) events in participants with mutation (n = 17)	3 (17.7)	0 (0.0)	3 (17.7)
N (%) events in participants without mutation (n = 390)	36 (9.2)	35 (9.0)	62 (15.9)
Crude OR (95% CI)	2.11 (0.58 to 7.68)	Not available‡	1.13 (0.32 to 4.06)
Stratified OR (95% CI)			
aPL+	Not available‡	Not available‡	Not available *
aPL-	4.32 (1.11 to 16.80)	Not available‡	2.15 (0.57 to 8.08)

* APCR phenotype is unknown for 4 aPL+ and 7 aPL– persons.

[†] Factor V Leiden genotype is unknown for 2 aPL+ and 7 aPL– persons.

[‡] OR could not be calculated because of insufficient numbers of events.

[§] Homocysteine levels are unknown for 2 aPL+ and 8 aPL– persons.

// MTHFR and prothrombin genotypes are unknown for 2 aPL+ and 6 aPL– persons.

Table 3

Regression analysis for thrombophilic features associated with venous thrombosis ^{*}.

Variable	OR (95% CI)
APCR	1.51 (0.73 to 3.13)
aPL-positive at baseline	2.19 (1.04 to 4.61)
APCR AND aPL-positive at baseline [†]	3.31 (1.30 to 8.41)
Study recruitment group	12.02 (3.59 to 40.31)

^{*} Thirty-nine events were observed in 404 persons. Eleven individuals were not included in the regression model because of missing APCR status. Of these, 2 had a venous thrombosis.

[†] OR for APCR with aPL = product of ORs for each. The corresponding 95% CI was calculated using a covariance matrix.

Table 4

Regression analysis for thrombophilic features associated with arterial thrombosis ^{*}.

Variable	OR (95% CI)
Elevated serum homocysteine (Hhcy)	2.98 (1.10 to 8.06)
aPL-positive at baseline	1.64 (0.70 to 3.86)
Hhcy AND aPL-positive at baseline [†]	4.90 (1.37 to 17.37)
Hypertension	4.56 (2.04 to 10.18)
Family history of CVD	3.00 (1.28 to 7.02)
Gender (female)	0.28 (0.12 to 0.64)
Study recruitment group	3.13 (1.29 to 7.60)

^{*} Thirty-six events were observed in 405 persons. Ten individuals were not included in the regression model because of missing information on Hhcy. Of these, 3 had arterial thrombosis.

[†] OR for Hhcy with aPL = product of ORs for each. The corresponding 95% CI was calculated using a covariance matrix.