Journal of Thrombosis and Haemostasis, **8**: 237–242 DOI: 10.1111/j.1538-7836.2009.03674.x

IN FOCUS

Clinical course of high-risk patients diagnosed with antiphospholipid syndrome

V. PENGO,* A. RUFFATTI,† C. LEGNANI,‡ P. GRESELE,§ D. BARCELLONA,¶ N. ERBA,** S. TESTA,†† F. MARONGIU,* E. BISON,* G. DENAS,* A. BANZATO,* S. PADAYATTIL JOSE* and S. ILICETO*

**Clinical Cardiology, University Hospital, Padova; †Rheumatology, University Hospital, Padova; ‡Department of Angiology and Blood Coagulation ''Marino Golinelli'', University Hospital, Bologna; §Internal and Vascular Medicine, University Hospital, Perugia; ¶Internal Medicine, University Hospital, Cagliari; **Transfusion Medicine, District Hospital, Merate; and ††District Hospital, Cremona, Italy*

To cite this article: Pengo V, Ruffatti A, Legnani C, Gresele P, Barcellona D, Erba N, Testa S, Marongiu F, Bison E, Denas G, Banzato A, Padayattil Jose S, Iliceto S. Clinical course of high-risk patients diagnosed with antiphospholipid syndrome. *J Thromb Haemost* 2010; **8**: 237–42.

See also Galli M. The antiphospholipid triangle. This issue, pp 234–6.

Summary. *Background:* The characteristics and the clinical course of antiphospholipid syndrome (APS) in high-risk patients that are positive for all three recommended tests that detect the presence of antiphospholipid (aPL) antibodies have not been described. *Methods:* This retrospective analysis of prospectively collected data examined patients referred to Italian Thrombosis Centers that were diagnosed with definite APS and tested positive for aPL [lupus anticoagulant (LA), anti-cardiolipin (aCL), and anti-β2-glycoprotein I (β2GPI) antibodies]. Laboratory data were confirmed in a central reference laboratory. *Results:* One hundred and sixty patients were enrolled in this cohort study. The qualifying events at diagnosis were venous thromboembolism (76 cases; 47.5%), arterial thromboembolism (69 cases; 43.1%) and pregnancy morbidity (11 cases; 9.7%). The remaining four patients (2.5%) suffered from catastrophic APS. The cumulative incidence of thromboembolic events in the follow-up period was 12.2% (95%CI, 9.6–14.8) after 1 year, 26.1% (95%CI, 22.3–29.9) after 5 years and 44.2% (95%CI, 38.6–49.8) after 10 years. This was significantly higher in those patients not taking oral anticoagulants as compared with those on treatment (HR = 2.4 95%CI 1.3–4.1; $P < 0.003$). Major bleeding associated with oral anticoagulant therapy was low (0.8% patient/years). Ten patients died (seven were cardiovascular deaths). *Conclusions:* Patients with APS and triple positivity for aPL are at high risk of developing future thromboembolic events. Recurrence remains frequent despite the use of oral anticoagulants, which significantly reduces the risk of thromboembolism.

Correspondence: Vittorio Pengo, Clinical Cardiology, Thrombosis Center, Via Giustiniani 2, 35128 Padova, Italy.
Tel.: +39 49 821 5658; fax: +39 49 821 5658.
E-mail: vittorio.pengo@unipd.it

Keywords: anticoagulants, diagnosis, lupus anticoagulant, phospholipids, thrombosis.

Introduction

Antiphospholipid syndrome (APS) is an acquired autoimmune disease characterized by thrombosis-related clinical disorders or pregnancy morbidity in the presence of antiphospholipid antibodies (aPL) [1]. Clinical features of APS are commonly encountered in clinical practice. The diagnosis of APS is entirely based on laboratory tests that detect the presence of aPL [i.e. lupus anticoagulant (LA), anti-cardiolipin (aCL) antibodies and anti-β2-Glycoprotein I (aβ2GPI) antibodies]. However, these tests are poorly standardized, which leads to low sensitivity [2–4]. Indeed, the LA test is positive in the presence of aβ2GPI and anti-prothrombin antibodies, the aCL ELISA is positive in the presence of antibodies directed against cardiolipin-binding proteins and the aβ2GPI ELISA may identify antibodies that are distinct to those relevant to the syndrome [5]. As just one positive test is sufficient for APS diagnosis, there is a substantial risk of diagnosing APS in patients with either one false-positive test or with antibodies that recognize a target that is irrelevant to the syndrome. This contention is supported by meta-analyses showing that the presence of aCL and aβ2GPI alone are poorly associated with thromboembolic events [6,7]. In contrast, multiple positivity shows a marked association with thromboembolic events and severe pregnancy morbidity [8–13]. Many clinical studies on APS have included patients with different laboratory profiles, and consequently, the results may lack consistency [14–17]. To our knowledge, no study has reported the characteristics and clinical course of a homogenous group of APS patients that are positive in all three laboratory diagnostic tests. In this study, we assessed the clinical characteristics and clinical course of a large series of triple positive high-risk patients with APS.

Methods

Study design and population

This retrospective analysis of prospectively collected data from Italian Thrombosis Centers, considered patients with definite APS [1] and that had positive diagnoses in all three aPL tests (LA, aCL and aβ2GPI antibodies), and in two laboratory controls at least 12 weeks apart. To confirm triple positivity for aPL, and to validate the laboratory diagnosis, patient plasma from each center was also tested in a reference laboratory. Patients were included in the cohort if both LA and aβ2GPI antibodies were positive and aCL antibodies, exceeded 40 IgG phospholipid (GPL) and/or 40 IgM phospholipid (MPL) units. The predominant isotype was considered G when GPL was more than 40 units and M when MPL was more than 40 units and GPL $<$ 40 units. The observation period was set from the date of the earliest diagnosis (March 1989) until March 2009. Patients were seen by the center physician in an outpatient setting once a year (or earlier depending their clinical status) or if discharged from hospital. Patients diagnosed with APS before aβ2GPI ELISA test results were available had the test performed subsequently on the first original stored plasma. All patients were informed about the study and gave signed consent about the treatment of their data.

Data collection and clinical features at diagnosis

Centers were asked to provide demographic, laboratory and clinical data for each patient. Both the type and site of any thromboembolic events at diagnosis were demonstrated objectively. Pregnancy morbidity was classified according to the latest update on diagnostic criteria for APS. Unsuccessful pregnancies were defined as either: one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus (Type I); eclampsia or severe pre-eclampsia defined according to standard definitions (Type II); and three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded (Type III) [1]. Patients were considered as having catastrophic APS if they presented with acute devastating APS involving multiple ($>$ 3) organs and presenting over a short period of time ($<$ 1 week), as previously defined [18].

Information about the presence of any associated autoimmune diseases [systemic lupus erythematosus (SLE) or other connective tissue diseases] and the presence of any additional risk factors for thrombosis was also collected. Risk factors for arterial thrombosis included: diabetes mellitus, hypertension, hypercholesterolemia, obesity, smoking habit and family history. Risk factors for venous thrombosis were: recent surgical intervention, peri-operative immobilization, oral estroprogestinic treatment, pregnancy, malignancy, family history, thrombophilia (antithrombin, protein C, or protein S; factor V Leiden; prothrombin G20210A mutation; hyperhomocysteinemia, high factor VIII levels) and previous venous thromboembolism (VTE).

Laboratory tests

Methods and results for laboratory diagnosis refer to tests performed in the reference laboratory. LA was determined using diluted Russell viper venom time (dRVVT) for screening, mixing and confirmatory tests [2]. Mixing tests are expressed as the ratio of dRVVT of 1:1 mixing of patient and normal pooled plasma, divided by dRVVT of pooled normal plasma. Values of mixing tests were used to classify LA as strong (ratio $>$ 2.0) or low-moderate (ratio $>$ 1.2 $\leq$ 2.0).

ACL and aβ2GPI antibodies were measured by ELISA, as previously described [19], according to the proposals of the Standardization Group of the European Forum on aPL antibodies [20,21]. ACL ELISA was considered positive when GPL or MPL exceeded 40 units. Laboratory data for aCL ELISA tests are reported as predominant IgG or IgM isotypes. The aβ2GPI ELISA was considered positive when IgG or IgM exceeded the cut-off value calculated using the 99th percentile of 40 normal age- and sex-matched controls.

Outcome events

Reports for objectively diagnosed *thromboembolic events* during follow-up included type, site and the status of antithrombotic treatment at the time of event. A patient was considered as not taking oral anticoagulants if no anticoagulant drug was administered at the time of event or if being treated with aspirin.

VTE was diagnosed using compression ultrasonography or venography for deep vein thrombosis and spiral tomography, ventilation-perfusion lung scan or pulmonary angiography for pulmonary embolism. Intracerebral thrombosis was assessed by computed tomographic scanning, magnetic resonance imaging or angiography; retinal thrombosis was diagnosed by ophthalmologic examination. Peripheral- or mesenteric-artery thrombosis was documented by arteriography or at surgery. Acute myocardial infarction was diagnosed in the presence of a typical clinical presentation associated with typical electrocardiographic features and elevated cardiac enzymes (CK-MB or Troponins I or T). Stroke/transient ischemic attack (TIA) was defined according to standard definitions (TIA was only considered for analysis if cerebral imaging revealed ischemia).

Major bleeding was defined as: fatal bleeding, clinically overt bleeding associated with a fall in haemoglobin level of $\geq$ 20 g/L in 24 h and/or requiring non-planned transfusion of $\geq$ 2 units of packed red blood cells or whole blood; intracranial bleeding (documented by imaging), retroperitoneal bleeding, intraocular bleeding causing blindness, joint hemorrhage, or need for surgery or angiographic intervention to stop hemorrhage. All other bleeding events that did not match the criteria for major bleeding were considered minor bleeding.

The causes of death were obtained from clinical or autopsy reports and/or death certificates.

Statistics

Descriptive statistics are reported as appropriate: categorical data are expressed as frequencies (percentage) and compared using Fisher's exact test; continuous data are reported as mean (± standard deviation). Kaplan–Meier survival analysis was used to determine the cumulative incidence of thromboembolic events at follow-up; data were compared using the log-rank test for comparisons. Cox regression survival analysis was used to detect possible predictors of the primary endpoint among the demographic factors, associated autoimmune diseases, aCL isotype, arterial and venous risk factors and anticoagulant treatment at the time of event. Statistical significance was considered for $P < 0.05$. All analyses were performed using SPSS (Rel. 17.0.1. 2008; SPSS Inc., Chicago, IL, USA).

Results

Of the 189 patients considered for this study, 29 were excluded; 11 because the laboratory data were not confirmed in the reference laboratory and 18 for having both GPL and MPL aCL titers of < 40 units. Thus, 160 patients with three positive APS tests were enrolled in this cohort study. Eighty-five patients (53%) were strongly positive in the dRVVT mixing test (dRVVT ratio > 2.0). IgG aCL was predominant in most patients (94%) whereas only 10 patients had a predominant IgM aCL isotype. Mean (± SEM) GPL and MPL units were 165 ± 17 and 75 ± 7, respectively. All patients were positive for IgG or IgM in the aß2GPI ELISA and the results were in accordance with the predominant isotype found in the aCL ELISA.

The demographic and clinical characteristics of patients are summarized in Table 1. The qualifying event at diagnosis was VTE in 76 cases (47.5%), arterial thromboembolism in 69 cases (43.1%) and pregnancy morbidity in 11 cases (6.9%). The remaining four patients (2.5%) suffered from catastrophic APS. Most patients had primary APS; additional associated risk factors for arterial or VTE were also frequent.

Table 1 Demographic and clinical characteristics at diagnosis of 160 high-risk APS patients*

Characteristics	
Age years	41.1 ± 15.0
Female – no. (%)	113 (70.6)
APS-related event at diagnosis – no. (%)	
VTE	76 (47.5)
Arterial Thrombosis	69 (43.1)
Obstetric complications	11 (6.9)
Catastrophic APS	4 (2.5)
Autoimmune disorders – no.(%)	
SLE	33 (18.8)
Other	34 (19.3)
Risk for arterial thrombosis† – no. (%)	87 (49.4)
Risk for venous thrombosis‡ – no. (%)	71 (40.3)

*Plus–minus values are means ± SD; VTE, venous thromboembolism; SLE, systemic lupus erythematosus. †Considered risk factors for arterial thrombosis: diabetes mellitus, hypertension, hypercholesterolemia, obesity, smoking habit and family history. ‡Considered risk factor for venous thrombosis: recent surgical intervention, peri-operative immobilization, oral estroprogestinic treatment, pregnancy, malignancy, family history, thrombophilia and previous VTE.

Table 2 Sites of thromboembolic events at presentation

Site	N
Venous sites	
Venous thrombosis of the lower limbs	54
Pulmonary embolism	14
Venous thrombosis of the upper limbs	2
Retinal thrombosis	2
Inferior vena cava thrombosis	1
Mesenteric vein thrombosis	1
Jugular vein thrombosis	1
Tricuspid valve thrombosis	1
Arterial sites	
Stroke	27
TIA	15
Arterial thrombosis of the lower limbs	15
Myocardial infarction	8
Renal artery thrombosis	2
Retinal artery thrombosis	1
Mesenteric ischemia	1
Cutaneous necrosis	3

TIA, transient ischemic attack.

Patients with a predominant IgM isotype ($n = 10$) differed from the rest of the study population as they were significantly older (66.4 ± 6.8 vs. 39.4 ± 13.8, $P < 0001$) and more frequently presented with arterial thrombosis as a qualifying event at diagnosis (six arterial vs. four venous).

Sites of venous and arterial thrombosis at diagnosis are described in Table 2. Most venous events occurred in the lower limbs or in the pulmonary arteries. Other sites included the retinal, caval, mesenteric and jugular veins. One patient presented with a thrombus on the tricuspid valve. Stroke/TIA and thrombosis in the lower limbs were the most frequent arterial events. Eight patients had myocardial infarction. Unusual sites of arterial thrombosis were renal, mesenteric and retinal arteries. Pregnancy morbidity was of Type I and III in five patients, respectively. Three out of four patients with catastrophic APS suffered from cutaneous necrosis at presentation. Other associated clinical disorders were autoimmune thrombocytopenia, epilepsy, headache, renal insufficiency and thrombotic endocarditis. Two patients suffered from cancer and one patient had chronic thromboembolic pulmonary hypertension.

Thromboembolic events during the followup period

One hundred and thirty-one (81.9%) patients completed the 1-year follow-up, 76 (47.5%) completed 5 years and 23 (14.4%) completed 10 years of follow-up. Mean follow-up was 6.0 (± 4.6) years (range 0.3–20 years). Out of the 160 patients, 55 (34.4%) had a thromboembolic event during the follow-up period; 25 were VTE and 30 were arterial thromboembolism. The cumulative incidence of thromboembolic events during follow-up was 12.2% (95%CI, 9.6–14.8) after 1 year, 26.1% (95%CI, 22.3–29.9) after 5 years and 44.2% (95%CI, 38.6–49.8) after 10 years (Fig. 1). The thromboembolic recurrence in

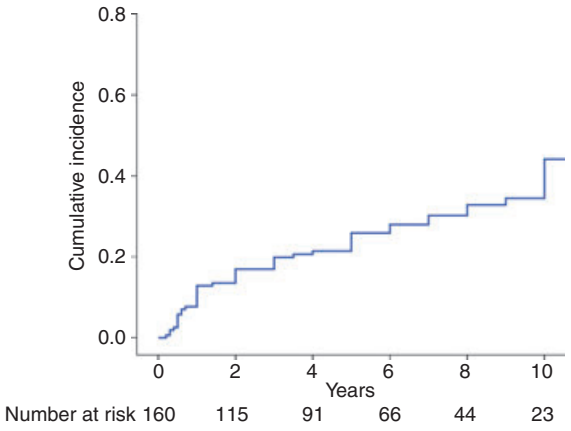

Fig. 1. Cumulative incidence of thromboembolic events [25 venous thromboembolism (VTE) and 30 arterial thromboembolism (ATE)] in 160 patients with antiphospholipid syndrome (APS) and triple laboratory positivity (figure reports data of 10-years follow-up).

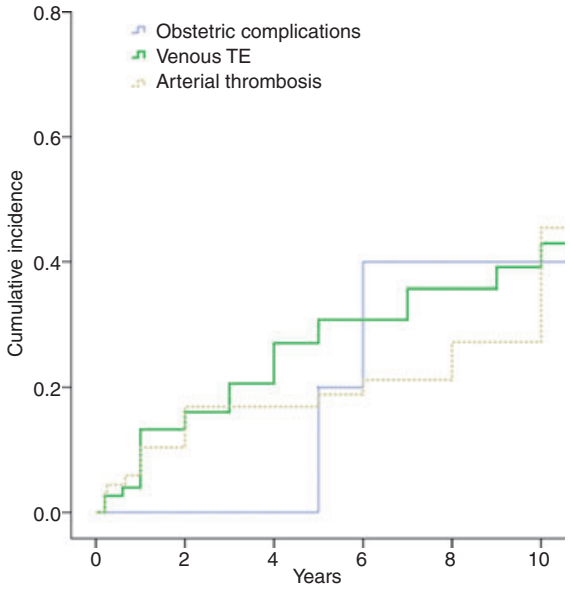

Fig. 2. Cumulative incidence of thromboembolic events in patients with antiphospholipid syndrome (APS) and triple laboratory positivity according to the clinical features at diagnosis.

patients with VTE at diagnosis was 16 out of 27 cases (59%). Likewise, recurrence of arterial thromboembolism (ATE) in patients was 15 of 22 cases (68%). Two patients with pregnancy morbidity at presentation subsequently suffered from an episode of cerebral ischemia. All four catastrophic APS patients suffered a recurrence during followup, two in the venous circulation (Budd–Chiari syndrome and pulmonary embolism) and two in the arterial circulation (myocardial infarction and peripheral artery thrombosis).

The incidence of events during follow-up was independent of the clinical manifestations at diagnosis. As shown in Fig. 2, the cumulative incidence of events after 10 years was 45%, 47% and 37% for venous, arterial and pregnancy morbidity as a qualifying event, respectively (P = ns).

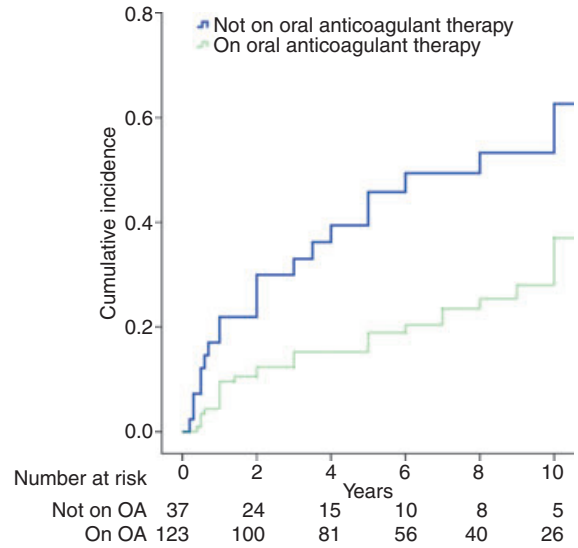

Fig. 3. Cumulative incidence of thromboembolic events in patients with antiphospholipid syndrome (APS) and triple laboratory positivity according to treatment with oral anticoagulants during follow-up (OA, oral anticoagulants).

Oral anticoagulant treatment and thromboembolic events in the follow-up period

Out of 160 patients, 123 received long-term oral anticoagulant treatment during the entire follow-up period, whereas 37 did not (13 did not take anticoagulants and 24 took aspirin alone). Subsequent thromboembolism occurred in 36 (16 VTE and 20 ATE) out of 123 anticoagulated patients [median International normalized ratio (INR) at the time of event 2.3], and in 19 (9 VTE and 10 ATE) out of the 37 non-anticoagulated patients. The cumulative incidence of events was significantly higher in patients not taking oral anticoagulants (P = 0.002, Fig. 3). Multivariate Cox regression analysis showed that the oral anticoagulant status was the only predictor of thromboembolic events during follow-up (HR = 2.4 95%CI 1.3–4.1; P < 0.003). Only one thromboembolic event recurred amongst the patients with a predominant IgM isotype. This was an acute myocardial infarction in a 64-year-old female receiving anticoagulant treatment.

Major bleeding and death

There were eight major bleeding events – seven in patients on oral anticoagulants (0.8% patient/years) – and 10 deaths during follow-up (0.8% patients/years; seven were cardiovascular and three were non-cardiovascular deaths). Among the cardiovascular deaths, two were as a result of cerebral bleeds (one patient was not anticoagulated), five were sudden deaths (one in a patient with dilated cardiomyopathy secondary to ischemic heart disease). Among non-cardiovascular deaths, one resulted from an acute respiratory failure and two patients died of neoplastic disease.

Discussion

Clinical studies on APS have generally enrolled patients with different laboratory profiles and consequently different risk profiles for VTE and ATE [6,7,10]. It is now recognized that patients positive for more than one laboratory test [11,12], and particularly those with three positive tests, are at higher risk of thrombosis [10,13,22]. To our knowledge, this is the first large-scale study looking at the clinical course of a homogenous cohort of APS patients with full positive laboratory profile. We applied rigid enrolment criteria: patients entered the study only if they had both clinical features of APS and positivity in the three laboratory tests (LA, aCL and aß2GPI). The laboratory data were confirmed by a reference laboratory. Importantly, data were confirmed in all patients and tests were repeated in recurrent cases or each year in the majority of patients. Those patients with aCL values of < 40 Units were excluded. This restriction criterion was introduced on the basis of the results of a previous clinical study showing a strong correlation between aCL titer and thrombosis [23]. We also recently observed that 40 units GPL rather than the 99th percentile is a valid cut-off to identify patients at risk of thrombosis [24]. The number of enrolled patients with obstetric complications alone was low (9.7%), highlighting that the majority of female patients with triple positivity in laboratory tests suffer from thromboembolic events [22]. This selected cohort of patients differed from the large cohort described by Cervera *et al*. [25,26] in several aspects. Less than half of the patients reported by Cervera *et al*. were LA positive. We had more male patients (29% vs. 18%) and primary APS cases (62% vs. 53%). Our data come close to the data from Cervera *et al*., if we only considered primary APS patients (female/male ratio drops to 3.5:1) [25]. The higher proportion of male patients in our cohort might underlie the higher prevalence of ATE events (43% vs. 26%) [25].

As this cohort of patients was monitored in Thrombosis Centers, we focused our attention on thromboembolic events during the follow-up period. There was a high cumulative incidence of events after 10 years (44.2%). The qualifying event at diagnosis did not predict the type of recurrence (arterial or venous). Indeed, patients with VTE, ATE or pregnancy morbidity had a similar incidence of recurrent thromboembolic events. This observation introduces the hypothesis that the full antibody profile, rather than the type of clinical onset, is more closely related to the recurrence of events. Our findings do not confirm the findings of previous studies [23,27] that have suggested that the recurrence of an event is more likely to occur in the same vascular district (i.e. venous or arterial).

The high incidence of recurrences highlights the need for adequate long-term antithrombotic treatment in high-risk patients. Oral anticoagulants are recommended after a first episode of VTE [28] to prevent recurrence [29–32]. As there is currently no evidence to support the superiority of oral anticoagulant therapy over antiplatelet therapy [15], antiplatelet drugs are generally recommended after an episode of stroke. However, in this cohort, patients treated with oral anticoagulants had significantly less VTE and ATE events. This finding encourages the use of long-term oral anticoagulant treatment not only in patients with VTE but also in patients with ATE at a stable INR between 2.0 and 3.0. This range can be increased to 3.0–4.5 after a recurrence during oral anticoagulant therapy, although the benefit of this approach has been questioned by some trials [16,17]. In some cases (particularly in ATE) adding low-dose aspirin to standard anticoagulation may be a valid option. The finding of a low incidence of major bleeding in our series also supports this contention. Alternatively, the use of long-term low-molecular weight heparin has also been proposed [33], but experience with this is limited. Other approaches that are aimed at the reduction of antiphospholipid titer in selected patients by using rituximab [34] or transplanting hematopoietic stem cells [35] are still under investigation.

In conclusion, in the present study – which to our knowledge is the first to examine patients that are positive for APS as determined by three separate tests – provides information on patient characteristics and on the clinical course of the disease. Triple positive patients have a substantial risk of recurrent thromboembolic events. These recurrences not only influence morbidity but are also the most common cause of death in APS [25]. Oral anticoagulant therapy significantly reduces recurrent thromboembolism, although it might prove insufficient in some cases. Therefore, there is need to now focus on determining prognostic factors and better treatment strategies for this group of high-risk patients.

Addendum

V. Pengo, A. Ruffatti and S. Iliceto participated in the planning, data collection, data analysis and writing of the manuscript. C. Legnani, P. Gresele, D. Barcellona, N. Erba, S. Testa and F. Marongiu participated in data collection, data analysis and writing of the manuscript. E. Bison and A. Banzato performed laboratory tests in reference laboratory, interpreted data and participated in writing of the manuscript. G. Denas and S. Padayattil Jose interpreted data, conducted the statistical analysis and participated in writing of the manuscript.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

References

1 Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, De Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; **4**: 295–306.

2 Pengo V, Biasiolo A, Gresele P, Marongiu F, Erba N, Veschi F, Ghirarduzzi A, de Candia E, Montaruli B, Testa S, Barcellona D, Tripodi A. Survey of lupus anticoagulant diagnosis by central evaluation of positive plasma samples. *J Thromb Haemost* 2007; **5**: 925–30.

3 Pengo V, Biasiolo A, Bison E, Chantarangkul V, Tripodi A. Antiphospholipid antibody ELISAs: survey on the performance of clinical laboratories assessed by using lyophilized affinity-purified IgG with

anticardiolipin and anti-beta2-Glycoprotein I activity. *Thromb Res* 2007; **120**: 127–33.
4 Pengo V, Biasiolo A. The risk of overdiagnosis of antiphospholipid antibody syndrome. *Thromb Haemost* 2001; **86**: 933.
5 de Laat B, Derksen RH, Urbanus RT, de Groot PG. IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. *Blood* 2005; **105**: 1540–5.
6 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**: 1827–32.
7 Galli M, Luciani D, Bertolini G, Barbui T. Anti-beta 2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. *Blood* 2003; **102**: 2717–23.
8 Detkova D, Gil-Aguado A, Lavilla P, Cuesta MV, Fontaan G, Pascual-Salcedo D. Do antibodies to beta2-glycoprotein 1 contribute to the better characterization of the antiphospholipid syndrome? *Lupus* 1999; **8**: 430–8.
9 Lee EY, Lee CK, Lee TH, Chung SM, Kim SH, Cho YS, Yoo B, Moon HB. Does the anti-beta2-glycoprotein I antibody provide additional information in patients with thrombosis? *Thromb Res* 2003; **111**: 29–32.
10 Pengo V, Biasiolo A, Pegoraro C, Cucchini U, Noventa F, Iliceto S. Antibody profiles for the diagnosis of antiphospholipid syndrome. *Thromb Haemost* 2005; **93**: 147–52.
11 Sailer T, Zoghlami C, Kurz C, Rumpold H, Quehenberger P, Panzer S, Pabinger I. Anti-beta2-glycoprotein I antibodies are associated with pregnancy loss in women with the lupus anticoagulant. *Thromb Haemost* 2006; **95**: 796–801.
12 Zoghlami-Rintelen C, Vormittag R, Sailer T, Lehr S, Rumpold H, Quehenberger P, Male C, Pabinger I. The presence of IgG antibodies against beta2-glycoprotein I predicts the risk of thrombosis in patients with the lupus anticoagulant. *J Thromb Haemost* 2005; **3**: 1160–5.
13 Ruffatti A, Tonello M, Cavazzana A, Bagatella P, Pengo V. Laboratory classification categories and pregnancy outcome in patients with primary antiphospholipid syndrome prescribed antithrombotic therapy. *Thromb Res* 2009; **123**: 482–7.
14 Khamashta MA, Cuadrado MJ, Mujic F, Taub NA, Hunt BJ, Hughes GRV. The management of thrombosis in the antiphospholipid-antibody syndrome. *N Engl J Med* 1995; **332**: 993–7.
15 Levine SR, Brey RL, Tilley BC, Thompson JL, Sacco RL, Sciacca RR, Murphy A, Lu Y, Costigan TM, Rhine C, Levin B, Triplett DA, Mohr JP. Antiphospholipid antibodies and subsequent thrombo-occlusive events in patients with ischemic stroke. *JAMA* 2004; **291**: 576–84.
16 Crowther MA, Ginsberg JS, Julian J, Denburg J, Hirsh J, Douketis J, Laskin C, Fortin P, Anderson D, Kearon C, Clarke A, Geerts W, Forgie M, Green D, Costantini L, Yacura W, Wilson S, Gent M, Kovacs MJ. A comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. *N Engl J Med* 2003; **349**: 1133–8.
17 Finazzi G, Marchioli R, Brancaccio V, Schinco P, Wisloff F, Musial J, Baudo F, Berrettini M, Testa S, D'Angelo A, Tognoni G, Barbui T. A randomized clinical trial of high-intensity warfarin vs. conventional antithrombotic therapy for the prevention of recurrent thrombosis in patients with the antiphospholipid syndrome (WAPS). *J Thromb Haemost* 2005; **3**: 848–53.
18 Asherson RA, Cervera R, de Groot PG, Erkan D, Boffa MC, Piette JC, Khamashta MA, Shoenfeld Y. Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus* 2003; **12**: 530–4.
19 Pengo V, Biasiolo A, Fior MG. Autoimmune antiphospholipid antibodies are directed against a cryptic epitope expressed when b2-glycoprotein-I is bound to a suitable surface. *Thromb Haemost* 1995; **73**: 29–34.
20 Tincani A, Allegri F, Balestrieri G, Reber G, Sanmarco M, Meroni P, Boffa MC. Minimal requirements for antiphospholipid antibodies ELISAs proposed by the European Forum on antiphospholipid antibodies. *Thromb Res* 2004; **114**: 553–8.
21 Reber G, Tincani A, Sanmarco M, de Moerloose P, Boffa MC. Proposal for the measurements of anti-b2-glycoprotein I antibodies. Standardization group of the European forum on antiphospholipid antibodies. *J Thromb Haemost* 2004; **2**: 1860–2.
22 Ruffatti A, Tonello M, Del RossT, Cavazzana A, Grava C, Noventa F, Tona F, Iliceto S, Pengo V. Antibody profile and clinical course in primary antiphospholipid syndrome with pregnancy morbidity. *Thromb Haemost* 2006; **96**: 337–41.
23 Finazzi G, Brancaccio V, Moia M, Ciaverella N, Mazzucconi MG, Schinco PC, Ruggeri M, Pogliani EM, Gamba G, Rossi E, Baudo F, Manotti C, D'Angelo A, Palareti G, De Stefano V, Berrettini M, Barbui T. 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**: 530–6.
24 Ruffatti A, Olivieri S, Tonello M, Bortolati M, Bison E, Salvan E, Facchinetti M, Pengo V. Influence of different IgG anticardiolipin antibody cut-off values on antiphospholipid syndrome classification. *J Thromb Haemost* 2008; **6**: 1693–6.
25 Cervera R, Piette JC, Font J, Khamashta MA, Shoenfeld Y, Camps MT, Jacobsen S, Lakos G, Tincani A, Kontopoulou-Griva I, Galeazzi M, Meroni PL, Derksen RH, de GrootPG, Gromnica-Ihle E, Baleva M, Mosca M, Bombardieri S, Houssiau F, Gris JC, *et al*. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum* 2002; **46**: 1019–27.
26 Cervera R, Khamashta MA, Shoenfeld Y, Camps MT, Jacobsen S, Kiss E, Zeher MM, Tincani A, Kontopoulou-Griva I, Galeazzi M, Bellisai F, Meroni PL, Derksen RH, de GrootPG, Gromnica-Ihle E, Baleva M, Mosca M, Bombardieri S, Houssiau F, Gris JC, *et al*. Morbidity and mortality in the antiphospholipid syndrome during a 5-year period: a multicenter prospective study of 1,000 patients. *Ann Rheum Dis* 2009; **68**: 1428–32.
27 Rosove MH, Brewer PM. Antiphospholipid thrombosis: clinical course after the first thrombotic event in 70 patients. *Ann Intern Med* 1992; **117**: 303–8.
28 Buller HR, Agnelli G, Hull RD, Hyers TM, Prins MH, Raskob GE. Antithrombotic therapy for venous thromboembolic disease: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004; **126**: 401S–28S.
29 Lim W, Crowther MA, Eikelboom JW. Management of antiphospholipid antibody syndrome: a systematic review. *JAMA* 2006; **295**: 1050–7.
30 Schulman S, Svenungsson E, Granqvist S. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. *Am J Med* 1998; **104**: 332–8.
31 Kearon C, Gent M, Hirsh J, Weitz J, Kovacs MJ, Anderson DR, Turpie AG, Green D, Ginsberg JS, Wells P, MacKinnon B, Julian JA. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. *N Engl J Med* 1999; **340**: 901–7.
32 Kearon C, Ginsberg JS, Kovacs MJ, Anderson DR, Wells P, Julian JA, MacKinnon B, Weitz JI, Crowther MA, Dolan S, Turpie AG, Geerts W, Solymoss S, van Nguyen P, Demers C, Kahn SR, Kassis J, Rodger M, Hambleton J, Gent M. Comparison of low intensity warfarin therapy with conventional-intensity warfarin therapy for long-term prevention of recurrent venous thromboembolism. *N Engl J Med* 2003; **349**: 631–9.
33 Dentali F, Manfredi E, Crowther M, Ageno W. Long-duration therapy with low molecular weight heparin in patients with antiphospholipid antibody syndrome resistant to warfarin therapy. *J Thromb Haemost* 2005; **3**: 2121–3.
34 Rubenstein E, Arkfeld DG, Metyas S, Shinada S, Ehresmann S, Liebman HA. Rituximab treatment for resistant antiphospholipid syndrome. *J Rheumatol* 2006; **33**: 355–7.
35 Statkute L, Traynor A, Oyama Y, Yaung K, Verda L, Krosnjar N, Burt RK. Antiphospholipid syndrome in patients with systemic lupus erythematosus treated by autologous hematopoietic stem cell transplantation. *Blood* 2005; **106**: 2700–9.