Lupus (2014) 23, 1468–1476
http://lup.sagepub.com

PAPER

Persistent antiphospholipid antibody (aPL) in asymptomatic carriers as a risk factor for future thrombotic events: a nationwide prospective study

P Mustonen[1], KV Lehtonen[2,3], K Javela[2] and M Puurunen[2]
[1]Department of Internal Medicine, Keski-Suomi Central Hospital, Jyväskylä, Finland; [2]Department of Haemostasis, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland; and [3]University of Helsinki, Faculty of Medicine, Helsinki, Finland

Objectives: The long-term prognosis of individuals fulfilling the laboratory criteria, but not clinical criteria, of antiphospholipid syndrome (APS) has not been widely investigated. The primary aim of this study was to evaluate the incidence of first thrombotic event (deep venous thrombosis (DVT), pulmonary embolism (PE), myocardial infarction (MI), stroke or transient ischaemic attack (TIA) in a nationwide antiphospholipid antibody (aPL) carrier cohort. **Design:** We conducted a prospective nationwide cohort study. **Setting:** The aPL profile of participants was recorded from the laboratory database. Information was collected about thrombotic and pregnancy complications, subsequent medical history, other risk factors for thrombosis, use of prophylactic antithrombotic medication and general health. **Participants:** Participants included adult asymptomatic aPL carriers recognized in Finland during 1971–2009. **Main outcome measure:** The main outcome measure was incidence of first thrombotic event. **Results:** A total of 119 (89% female) aPL carriers were followed for mean (SD) of 9.1 (7.5) years (range 3–41 years). Sixty-one per cent of the study participants had autoimmune disease, most often systemic lupus erythematosus (SLE). Thirty-six of 119 (30%) were either double or triple positive, 56% single lupus anticoagulant (LA) positive, and 8% and 5% single anticardiolipin antibodies (aCL) and anti-β2glycoprotein I antibodies (aβ2GPI) positive, respectively. Nine (7.6%) study patients experienced a first thrombotic event (five DVT, one PE, two MI, one TIA) mean (SD) 7.2 (8.3) years (range 1–26 years) after aPL detection (annual incidence rate 0.8%). All individuals who developed thrombotic complications had autoimmune disease. Annual rate of first thrombotic event in carriers of single positivity (0.65%) was equal to the known risk of thrombosis in the healthy Caucasian population, whereas the rate was two times higher in carriers of double or triple positivity (1.27%). Sixteen of 79 (20%) women experienced pregnancy complications. **Conclusions:** Double or triple positivity for aPL is a risk factor for future thrombotic events, especially in individuals with an underlying autoimmune disease, whereas single positivity does not seem to carry an elevated risk of thrombosis. *Lupus* (2014) **23,** 1468–1476.

Key words: Antiphospholipid antibodies; lupus anticoagulant; anticardiolipin antibodies; β_2-glycoprotein I antibodies; thrombosis

Introduction

Antiphospholipid antibodies (aPL) are a wide group of autoantibodies detected in serum or plasma by diverse laboratory tests employing phospholipids. The association of these antibodies with thrombotic events (deep venous thrombosis (DVT), pulmonary embolism (PE), myocardial infarction (MI), stroke or transient ischaemic attack (TIA) and pregnancy complications is well established. The combination of aPL with either venous or arterial thrombosis or obstetric complications is referred to as antiphospholipid syndrome (APS). To fulfil the laboratory criteria of definite APS according to the most recent revised classification (Sydney consensus conference), either lupus anticoagulant (LA), anticardiolipin antibodies

Correspondence to: Marja Puurunen, Finnish Red Cross Blood Transfusion Service, Kivihaantie 7, FIN-00310 Helsinki, Finland.
Email: marja.puurunen@veripalvelu.fi
Received 9 March 2014; accepted 8 July 2014

10.1177/0961203314545410

(aCL) or anti-β2glycoprotein I antibodies (aβ2GPI) should be persistently positive on two occasions at least 12 weeks apart.[1]

It has turned out that these various aPL, their titre, as well as single vs. double or triple positivity, have different clinical significance.[2–6] The Sydney consensus conference recommended that in research settings patients should be classified into the following categories according to the presence of laboratory criteria: 1) more than one laboratory criteria (any combination), 2(a)) LA present alone, 2(b)) aCL present alone or 2(c)) aβ2GPI present alone.[1,7]

The association of aPL with recurrence of a thrombotic event or pregnancy complication is evident. aPL predict the risk of recurrence of DVT or PE after an unprovoked thrombotic event by the risk ratio (RR) of ≈ 2[8–10] whereas the risk of recurrence of arterial thrombosis or pregnancy complications is not equally well defined.[11–13] However, the association of the presence of aPL with a first thrombotic event or first pregnancy complication in aPL carriers, i.e. individuals with positive laboratory criteria of APS, but without clinical complications at the time of aPL detection, seems to be much weaker and is less investigated.

In Finland (population 5.4 million) the laboratory testing for thrombophilia has been centralized to a single reference laboratory for almost four decades, and all positive findings during this period have been entered into a registry. The aim of this study was to assess the incidence of a first thrombotic event in asymptomatic carriers of aPL.

Methods

Study population

Nearly all individuals diagnosed with confirmed aPL in Finland during 1971–2009 have been included in the Register for Coagulation Disorders of the Finnish Red Cross Blood Service. The criteria for inclusion into the registry have been the fulfilment of the laboratory criteria of APS, i.e. at least two consecutive positive results for at least one of the aPL antibodies (LA, aCL, aβ2GPI) analysed on two separate occasions. During the earlier years the time interval was six weeks,[14,15] and after implementation of the Sydney update of the classification criteria for APS,[1] at least 12 weeks. Originally, these individuals had been referred to testing based on either 1) clinical manifestation (DVT, PE MI, stroke, TIA) or pregnancy complication), 2) suspicion of systemic lupus erythematosus (SLE) or other autoimmune disease or 3) family history of thrombosis. At the time of the study, there were 830 people in the registry. The individuals still alive, ≥18 years old and living in Finland ($n = 720$), were approached with questionnaires (Figure 1). Information was collected about their subsequent thrombosis history (DVT, PE, MI, stroke, TIA), pregnancy history, pregnancy complications (spontaneous miscarriages before and after week 10), general health and medication, and known risk factors and predisposing agents for thrombosis, both in general and at the time of the past incidents. The duration and type of possible

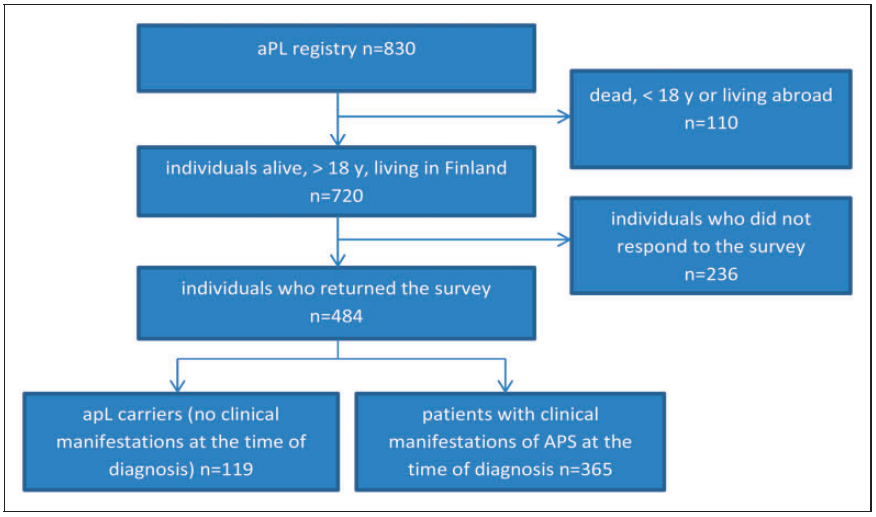

Figure 1 Number of individuals with persistently positive antiphospholipid (aPL) antibodies in the Register for Coagulation Disorders of the Finnish Red Cross Blood Service during 1971–2009, total number of survey participants, and asymptomatic aPL carriers among survey participants. The criteria for inclusion into the registry have been the fulfilment of the laboratory criteria of antiphospholipid syndrome (APS).

prophylactic antithrombotic therapy, such as aspirin, warfarin or heparin, both long-term use and administration during high-risk periods, were asked for separately.

In total, 484 (67.2%) people filled the survey and gave informed consent. Individuals were defined as aPL carriers if they were without clinical manifestations of APS whilst aPL were detected. A total of 119 out of 484 were aPL carriers and formed the study cohort of the present study (Figure 1). The judgement was based on clinical data reported by the referring physician at the time of aPL testing. The absence of baseline clinical manifestations of APS was further ensured directly from the study participants as part of the survey.

aPL detection

All aPL antibodies were determined in a single specialized national reference laboratory (Finnish Red Cross Blood Service, Laboratory of Haemostasis), which also administered the collection and processing of blood samples. LA was analysed by two different screening tests (Staclot® DVV Screen and PTT-LA (Lupus Anticoagulant-Sensitive APTT Reagent), Diagnostica Stago) according to internationally accepted guidelines[16] using directly mixed tests in the first step. Positive screening results have been confirmed by the dilute Russell's viper venom time (DRVVT) confirming test since 1995 (American Diagnostics Inc up to 2007 and DRVV Confirm, Diagnostica Stago after that) and by the partial thromboplastin time lupus anticoagulant (PTT-LA) confirming test since 2008 (Staclot® LA, Diagnostica Stago). The interference of anticoagulation therapy on LA was excluded by 1) requiring information of medication from the referring clinician before testing and 2) simultaneous prothrombin time/INR and thrombin time assessments. aCL testing was included into the aPL panel in 1994. aCL of immunoglobulin (Ig)G and IgM isotypes were determined by homemade enzyme-linked immunosorbent assay (ELISA) procedures until 2009,[17] and thereafter by a commercial kit (QUANTA Lite™ ACA IgG III, INOVA and QUANTA Lite™ ACA IgM III, INOVA Diagnostics Inc). Since 2001 aβ2GPI of IgG isotype was added to the aPL panel and analysed by a standardized ELISA (Quanta Lite™2 GPI IgG ELISA, Inova Diagnostics Inc) following the manufacturer's instructions. The cutoffs have been determined throughout the years according to internationally accepted guidelines. The cutoffs have been measured using a local reference population.

Statistical analyses

Categorical data are expressed as frequencies (percentage) and continuous data are reported as mean (SD). Between-group differences were assessed by Pearson's chi-square test for independence. Logistic regression was used to detect possible risk factors for thromboembolism. A Kaplan-Maier survival analysis was carried out to analyse the cumulative incidence of events. A p value of <0.05 was considered statistically significant. All analyses were performed using IBM SPSS 21 (SPSS Inc, Chicago, IL, USA).

The study was approved by Helsinki and Uusimaa Hospital District Ethics Committee, reference number DNRO 276/13/03/01/09.

Results

The mean (SD) follow-up time was 9.1 (7.5) years (median seven years) after the detection of aPL. The mean age of the carriers at the time of aPL detection was 35.0 (14.2) years (range 10–70 years, median 32 years). The mean age at the time of the survey was 44.7 (14.3) years (range 20–79 years, median 43 years). A total of 106 (89%) members of the study cohort were female. Demographic and clinical characteristics of the aPL carriers are summarized in Table 1.

Thrombotic events

Nine (7.6%) study patients experienced a thrombotic event during the follow-up. The mean (SD) age at presentation of thrombosis was 38.7 (11.8) years (range 27–55 years). Thrombosis occurred 7.2 (8.3) years (range 1–26 years) after aPL detection. The incidence rate of any thrombosis (venous or arterial) was 0.8/100 patient-years; cumulative incidence of all thrombotic events is shown in Figure 2.

Venous thrombosis was the first thrombotic event in six of nine cases (five DVT, one PE). The incidence rate of DVT or PE was 0.6/100 patient-years. Venous thrombosis was idiopathic in half of the cases. Arterial thrombosis (two MI, one TIA) was the first thrombotic event in three study individuals, the incidence rate of arterial thrombosis being 0.3/100 patient-years. All three patients with arterial thrombosis had at least one additional risk factor (smoking, hypertension, hyperlipidaemia or positive family history).

Sixty-one per cent (72/119) of the study patients had autoimmune disease, most often SLE (44/119)

Table 1 Demographic and clinical characteristics of asymptomatic aPL carriers ($n = 119$)

	All (n = *119*)	*No thrombotic events* (n = *110*)	*Thrombotic events* (n = *9*)	p Value
Female	106/119 (89%)	99/110 (90%)	7/9 (78%)	**0.258***
Smoking	24/119 (20%)	19/110 (17%)	5/9 (56%)	**0.006****
Hypercholesterolemia	24/119 (20%)	22/110 (20%)	2/9 (22%)	**0.873***
Diabetes	3/119 (3%)	3/110 (3%)	0/9 (0%)	**0.616***
Hypertension	18/119 (15%)	16/110 (15%)	2/9 (22%)	**0.537***
Indication for aPL testing				
Diagnostic workup of autoimmune disease	82/119 (69%)	74/110 (67%)	8/9 (89%)	
Positive family history of thrombosis	27/119 (23%)	26/110 (24%)	1/9 (11%)	
Thrombosis risk assessment without positive family history of thrombosis	8/119 (7%)	8/110 (7%)	0/9 (0%)	
Unknown	2/119 (2%)	2/110 (2%)	0/9 (0%)	
Autoimmune disease				
Individuals with autoimmune disease[a]	72/119 (61%)	63/110 (57%)	9/9 (100%)	**0.012*****
SLE	44/119 (37%)	40/110 (36%)	4/9 (44%)	**0.629***
Sjögren's syndrome	11/119 (9%)	9/110 (8%)	2/9 (22%)	
CREST	3/119 (3%)	3/110 (3%)	0/9 (0%)	
Rheumatoid arthritis	8/119 (7%)	7/110 (6%)	1/9 (11%)	
Other	16/119 (13%)	14/110 (13%)	2/9 (22%)	
Primary prophylaxis with ASA (long term)	45/119 (38%)	44/110 (40%)	1/9 (11%)	**0.086***
Short-term prophylaxis with ASA (high-risk situations)	29/119 (25%)	27/110 (25%)	2/9 (22%)	

[a]Some individuals had more than one autoimmune disease. aPL: antiphospholipid antibodies; SLE: systemic lupus erythematosus; ASA: acetylsalicylic acid; CREST: calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly and telangiectasia. * = ns; ** = $p < 0.01$; *** = $p < 0.05$.

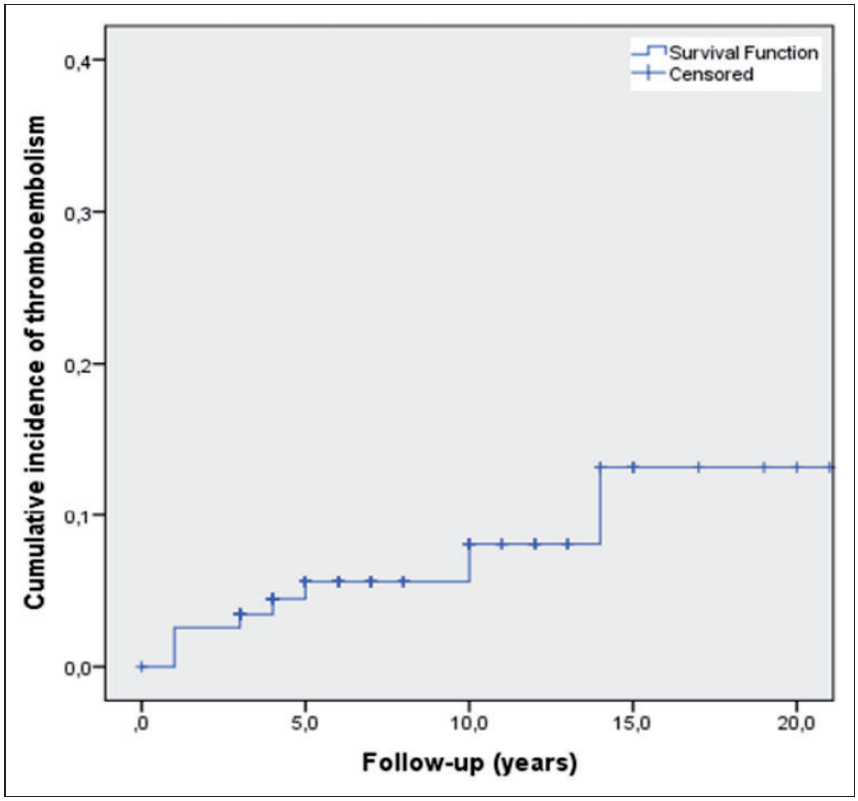

Figure 2 Kaplan-Meier survival analysis showing the cumulative incidence of thrombotic events (DVT, PE, MI, stroke/TIA) in a Finnish antiphospholipid (aPL) carrier cohort. DVT: deep venous thrombosis; PE: pulmonary embolism, MI: myocardial infarction; TIA: transient ischaemic attack.

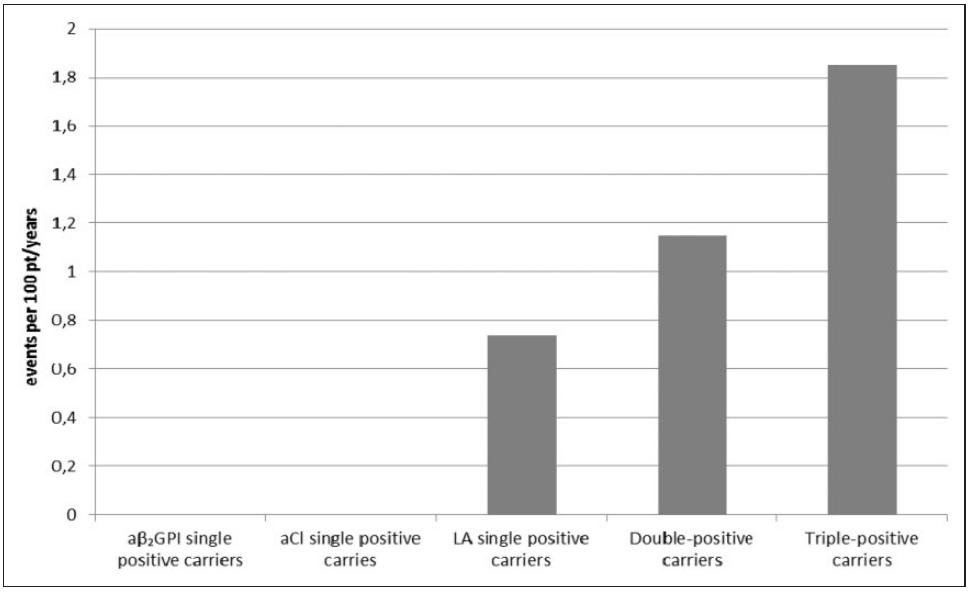

Figure 3 Average annual rates of first thrombotic events (DVT, PE, MI, stroke/TIA) in single aPL-positive, double and triple aPL-positive carriers in a Finnish aPL carrier cohort. DVT: deep venous thrombosis; PE: pulmonary embolism, MI: myocardial infarction; TIA: transient ischaemic attack.

Table 2 aPL profile and the rate of thrombotic complications (DVT, PE, myocardial infarction or stroke/TIA)

aPL	*All carriers* (n = *119*)	*No thrombotic events* (n = *110*)	*Thrombotic events* (n = *9*)	p Vaule
LA only	67/119 (56%)	62/110 (56%)	5/9 (56%)	**0.963***
aCL only	10/119 (8%)	10/110 (9%)	0/9 (0%)	**0.345***
Anti-β2GPI only	6/119 (5%)	6/110 (5%)	0/9 (0%)	**0.472***
Double-positive (LA + aCL or anti-β2GPI)	29/119 (24%)	23/110 (21%)	3/9 (33%)	**0.515***
Triple-positive	7/119 (6%)	6/110 (5%)	1/9 (11%)	**0.488***

aPL: antiphospholipid antibodies; DVT: deep venous thrombosis; PE: pulmonary embolism, TIA: transient ischaemic attack; LA: lupus anticoagulant; aCL: anticardiolipin antibodies; anti-β2GPI: anti-β2glycoprotein I antibodies. * = ns.

(Table 1). All individuals who developed thrombotic events (nine of nine) had autoimmune disease, in comparison to 57% (63/110) of those who did not ($p = 0.012$). Twenty-six patients with autoimmune disease were on hydroxychloroquine treatment; three of them had a thrombotic event (odds ratio (OR) 1.81, $p = 0.386$, confidence interval (CI) 95% 0.44–8.15). Smoking was more common in aPL carriers with thrombosis ($p = 0.006$). No difference was seen in those with diabetes, hypertension or hypercholesterolemia (Table 1).

There were only 13 men among our study group (11%), but two of nine (22%) individuals with thrombotic events were male. OR was 2.571, but male sex was not statistically significantly associated with the risk of thrombosis ($p = 0.258$, CI 95% 0.474–13.94). Interestingly, four of seven (56%) triple-positive individuals were male.

aPL profiles and thrombotic events

Not all of the 119 patients were studied in all aPL subclasses as this was not routine practice in the early years of the registry. All three aPL classes were determined in 83/119 patients, LA + aCL in 23/119 and LA only in 13/119 patients. Nine of the 23 patients (39%) studied for LA + aCL only were double positive. The percentage of positivity of each single aPL and different aPL combinations are presented in Table 2. The percentage of single positivity of LA was high in the study population (56%), whereas single positivity of aCL and aβ2GPI was low (8% and 5%, respectively).

Table 3 Triple-positive (LA, aCL and aβ2GPI) aPL carriers: the characteristics, detailed aPL results, duration of follow-up and complications depicted individually

Gender	*Age (y)*	*Follow-up time (y)*	*LA (PTT-LA)*	*LA (DRVVT)*	*aCL (GPL)*	*aCL (MPL)*	*aβ2GPI (SGU)*	*Thrombosis*	*Treatment*	*SLE*
Female	35	6	1.49/1.16[a]	1.77/1.06[a]	55	490	55	No	Aspirin	Yes
Female	34	7	1.29/1.23[a]	1.14/1.12[a]	80	50	36	No	No	No
Male	67	7	2.53/1.23[a]	1.13/1.12[a]	85	20	111	No	Aspirin	No
Male	30	15	1.36/1.23[a]	1.15/1.07[a]	Positive	Positive	150	No	Aspirin	Yes
Female	29	8	Positive	Positive	Positive	20	31	No	No	Yes
Male	64	6	1.27/1.16[a]	1.13/1.12[a]	Positive	75	39	No	No	No
Male	32	7	1.70/1.22[a]	2.07/1.12[a]	85	88	57	DVT (age 37)	Aspirin	No
								DVT (age 38)	Aspirin	
								DVT (age 39)	LMWH	

[a]Upper limit of the up-to-date reference value at the time of the analyses. LA: lupus anticoagulant; aCL: anticardiolipin antibodies; aβ2GPI: anti-β2glycoprotein I antibodies; PTT-LA: partial thromboplastin time lupus anticoagulant; DRVVT: dilute Russell's viper venom time; Ig: immunoglobulin; GPL: IgG phospholipid units; MPL: IgM phospholipid units; SGU: standard IgG aB2GPI unit; y: years; DVT: deep venous thrombosis; SLE: systemic lupus erythematosus; LMWH: low molecular-weight heparin.

The percentage of those with single LA positivity was similar in individuals with and without thrombotic events (DVT, PE, MI, stroke or TIA). None of the individuals with single aPL or aβ2GPI developed thrombosis. Thirty-six of 119 (30%) aPL carriers were either double (LA and aCL or aβ2GPI) or triple positive. None of the individuals was aCL and aβ2GPI positive but LA negative. Average annual rate of first thrombotic event in carriers of any single positivity was 0.74%, of double positivity 1.15%, and of triple positivity 1.85% (Figure 3).

Among the study population there were seven aPL carriers with triple positivity; details of these subjects are presented in Table 3. One triple-positive individual had thrombotic events (three DVTs) during the follow-up of seven years, whereas six of seven individuals during the follow-up of 8.2 (3.4) (mean (SD)), range 6–15 years, did not. Four of seven (57%) triple-positive carriers had been on long-term prophylactic acetylsalicylic acid (ASA) medication.

Antithrombotic medication

Thirty-eight per cent (45/119) of all aPL carriers had been on long-term prophylaxis with low-dose aspirin (Table 1); only one of these 45 individuals had a thrombotic event. Primary thromboprophylaxis with long-term ASA was trendwise, but not statistically significantly associated with reduced risk of thrombosis ($p = 0.086$, CI 95% 0.023–1.552, OR 0.188). Twenty-nine individuals (24%) had used short-term prophylaxis (ASA and/or low molecular-weight heparin (LMWH)) during high-risk situations (i.e. surgery); despite that two of 29 (one triple positive and one with strongly positive LA) had a thrombotic event.

Pregnancy complications

Seventy-nine of 106 women (74.5%) had been pregnant. These 79 women had altogether 169 pregnancies, out of which 147 (89.6%) succeeded without complications. Sixteen of 79 (20%) women had had a total of 22 pregnancy complications (13% of all pregnancies in the whole study population). The complications were as follows: 18 spontaneous miscarriages before week 10 (16 while without, and two while on prophylactic medication) and four miscarriages after week 10 (two while without, and two while on prophylactic medication). All four women who experienced miscarriage after week 10 were single positive (three LA and one aCL). The Sydney aPL criteria of pregnancy complications were fulfilled only in the four women with late miscarriages, since none of the survey participants had had more than two early miscarriages. Of 169 pregnancies, 101 (60%) were successful without any medication. Prophylactic antithrombotic medication was used in 46/147 (31%) successful and in four of 22 (18%) unsuccessful pregnancies (all four were on low-dose aspirin).

Discussion

The results of this study strengthen the previous data showing that double or triple positivity of aPL antibodies (LA, aCL, aβ2GPI) carries a much higher risk for future thrombotic events (either venous or arterial) in asymptomatic aPL carriers than any single positive test. Single aCL or aβ2GPI positivity was not associated with thrombosis in our study, also supporting earlier data. There is still controversy surrounding the

significance of single-positive LA in this setting: In our study it was not associated with increased risk. The annual rate of first thrombotic event in our aPL carrier population ($n = 119$) was 0.8% during a mean follow-up of 9.4 years. The annual incidence of thrombosis was only slightly elevated and not more than 1.5 times higher than reported in a comparable age group of a healthy Caucasian population.[18–21] When compared to previous studies assessing the risk of thrombosis in asymptomatic aPL carriers, the annual incidence of thrombosis in our study was within the reported range 0%–3.8%.[22–31] More recently, two important prospective follow-up studies have been published. Pengo et al. followed 104 asymptomatic aPL carriers for a mean of 4.5 years and reported the annual risk of thrombosis to be 1.36%.[32] The main difference in comparison to the present study is that the study population of Pengo et al. (2011) consisted solely of triple-positive individuals with a high-risk profile. In another recent prospective multicentre study with 258 participants and a mean follow-up of 2.9 years,[33] the respective annual incidence rate was 1.86%; in this study individuals with single, double and triple positivity were followed. The smaller incidence rate in our study could be partly explained by differences in study populations. In our study we did not divide the population according to antibody titre.

Single test positivity

In our study the incidence of a first thrombotic event in the carriers of any single positivity did not differ from the known incidence of a first thrombotic event in the healthy population.[18–21] Furthermore, single positivity of any aPL (LA, aCL or aβ2GPI) was not associated with increased risk of future thrombotic events. Previous studies support this observation for sole aCL or aβ2GPI positivity and thrombosis.[2,4,7,32,33] However, contradictory data exist regarding the single positivity of LA (when aCL and aβ2GPI are negative) and thrombosis. According to some previous studies[2] positive LA is a strong risk factor for thrombosis, whereas this has been disputed by others.[7] In the study by Ruffatti et al., LA positivity was an independent risk factor for thrombosis in asymptomatic aPL carriers. However, single or multiple positivity was not depicted separately.[33]

Double or triple positivity

Multiple positivity in aPL tests is known to identify APS patients with a high risk of recurrent thrombotic events.[4,7,34,35] When asymptomatic aPL carriers are concerned, the existing evidence is less abundant. An aPL carrier case-control study by De Groot et al. showed that positivity of more than one aPL was associated with a higher risk of first thrombotic event than single positivity.[6] In our study the annual rate of first thrombotic event in the carriers of double or triple positivity was two-fold higher than in individuals with a single positive test, which is in line with previous aPL carrier studies. In a recent multicentre prospective study, triple positivity (LA, aCL and aβ2GPI) increased the risk of first episode of thrombosis 3.9-fold when compared to single positivity.[32] In our small triple-positive subgroup the annual rate of first thrombotic event was 1.3%, whereas in the study by Pengo et al. it was 5%.[32] Sixty-seven per cent of our triple-positive patients used long-term, low-dose aspirin and only 33% were without any antithrombotic prophylaxis; the corresponding figures in the study by Pengo et al.[32] were 36% and 64%, respectively.

The effect of antithrombotic medication

Thirty-eight per cent of our population was on long-term, low-dose aspirin treatment. Use of aspirin did not result in a statistically significant reduction of thrombotic events. Of the seven triple-positive patients, four were on prophylactic aspirin treatment. It is impossible to determine whether this has affected the low incidence of thrombotic events in this high-risk population. Aspirin did not reduce thrombotic events in aPL carrier populations in previous prospective follow-up studies.[27,32,33] However, in a recent meta-analysis it seems that in the subgroup of asymptomatic aPL carriers there might be a protective effect of low-dose aspirin, especially in patients with SLE.[36]

SLE or other autoimmune disease

The dominant reason (61%) for aPL testing in asymptomatic individuals in our cohort was the presence of autoimmune disease, most often SLE (37%). In a study by Ruffatti, which was performed solely in rheumatology centres, the prevalence of autoimmune disorder was very similar (68%).[33] SLE and other autoimmune diseases are associated with aPL. aPL have been reported to be present in 2% of the general population[37] with LA around 1%.[6] The prevalence of aPL in SLE patients without history of thrombosis is about 40%.[38]

It is notable that in our study all nine aPL carriers who developed thrombotic events had autoimmune disease; out of these, four had SLE.

In patients with SLE the association of aPL with thrombosis has been firmly established.[2,39,40]

Sex

Women are more prone to autoimmune phenomena. About 80% of patients with APS are female.[6] Asymptomatic aPL carriers also are predominantly female.[32,6,33] The same phenomenon was observed in our study population with 89% being females. Male sex has been reported to be a strong independent predictor of thrombotic events in aPL carriers in some[32] but not in all studies.[33] In our cohort males had thrombotic events more often than females (OR 2.6), but this difference was not statistically significant.

Pregnancy complications

In our study, pregnancy complications occurred in 16 out of 79 women (20%) who became pregnant during the follow-up. A higher frequency of pregnancy morbidity in triple-positive asymptomatic aPL carriers has been reported previously.[32] In our study the number of triple-positive women was too low to allow for any firm conclusions on pregnancy-associated risk.

Study limitations

The present study has some advantages when compared to previous aPL carrier studies. The study population consists of a nationwide cohort with a very long follow-up period. All aPL testing was performed in a single reference laboratory specializing in haemostasis, where throughout the years testing was performed according to up-to-date good laboratory practice, including vigorous quality testing and careful preanalytical consideration. However, there are some limitations in our study. The data on clinical events are based on information from the reference letters and patients' answers in the survey but not objectively confirmed from patient records. Also, the causes of death of aPL carriers are not known, as the survey was sent to alive patients in the registry. Third, although the response rate was good (67%), it was not comprehensive, which might affect the results.

Conclusions

Double or triple positivity for aPL is a risk factor for future thrombotic events especially in patients with an underlying autoimmune disease, whereas single positivity does not seem to carry an elevated risk of thrombosis. Screening for aPL might be considered in patients with autoimmune diseases. Modifiable risk factors should be actively treated in these patients. The role of aspirin in primary prevention can be questioned. Prospective studies in autoimmune patients are needed to determine the role of aPL testing and prophylactic treatment.

Funding

This work was supported by the Finnish Red Cross Blood Service and University of Helsinki, Faculty of Medicine.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgement

The excellent technical assistance of Petra Makkonen is gratefully acknowledged.

References

1 Miyakis S, Lockshin MD, Atsumi T, *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
2 Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: A systematic review of the literature. *Blood* 2003; 101: 1827–1832.
3 Zochlami-Rintelen C, Vormittag R, Sailer T, *et al*. The presence of IgG antibodies against β2-glycoprotein I predicts the risk of thrombosis in patients with the lupus anticoagulant. *J Thromb Haemost* 2005; 3: 1160–1165.
4 Pengo V, Biasiolo A, Pegoraro C, Cucchini U, Noventa F, Ilioceto S. Antibody profiles for the diagnosis of antiphospholipid syndrome. *Thromb Haemost* 2005; 93: 1147–1152.
5 Pengo V, Biasiolo A, Gresele P, *et al*. A comparison of lupus anticoagulant-positive patients with clinical picture of antiphospholipid syndrome and those without. *Arterioscler Thromb Vasc Biol* 2007; 27: e309–e310.
6 de Groot PG, Lutters B, Derksen RH, Lisman T, Meijers JC, Rosendaal FR. Lupus anticoagulants and the risk of a first episode of deep venous thrombosis. *J Thromb Haemost* 2005; 3: 1993–1997.
7 Pengo V, Banzato A, Bison E, Bracco A, Denas G, Ruffatti A. What have we learned about antiphospholipid syndrome from patients and antiphospholipid carrier cohorts? *Semin Thromb Hemost* 2012; 38: 322–327.
8 Kearon C, Akl EA, Comerota AJ, *et al*. Antithrombotic therapy for VTE disease. Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; 141: e419S–e494S.
9 Schulman S, Svenungsson E, Granqvist S. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. *Am J Med* 1998; 104: 332–338.

10 Rodger MA, Kahn SR, Wells PS, *et al*. Identifying unprovoked thromboembolism patients at low risk for recurrence who can discontinue anticoagulant therapy. *CMAJ* 2008; 179: 417–426.
11 Lim W. Antiphospholipid antibody syndrome. *Hematology Am Soc Hematol Educ Program* 2009: 233–239.
12 Pengo V, Ruiz-Irastorza G, Denas G, Andreoli L, Khamashta M, Tincani A. High intensity anticoagulation in the prevention of the recurrence of arterial thrombosis in antiphospholipid syndrome: 'PROS' and 'CONS'. *Autoimmun Rev* 2012; 11: 577–580.
13 Ruiz-Irastorza G, Hunt BJ, Khamastha MA. A systematic review of secondary thromboprophylaxis in patients with antiphospholipid antibodies. *Arthritis Rheum* 2007; 57: 1487–1495.
14 Wilson WA, Gharavi AE, Koike T, *et al*. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: Report of an international workshop. *Arthritis Rheum* 1999; 42: 1309–1311.
15 Lockshin MD, Sammaritano LR, Schwartzman S. Validation of the Sapporo criteria for antiphospholipid syndrome. *Arthritis Rheum* 2000; 43: 440–443.
16 Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulant: An update. *Thromb Haemost* 1995; 74: 1185–1190.
17 Vaarala O. Binding profiles of anticardiolipin antibodies in sera from patients with SLE and infectious diseases. *J Autoimmun* 1991; 4: 819–830.
18 Roger VL, Go AS, Lloyd-Jones DM, *et al*. Heart disease and stroke statistics: 2011 update. A report from the American Heart Association. *Circulation* 2011; 123: e18–e209.
19 Kissela BM, Khoury JC, Alwell K, *et al*. Age at stroke: Temporal trends in stroke incidence in a large, biracial population. *Neurology* 2012; 79: 1781–1787.
20 Mannsverk J, Wilsgaard T, Njølstad I, *et al*. Age and gender differences in incidence and case fatality trends for myocardial infarction: A 30-year follow-up. The Tromso Study. *Eur J Prev Cardiol* 2012; 19: 927–934.
21 Go AS, Mozaffarian D, Roger VL, *et al*. Executive summary: Heart disease and stroke statistics – 2013 update: A report from the American Heart Association. *Circulation* 2013; 127: 143–152.
22 Finazzi G, Brancaccio V, Mola A, *et al*. Natural history and risk factors for thrombosis in 360 patients with antiphospholipid antibodies: A four-year prospective study from the Italian registry. *Am J Med* 1996; 100: 530–536.
23 Shah NM, Khamashta MA, Atsumi T, Hughes GR. Outcome of patients with anticardiolipin antibodies: A 10 year follow-up of 52 patients. *Lupus* 1998; 7: 3–6.
24 Somers E, Magder LS, Petri M. Antiphospholipid antibodies and incidence of venous thrombosis in a cohort of patients with systemic lupus erythematosus. *J Rheumatol* 2002; 29: 2531–2536.
25 Girón-González JA, García del Río E, Rodríguez C, Rodríguez-Martorell J, Serrano A. Antiphospholipid syndrome and asymptomatic carriers of antiphospholipid antibody: Prospective analysis of 404 individuals. *J Rheumatol* 2004; 31: 1560–1567.
26 Forastiero R, Martinuzzo M, Pombo G, *et al*. A prospective study of antibodies to beta2-glycoprotein I and prothrombin, and risk of thrombosis. *J Thromb Haemost* 2005; 3: 1231–1238.
27 Erkan D, Harrison MJ, Levy R, *et al*. Aspirin for primary thrombosis prevention in antiphospholipid syndrome: A randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody-positive individuals. *Arthritis Rheum* 2007; 56: 2382–2391.
28 Hereng T, Lambert M, Hachulla E, *et al*. Influence of aspirin on the clinical outcomes of 103 antiphospholipid antibodies-positive patients. *Lupus* 2008; 17: 11–15.
29 Tektonidou MG, Laskari K, Panagiotakos DB, Moutsopoulos HM. Risk factors for thrombosis and primary thrombosis prevention in patients with systemic lupus erythematosus with or without antiphospholipid antibodies. *Arthritis Rheum* 2009; 61: 29–36.
30 Ruffati A, Del Ross T, Ciprian M, *et al*. Risk factors for a first thrombotic event in antiphospholipid antibody carriers. A multicentre, retrospective follow-up study. *Ann Rheum Dis* 2009; 68: 397–399.
31 Barbhaia M, Erkan D. Primary thrombosis prophylaxis in antiphospholipid antibody-positive patients: Where do we stand? *Curr Rheumatol Rep* 2011; 13: 59–69.
32 Pengo V, Ruffari A, Legnani C, *et al*. Incidence of a first thromboembolic event in asymptomatic carriers of high-risk antiphospholipid antibody profile: A multicenter prospective study. *Blood* 2011; 118: 4714–4718.
33 Ruffatti A, Del Ross T, Ciprian M, *et al*. Risk factors for a first thrombotic event in antiphospholipid antibody carriers: A prospective multicentre follow-up study. *Ann Rheum Dis* 2011; 70: 1083–1086.
34 Ruffatti A, Tonello M, Del Ross T, *et al*. Antibody profile and clinical course in primary antiphospholipid syndrome with pregnancy morbidity. *Thromb Haemost* 2006; 96: 337–341.
35 Ruffatti A, Tonello M, Cavazzana A, Bagotella P, Pengo V. Laboratory classification categories and pregnancy outcome in patients with primary antiphospholipid syndrome prescribed antithrombotic therapy. *Thromb Res* 2009; 123: 482–487.
36 Arnaud L, Mathian A, Ruffatti A, *et al*. Efficacy of aspirin for the primary prevention of thrombosis in patients with antiphospholipid antibodies: An international and collaborative meta-analysis. *Autoimmun Rev* 2014; 3: 281–291.
37 Ginsberg JS, Wlls PS, Brill-Edwards P, *et al*. Antiphospholipid antibodies and venous thromboembolism. *Blood* 1995; 86: 3685–3691.
38 Ruiz-Irastorza G, Egurbide MW, Ugalde J, Aquirre C. High impact of antiphospholipid syndrome on irreversible organ damage and survival of patients with systemic lupus erythematosus. *Arch Intern Med* 2004; 164: 77–82.
39 Tarr T, Lakos G, Bhattoa HP, Shoenfeld Y, Szegedi G, Kiss E. Analysis of risk factors for the development of thrombotic complications in antibody positive lupus patients. *Lupus* 2007; 16: 39–45.
40 Palatinus A, Adams M. Thrombosis in systemic lupus erythematosus. *Semin Thromb Haemost* 2009; 35: 621–629.