



Original article

Persistent thrombocytopenia predicts poor long-term survival in patients with antiphospholipid syndrome: a 38-year follow-up study

José Pardos-Gea ¹, Joana R. Marques-Soares¹, Segundo Buján¹, José Ordi-Ros¹ and Jaume Alijotas-Reig ¹

Abstract

Objectives. To investigate the impact of thrombocytopenia on survival in patients with APS.

Methods. Thrombocytopenia and other predictors of outcome were retrospectively evaluated in an aPL-positive and APS cohort with 38-year follow-up (1980–2018). Thrombocytopenia was defined as $<150 \times 10^9$ platelets/l. Hazard ratios (HR) of mortality were calculated using Cox-regression models.

Results. Among 114 patients, 64% had primary APS, 25% secondary APS and 10% asymptomatic aPL. Mean follow-up was 19 (range 5–38) years. ANA [hazard ratio (HR) 1.8, 95% CI 0.8, 3.6, $P=0.10$], arterial thrombotic events (HR 7.0, 95% CI 1.4, 3.5, $P=0.016$), myocardial infarction (HR 8.3, 95% CI 1.1, 59, $P=0.03$), intracardiac thrombosis (HR 17, 95% CI 1, 279, $P=0.04$) and thrombocytopenia (HR 2.9, 95% CI 1.4, 6.1, $P=0.004$) were risk factors for all-cause mortality, but in multivariate analysis only thrombocytopenia (HR 2.7, 95% CI 1.3, 6.0, $P=0.01$) remained significant. Persistent (HR 4.4, 95% CI 2.1, 9.2, $P=0.001$) and low–moderate thrombocytopenia (HR 2.8, 95% CI 1.2, 6.4, $P=0.01$) were associated with a significant increase in mortality compared with acute (HR 1.6, 95% CI 0.5, 5.3, $P=0.40$) and severe (HR 2.1, 95% CI 0.5, 9.2, $P=0.30$) forms. APS patients with vs without thrombocytopenia were more frequently male (58 vs 24%, $P=0.001$) with arterial thrombosis (55 vs 32%, $P=0.04$), LA positivity (100 vs 87%, $P=0.04$), type I aPL profile (89% vs 71%, $P=0.05$) and anticoagulant treatment (89 vs 63%, $P=0.01$). Thrombosis caused 13% of deaths in thrombocytopenic patients and 1% in those without ($P=0.01$).

Conclusion. Thrombocytopenia is an aPL-related manifestation that identifies patients with severe disease phenotype and high thrombotic risk. Persistent low–moderate thrombocytopenia is associated with a reduced long-term survival.

Key words: antiphospholipid syndrome, thrombocytopenia, platelet count, anti-platelet antibodies, systemic lupus erythematosus, mortality, aPL profile.

Rheumatology key messages

- Persistent low–moderate thrombocytopenia identifies a subgroup of APS patients at high risk of thrombosis.
- Persistent low–moderate thrombocytopenia is associated with the poor long-term survival of APS patients.
- Persistent low–moderate thrombocytopenia predicts thrombosis-related mortality in APS.

Introduction

Thrombocytopenia is a prevalent manifestation associated with the presence of aPL antibodies or APS, with

rates ranging from 15% to 53% [1, 2], but it is currently not included as a classificatory criterion in the definition of APS [3]. Episodes of severe thrombocytopenia ($<50 \times 10^9/l$) associated with APS and developing from an immune-mediated (aPL-related, secondary immune thrombocytopenia, anti-platelet or heparin-related auto-antibodies) or thrombotic [catastrophic APS (CAPS), HELLP (haemolysis, elevated liver enzymes, low platelet count) syndrome] micro-angiopathy have been described in some patients, with potential clinical consequences requiring prompt treatment. However, in most cases, thrombocytopenia in APS is mild to moderate

¹Department of Internal Medicine, Autoimmune Diseases Unit, Universidad Autonoma de Barcelona, Vall d'Hebron University Hospital, Barcelona, Spain

Submitted 20 December 2020; accepted 25 May 2021

Correspondence to: José Pardos-Gea, Department of Internal Medicine, Autoimmune Diseases Unit, Universidad Autonoma de Barcelona, Vall d'Hebrón Research Institute (VHIR), Vall d'Hebrón University Hospital, Barcelona, Spain. E-mail: jpardos@vhebron.net

($50\text{--}150 \times 10^9$ platelets/l), usually detected at diagnosis, and stable over the long-term.

The association of thrombocytopenia with the clinical manifestations of APS has been investigated in a few studies [4–13], which found no evidence of a relationship to bleeding, but the clinical relevance of thrombosis in APS remains a matter of controversy. In most studies, thrombocytopenia correlated with high-risk aPL antibody profiles (LA, double-triple positivity) and higher APS risk scores [abbreviated global anti-phospholipid syndrome score (aGAPSS), aPL] [4, 10, 12], although this has not been uniformly demonstrated [7, 9, 11, 13]. In addition, higher thrombotic rates, and specifically arterial thrombosis, have been described in a subset of APS patients with mild to moderate thrombocytopenia [5, 8]. Thus, while thrombocytopenia in APS seems to result in a more severe phenotype of the disease, supporting evidence is thus far lacking. Therefore, the aim of this study was to assess thrombocytopenia and other clinical variables as potential predictors of outcome in patients with APS. For this purpose, we analysed the data of a large and well-defined cohort of APS patients followed at our centre over a period of 38 years.

Methods

Study cohort

The cohort of 114 patients had been diagnosed and followed at our center from 1980 to 2018. Patients were diagnosed either with primary APS (PAPS) and secondary associated APS (SAPS) according to the APS classification criteria of the respective time [3, 14, 15], or with non-criteria-positive aPL. Patients with pure obstetric APS without vascular thrombosis were excluded. Follow-up was conducted on an outpatient basis at our centre every 3–6 months and by the same medical team.

Baseline demographics and the medical history of the patients were assessed by interview and/or review of the historical documentation. The decade in which the patient was diagnosed was documented. The presence of classical atherosclerotic risk factors (hypertension, diabetes, dyslipidaemia, age >65 years, smoking) and venous thrombotic risk factors (obesity, immobilization, neoplasm, hormonal treatment) was also recorded. According to The National Code on Clinical Trials, ethical approval is not necessary for retrospective studies and was therefore not sought for this study.

Definitions

The occurrence of arterial-, venous- or micro-thrombotic events or pregnancy morbidity was reviewed. Deep venous thrombosis and pulmonary embolism were diagnosed according to appropriate methods (Doppler echography, CT angiography, ventilation/perfusion scan). Acute ischaemic stroke was diagnosed based on clinical (>24 h of focal neurological symptoms) and imaging (brain lesion on CT, magnetic resonance,

arterial Doppler US) features. Acute coronary syndrome with clinical and electrocardiographic features occurring together with an elevation of myocardial enzymes was classified as myocardial infarction. Peripheral artery embolism was defined as ischaemic symptoms detected as thrombosis by arteriography or arterial Doppler US. Micro-thrombotic aPL-associated disease included livedoid vasculopathy (painful ulcerations with capillary thrombosis), digital ischaemic lesions, nephrogenic hypertension (renal failure, glomerular micro-thrombosis) and CAPS (simultaneous micro-thrombotic involvement of more than three organs in an aPL or APS patient) [16]. Pulmonary capillaritis or aPL-associated alveolar haemorrhage was diagnosed by CT scan and bronchoscopy based on histological features. Pregnancy complications were defined following established APS criteria: one or more unexplained deaths at or beyond the 10th week of gestation, with normal foetal morphology by US or direct examination of the fetus, or one or more premature (before the 34th week of gestation) births in a mother with eclampsia or severe pre-eclampsia, or recognized features of placental insufficiency, or three or more unexplained consecutive spontaneous abortions before the 10th week of gestation.

Echocardiography was performed in all patients at the initial assessment to detect APS valvulopathy (Libman-Sacks valvular thickening and vegetations), pulmonary hypertension, intra-atrial thrombosis and/or myocardial pathology (diastolic or systolic failure). Non-criteria neurologic manifestations such as epilepsy, migraine, cognitive dysfunction and chorea were defined on clinical grounds. Dermatologic manifestations such as livedo and RP were documented.

Laboratory determinations were performed at diagnosis and every 3–6 months during follow-up. Although the consensus regarding laboratory values changed over the course of the follow-up period, the data were considered valid and included in our analysis. The presence of positive ANA, anti-DNA antibodies and hypocomplementemia (low C3 and/or C4 levels) was also evaluated. In this study, patients were included in the thrombocytopenia subgroup if they exhibited persistent thrombocytopenia, defined as platelet count of $<150 \times 10^9/l$ in >90% of the determinations along all follow-up, or acute thrombocytopenia, defined as a sudden decrease in the platelet count to below this level sustained (>90% of determinations) during a period of days or weeks. The mean platelet count of each patient was calculated based on trimestral or semestral determinations during every year of follow-up. Anti-platelet antibodies were measured by ELISA and/or flow cytometry techniques and in accordance with clinical criteria.

LA was determined according to international consensus criteria [17, 18]. LA was assessed in all our patients in stable clinical situation and without any anticoagulant treatment or heparin bridging therapy (last dose of low molecular heparin 12 h before) withdrawing temporarily oral anticoagulants. We performed DRVVT and aPTT Silica clotting time in all patients. DRVVT and aPTT

Silica screening tests were considered positive if cut-off ratios were above the 99th percentile of the distribution of the local healthy population, that is >1.20. In those cases, a confirmatory test was performed increasing the concentration of phospholipids to check the correction of clotting times considering positive cut-off ratios compared with local controls >1.20. aCL IgG or IgM was measured by standardized ELISA or chemiluminescence assay depending on the study period, with positivity defined as >40 GPL/MPL or >20 U/ml, respectively. Anti-beta2-GPI (Anti-β2GPI) IgG and IgM isotypes were measured in an ELISA, with positivity defined as absorbance values above the 99th percentile or chemiluminescence assay values >20 U/ml. For all positive aPL detections, positivity was confirmed on at least two occasions separated by 12 weeks.

The thrombotic risk associated with APS was stratified based on the serological aPL profile (type I: double or triple positivity; type II: single positivity) and the aGAPSS according to a literature description [19]. Death and its causes were assessed using medical and pathological certificates as needed.

Statistics

Quantitative variables are presented as the mean (s.d.), and categorical variables as a percent. Inferential comparative tests (chi-squared, Student's *t*-test, Mann-Whitney test, analysis of variance) were used in comparisons between groups. The number of deaths at the final follow-up was calculated as a percent. The overall survival time was defined as the interval from the time of diagnosis until death by any cause (all-cause mortality), and the APS-specific survival time until death due to thrombotic complication (APS thrombotic mortality). A standardized mortality rate (SMR) was used to compare the mortality risk of patients with APS vs that of the general population. The SMR was calculated as observed deaths (O)/expected deaths (E). Expected deaths were calculated using the annual sex- and age-specific mortality rates per 1000 individuals in the Spanish population. The 95% CI was calculated as (SMR - 1.96 × √O/E, SMR + 1.96 × √O/E).

A Cox proportional hazard model was used to determine the associations of all-cause and thrombotic APS-specific mortality (dependent variables) with the different independent variables (sex, age, decade of diagnosis, PAPS or SAPS, presence and type of thrombosis, arterial and venous thrombotic risk factors, aPL profile, type and subtype aPL, prolonged aPTT, aGAPSS, non-criteria APS manifestations, thrombocytopenia, ANA, anti-DNA antibodies, hypocomplementemia, anti-thrombotic treatment and immunosuppressants). Hazard ratios (HRs) and 95% CIs were computed independently for each predictor in the univariate analysis and all variables that reached a *P*-value <0.10 were included in the multivariate assessment. Multivariate Cox regression model using backward stepwise approach was used and a *P*-value <0.05 was considered significant.

Statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA).

Results

Baseline population

The mean (S.D.) follow-up time of our cohort of 114 patients was 19 (7) years (range 5–38 years), corresponding to 2202 person-years. The mean age of the patients was 38 (15) years and 66% (76/114) were

TABLE 1 Baseline characteristics

Total cohort, <i>n</i>	114
Age at diagnosis, years	38 (15)
Decade of diagnosis	
1980–1989	19 (16)
1990–1999	54 (47)
2000–2009	41 (36)
Follow-up time, years	19 (7)
Female	76 (66)
PAPS	73 (64)
SLE-SAPS	29 (25)
aPL non-criteria manifestations	12 (10)
Thrombosis, any	100 (87)
Arterial	44 (38)
Venous	58 (51)
Microvascular	13 (11)
Pregnancy pathology	36 (14)
>1 APS clinical criteria	43 (37)
aGAPSS score	11 (6)
Non-criteria clinical manifestations	
Migraine	34 (29)
Livedo	23 (20)
RP	13 (11)
Seizure	8 (7)
APS heart valvulopathy	42 (36)
aPL positivity	
LA	103 (90)
aCL	84 (73)
Anti-β2GPI	70/97 (72)
aPL profile	
Type I	86/97 (88)
Type IIa	18 (15)
Type IIb	8 (7)
Type IIc	2/97 (2)
ANA	59 (51)
Anti-DNA	24 (21)
Thrombocytopenia	29 (25)
Treatment	
Anticoagulant	80 (70)
Antiplatelet	42 (36)
CS	33 (28)
Immunosuppressants	20 (17)
Deaths	35 (30)

Data are presented as means (s.d.) or numbers (percentage). PAPS: primary APS; SAPS: secondary APS; aGAPSS: abbreviated global anti-phospholipid syndrome score; anti-β2GPI: anti-beta2-GPI; RP: Raynaud phenomenon; LA: lupus anticoagulant; aCL: anticardiolipin; CS: Corticoids.

female (Table 1). PAPS was diagnosed in 64% (73/114) and SAPS in 25% (29/114), while 10% (12/114) were aPL-positive but without classificatory manifestations. Thrombotic events occurred in 87% of the patients (100/114), 51% of which were venous, 38% arterial and 11% microvascular. Pregnancy APS manifestations developed in 47.3% (36/76) of the women in the cohort. The most frequent non-criteria APS clinical manifestations were cardiac valvulopathy (36%, 42/114 patients), migraine (29%, 34/114) and livedo reticularis (20%, 23/114). Antibody profiling revealed LA positivity in 90% (103/114), aCL positivity in 73% (84/114) and anti- β 2GPI positivity in 72% (70/97) of the patients, with a predominantly type I aPL profile in 88% (86/97) and triple positivity in 59% (58/97), with double positivity in 28% (28/97) of the cohort. Thrombocytopenia was detected in 25% (29/114) of the entire cohort, including 23 patients with the persistent form: 17 with idiopathic APS-related forms and 6 with thrombocytopenia secondary to liver cirrhosis (2 patients), splenomegaly associated with portal thrombosis cavernomatosis (1 patient), or autoimmune-based with anti-platelet autoantibodies (3 patients). The remaining six patients had acute thrombocytopenia induced by microangiopathy-CAPS (four patients), splenic lymphoma (one patient) and immune based anti-platelet antibodies (one patient).

Survival outcomes

Among the 114 patients there were 35 deaths (30%), which occurred at a mean age of 65 (8) years. The SMR of our APS cohort compared with the reference local Spanish population was 2.2 (95% CI 0.7, 5.1). Cumulative overall survival after diagnosis was 98% at 5 years, 93.8% at 10 years, 70% at 20 years and 46% at 30 years. The main causes of death were neoplasia (8/35, 23%) and infection (8/35, 23%) followed by baseline chronic diseases (6/35, 17%) and senility (5/35, 14%). Among the eight patients with APS-related mortality, five (14%) suffered a fatal thrombosis (stroke in two patients, CAPS in two patients and pulmonary embolism in one patient), two (6%) developed alveolar capillaritis and one patient (3%) had a fatal haemorrhage.

The univariate Cox proportional hazard model (Table 2) identified presence of ANA (HR 1.8, 95% CI 0.8, 3.6, $P=0.10$), arterial thrombotic events (HR 7.0, 95% CI 1.4, 3.5, $P=0.016$), myocardial infarction (HR 8.3, 95% CI 1.1, 59, $P=0.03$), intracardiac thrombosis (HR 17, 95% CI 1, 279, $P=0.04$) and thrombocytopenia (HR 2.9, 95% CI 1.4, 6.1, $P=0.004$) as all-cause mortality risk factors in the APS cohort, but in the multivariate analysis only thrombocytopenia (HR 2.7, 95% CI 1.3, 6.0, $P=0.01$) remained significant (Fig. 1A). In a sub-analysis, thrombotic APS-specific mortality occurred more often in thrombocytopenic patients (Fig. 1B) than in the rest of the cohort (HR 8.9, 95% CI 1.8, 44, $P=0.007$). In an analysis of the different types of thrombocytopenia, the Cox hazard model showed a higher rate of all-cause mortality in patients with persistent thrombocytopenia (HR 4.4, 95% CI 2.1, 9.2, $P=0.001$) than in APS patients with normal platelet counts, whereas this was not the case in patients with acute thrombocytopenia (HR 1.6, 95% CI 0.5, 5.3, $P=0.40$) (Fig. 2). An analysis of the different cut-offs of thrombocytopenia allowed patients to be divided into those with low-moderate ($50\text{--}150 \times 10^9$ platelets/l) and severe ($<50 \times 10^9$ platelets/l) forms. All-cause mortality was increased for both cut-offs of thrombocytopenia (Fig. 3) with similar HRs, although statistical significance was reached in low-moderate thrombocytopenia (HR 2.8, 95% CI 1.2, 6.4, $P=0.01$) compared with severe thrombocytopenia (HR 2.1, 95% CI 0.5, 9.2, $P=0.30$).

Differential characteristics of APS patients with thrombocytopenia

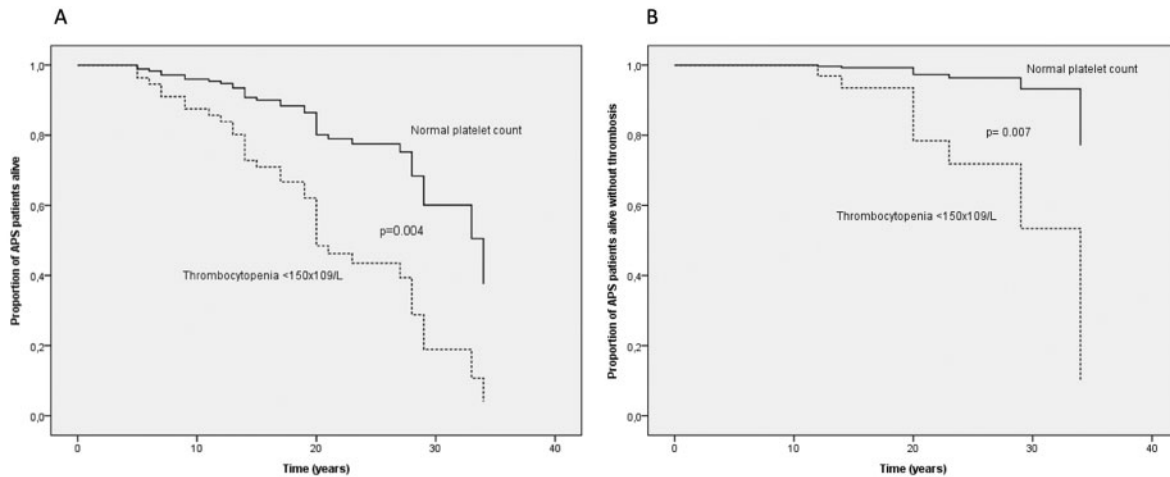
A comparative analysis of the subgroup of patients with thrombocytopenia showed differences in their clinical characteristics (Table 3). The mean platelet count of these patients was $112 (52) \times 10^9/l$ compared with $230 (59) \times 10^9/l$ ($P=0.0001$) in patients without thrombocytopenia. APS patients with thrombocytopenia were more frequently male (58 vs 24%, $P=0.001$) and had higher rates of arterial thromboembolic events (55 vs 32%, $P=0.04$), whereas there were no significant differences in either arterial thrombotic risk factors ($P=0.57$) or the presence of primary/secondary APS ($P=0.47$).

TABLE 2 Predictors of all-cause mortality in our APS cohort: univariate and multivariate analysis

Baseline variable	HR (95% CI)	P-value	aHR (95% CI) ^a	P-value
ANA	1.8 (0.8, 3.6)	0.10	2.2 (0.9, 4.0)	ns
Arterial thrombotic event, any	7.0 (1.4, 35)	0.01	1.5 (0.6, 3.5)	ns
Myocardial infarct	8.3 (1, 59)	0.03	1.3 (0.4, 3.8)	ns
Intracardiac thrombosis	17 (1, 279)	0.04	2.9 (0.3, 24)	ns
Thrombocytopenia	2.9 (1.4, 6.1)	0.004	2.7 (1.3, 6.0)	0.01

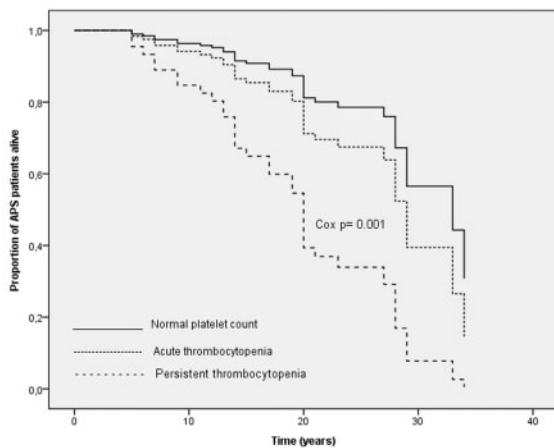
^aCox proportional hazard model of overall survival adjusted (aHR) with sex, age and decade of APS diagnosis, PAPS or SAPS, presence and type of thrombosis, arterial and venous thrombotic risk factors, aPL profile, type and subtype of aPL, aGAPSS score, APS non-criteria manifestations, thrombocytopenia, ANA, anti-DNA, hypocomplementemia, antithrombotic treatment and immunosuppressants. ns $P < 0.05$. HR: hazard ratio; aHR: adjusted hazard ratio; PAPS: primary APS; SAPS: secondary APS; aGAPSS: abbreviated global anti-phospholipid syndrome score; ns: not significant.

Fig. 1 Mortality according to the presence of thrombocytopenia



All-cause (A) and thrombotic APS-specific (B) mortality according to the presence of thrombocytopenia at any time in the disease.

Fig. 2 Cox proportional hazard model of all-cause mortality in APS patients according to the type of thrombocytopenia during the disease

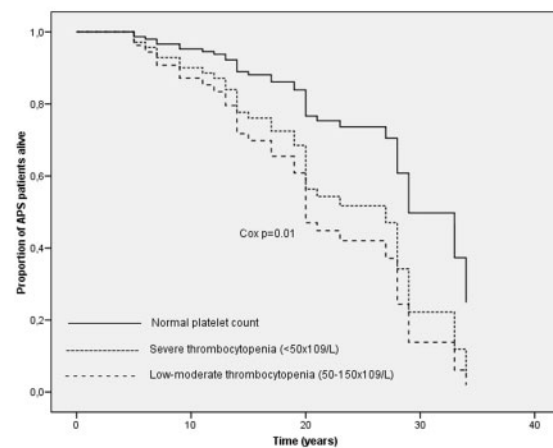


Persistent (dashed line), acute (dotted line) and without thrombocytopenia (solid line).

In the laboratory evaluation of APS patients with and without thrombocytopenia, higher rates of LA positivity (100 vs 87%, $P=0.04$) and a type I aPL profile (89 vs 71%, $P=0.05$) were determined in the former. The differences in the frequency of aCL (79 vs 71%, $P=0.42$) and anti- β 2GPI (62 vs 61%, $P=0.82$) positivity, the aGAPSS (12 vs 10 points, $P=0.15$), and a triple-positive profile (65% vs 45%, $P=0.09$) were not significant.

The percentage of patients under anti-coagulant treatment was significantly higher in patients with thrombocytopenia compared with those APS with normal platelets (89 vs 63%, $P=0.01$), whereas anti-platelet agents did not differ between groups (27 vs 40%, $P=0.18$). During the study period, 62% of patients with

Fig. 3 Cox proportional hazard model of all-cause mortality in APS patients according to the type of thrombocytopenia during the disease



Low-moderate (dashed line), severe (dotted line) and without thrombocytopenia (solid line).

thrombocytopenia (18/29) and 20% (17/85) of those without died ($P < 0.001$). A sub-analysis of the causes of death showed that thrombosis accounted for 13% of the fatal events in the group with thrombocytopenia but only 1% in the group without ($P=0.01$); the other identified causes of death did not differ between the two groups.

Discussion

Current knowledge on the long-term prognosis and survival of APS patients is limited. The results of available APS cohort studies, with a maximum of 10–15 years of

TABLE 3 Comparative characteristics of APS subgroups with and without thrombocytopenia

	Thrombocytopenia (n = 29)	Without thrombocytopenia (n = 85)	P-value
Age, years	41 (16)	37 (14)	0.21
Male, years	17 (58)	21 (24)	0.001
PAPS	21 (72)	52 (61)	
SAPS	7 (24)	22 (25)	0.47
Arterial thrombosis	16 (55)	28 (32)	0.04
Venous thrombosis	19 (65)	39 (45)	0.06
Microvascular thrombosis	5 (17)	9 (10)	0.34
Arterial thrombosis risk factors	14 (48)	36 (42)	0.57
Pregnancy pathology	5 (41)	31 (49)	0.63
Non-criteria manifestations	18 (62)	49 (59)	0.82
aGAPSS score	12 (3)	10 (3)	0.15
LA	29 (100)	74 (87)	0.04
aCL	23 (79)	61 (71)	0.42
Anti- β 2GPI	18 (62)	52 (61)	0.82
Triple positive	19 (65)	39 (45)	0.09
Type I	26 (89)	60 (71)	0.05
Platelet count, $\times 10^9/l$	112 (52)	230 (59)	0.001
Anticoagulant	26 (89)	54 (63)	0.01
Antiplatelet	8 (27)	34 (40)	0.18
Deaths	18 (62)	17 (20)	0.001
Thrombosis	4 (13)	1 (1)	0.01
Haemorrhage	1 (3)	–	0.21
Pulmonary capillaritis	1 (3)	1 (1)	0.14
Infection	4 (13)	4 (4)	0.12
Neoplasia	4 (13)	4 (4)	0.12

Data are presented as means (s.d.) or numbers (percentage). PAPS: primary APS; SAPS: secondary APS; aGAPSS: abbreviated global anti-phospholipid syndrome score; anti- β 2GPI: anti-beta2-GPI; LA: lupus anticoagulant; aCL: anticardiolipin.

follow-up, are contradictory, as they report mortality rates similar to or higher than those of the local reference populations [2, 20]. Our study, with a much longer follow-up of 38 years, found an increased SMR of our APS patients compared with the local Spanish reference population, although not statistically significant. The identification of predictors of mortality in APS is essential to improve the clinical management of these patients. Studies assessing morbidity in APS patients have identified chronic disability in 20% and demonstrated that it predicted APS-related mortality [21, 22]. Arterial thrombotic events in patients with both APS and SLE are also associated with a worse prognosis [23, 24]. Nevertheless, the largest international APS cohort [2] failed to detect any parameter with prognostic significance for mortality.

In our study, the univariate analysis identified arterial thrombotic events, myocardial infarction and intracardiac thrombosis as significant risk factors of mortality, thus pointing to ischaemic processes in the arterial system as a leading cause of death in APS. However, in the multivariate analysis those risk variables were dependent on thrombocytopenia. According to this result, persistent low-moderate aPL-associated thrombocytopenia is associated with a poor long-term survival of patients with APS. A limited number of studies have evaluated prognostic factors of mortality in APS, and fewer still have focused on thrombocytopenia. In the

first reports including SLE patients [25, 26], the authors concluded that thrombocytopenia ($<150 \times 10^9$ platelets/l) at diagnosis or any time during the disease increased mortality; after 10 years, 67% of the SLE patients with thrombocytopenia were still alive compared with 83% of those without. However, those early studies did not include data on the presence of APS or the specific causes of thrombocytopenia. Drenkard *et al.* [27] followed a cohort of SLE patients (including 412 patients with SAPS) over 10 years and found that arterial thrombosis, APS diagnosis and thrombocytopenia ($<100 \times 10^9$ platelets/l) increased mortality. Specifically, they found that HR for death was three times higher for SLE patients with thrombocytopenia (95% CI 1.7, 5.3, $P=0.001$), a quantitative risk close to that determined in our study. A relevant limitation was the lack of information on the specific causes of thrombocytopenia. In 2005, To and Petri [28] reported that the cluster of SLE patients with aCL and LA was the only one associated with vascular thrombosis and cerebrovascular events. That finding was later confirmed by Artim-Esen *et al.* [5], who evaluated a large cohort of SLE patients. Patients with associated aCL/LA had the highest rate of thrombocytopenia (36%) and in the 20-year follow-up also the highest rates of vasculopathy and nephropathy, and a significantly reduced survival. By contrast, a more recent study [13] of PAPS and aPL carriers showed that an elevated platelet count was associated with an

increased APS-specific vascular mortality (HR 1.0, 95% CI 1.0, 1.02, $P = 0.003$); however, the platelet count was measured only once, at the time of the thrombotic event, two potential sources of bias influencing the reliability of the platelet count and, possibly, the conclusions of the study. Mortality in our subgroup of APS patients with thrombocytopenia, and especially APS-specific mortality, began to increase significantly starting from the 10th year of follow-up, which may explain why other studies with larger populations, but shorter follow-ups, were unable to identify prognostic variables related to outcome.

Unlike the persistent form, acute thrombocytopenia did not affect long-term survival in our APS cohort. In fact, for patients with severe thrombocytopenia ($<50 \times 10^9$ platelets/l), as was the case in most of those with acute forms, long-term mortality was not affected. The causes of acute thrombocytopenia included CAPS in three patients, two of whom died despite plasmapheresis, CYC and CS; septic endocarditis in one patient, who also died; splenic lymphoma in one patient, who died due to sepsis; and one patient with anti-platelet antibodies that responded to CS. It therefore seems clear that those conditions are associated with a high mortality, although overall survival could not be statistically analysed due to the limited number of cases. However, a study from the Italian Registry of APS reported a significantly higher rate of thrombotic complications in patients with platelet counts in the range of $50\text{--}100 \times 10^9$ /l than in those with a platelet counts $<50 \times 10^9$ /l [11]. Finally, it should be noted that among patients with persistent thrombocytopenia, only those with anti-platelet or immune-based forms were treated (corticoids, immunoglobulins) when clinically needed.

Our results reinforce the link between thrombocytopenia and a subgroup of high-risk APS patients. It should be noted that ours is comparable to other APS cohorts in terms of its demographics, clinical characteristics and the rate of thrombocytopenia [1, 2]. Among thrombocytopenic patients, our study identified distinctive characteristics that define a thrombotic risk profile: male patient with arterial thrombotic events, predominance of LA and a type I aPL profile, tendencies of a higher aGAPSS and triple-positivity profile, and a significantly greater requirement for anti-coagulant therapy. Other studies have reported a relationship between thrombocytopenia and arterial thrombosis and LA [5, 8, 10], a triple-positive aPL profile [4], and thrombotic prognostic scores (aGAPSS and the aPL score) [8, 12]. The predominance of males in our subgroup of APS patients with thrombocytopenia also merits comment. Since females accounted for most of the total cohort (66%), most likely due to the association of APS with SLE, it is very unlikely that a male selection bias occurred in our population with thrombocytopenia. Moreover, there were no differences in age and classical arterial thrombotic risk factors between patients with and without thrombocytopenia, nor were there differences in less frequent thrombotic risk factors such as

primary Cushing disease, chronic inflammatory processes, myelodysplastic disease or morbid obesity.

Our APS patients with thrombocytopenia died more frequently from thrombosis than did those without; in fact, this was the only cause of death that differed between the two groups and it suggests a higher prothrombotic tendency in patients with this APS phenotype. Bleeding associated with APS thrombocytopenia is rare. This was demonstrated in a study of 305 PAPS patients [4], in whom the rate of major bleedings (3%) did not differ between those with and without thrombocytopenia. In a study with SLE cases, Reveille *et al.* [25] determined that bleeding was an infrequent cause of death among those who were thrombocytopenic. These findings raise several issues regarding the management of anti-coagulants. Standardized anti-coagulant therapy is typically administered to APS patients with mild to moderate thrombocytopenia, but a platelet count $<50 \times 10^9$ /l is considered to warrant a dose correction or even the withdrawal of anti-coagulants; however, similar data for APS patients are lacking.

In APS patients, platelets play a crucial role in thrombosis. Platelet activation by aPL and the anti- $\beta 2$ GPI- $\beta 2$ GPI complex increases the expression of platelet membrane glycoproteins, especially GPIIb/IIIa [29], thus initiating platelet aggregation and thromboxane synthesis [30]. In turn, aPL-activated platelets enhance the endothelium and increase the levels of fibrinogen and tissue factor [31, 32]. Thrombocytopenia in APS has also been attributed to the presence of autoantibodies against the platelet receptors GPIIb/IIIa GPIb/IX and GPIa/IIa, thus resulting in a secondary immune thrombocytopenia [33–35]. Persistent thrombocytopenia in APS may be the product of a combination of yet not fully established mechanisms, but its association with thrombosis and thus with mortality seems clear.

Our study had several limitations. Its retrospective design may have resulted in missing data or errors in some variables as well as in group selection bias or underreporting of clinical issues in our patients. Another concern is the definition of thrombocytopenia. An APS Task Force consensus proposed a platelet count of 100×10^9 /l as the upper cut-off defining thrombocytopenia [1]. However, in our study the cut-off was 150×10^9 /l, based on the standard definition of thrombocytopenia. This higher value allowed the inclusion of a broader, more representative sample of APS patients with mild–moderate thrombocytopenia. Furthermore, the cut-off was consistent with other studies of APS [4, 7, 9, 11]. The different fluctuations in the number of platelets could give complex ascending or descending patterns occurring in the same patient that could be studied with latent trajectory models and correlate with mortality, an approach that we did not use with our cohort and could be a matter of criticism. We thought of a qualitative approximation classifying patients with thrombocytopenia and being strict in its definition to increase the reliability of our results, however using only two patterns of longitudinal platelet

levels fluctuation (stable thrombocytopenia and acute decrease from normal levels constituting acute form).

In conclusion, thrombocytopenia, a relatively common manifestation of APS and a feature of aPL-positive patients, likely identifies the subgroup with a more severe disease phenotype and a high thrombotic risk. Persistent mild–moderate thrombocytopenia is associated with a reduced long-term survival in APS and should be considered a relevant risk factor to stratify patients. It should also be taken into account in the design of studies on disease pathogenesis and therapeutic strategies, as well as in revisions to the current APS classification criteria.

Funding: No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

Disclosure statement: The authors have no conflicts of interest to declare.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- Cervera R, Tektonidou MG, Espinosa G *et al.* Task Force on Catastrophic Antiphospholipid Syndrome (APS) and Non-criteria APS Manifestations (II): thrombocytopenia and skin manifestations. *Lupus* 2011;20:174–81.
- Cervera R, Serrano R, Pons-Estel GJ *et al.*; Euro-Phospholipid Project Group (European Forum on Antiphospholipid Antibodies). Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis* 2015;74:1011–8.
- 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.
- Yelnik CM, Nguyen Y, Le Guern V, Hachulla E, Lambert M. Thrombocytopenia in primary antiphospholipid syndrome, a marker of high-risk patients? *Eur J Intern Med* 2020;74:106–7.
- Artim-Esen B, Çene E, Şahinkaya Y *et al.* Cluster analysis of autoantibodies in 852 patients with systemic lupus erythematosus from a single center. *J Rheumatol* 2014;41:1304–10.
- Krause I, Blank M, Fraser A *et al.* The association of thrombocytopenia with systemic manifestations in the antiphospholipid syndrome. *Immunobiology* 2005;210:749–54.
- Cuadrado MJ, Mujic F, Munoz E, Khamashta MA, Hughes GR. Thrombocytopenia in the antiphospholipid syndrome. *Ann Rheum Dis* 1997;56:194–6.
- Hisada R, Kato M, Sugawara E *et al.* Thrombotic risk stratification by platelet count in patients with antiphospholipid antibodies: a longitudinal study. *J Thromb Haemost* 2017;15:1782–7.
- Pontara E, Banzato A, Bison E *et al.* Thrombocytopenia in high-risk patients with antiphospholipid syndrome. *J Thromb Haemost* 2018;16:529–32.
- Demetrio Pablo R, Muñoz P, López-Hoyos M *et al.* Thrombocytopenia as a thrombotic risk factor in patients with antiphospholipid antibodies without disease criteria. *Med Clin (Barc)* 2017;148:394–400.
- Finazzi G, Brancaccio V, Moia M *et al.* Natural history and risk factors for thrombosis in 360 patients with antiphospholipid antibodies: four-year prospective study from the Italian Registry. *Am J Med* 1996;100:530–6.
- Radin M, Ugolini-Lopes MR, Sciascia S, Andrade D. Extra-criteria manifestations of antiphospholipid syndrome: risk assessment and management. *Semin Arthritis Rheum* 2018;48:117–20.
- Ames PR, Merashli M, Chis Ster I *et al.* Survival in primary antiphospholipid syndrome. A single-centre cohort study. *Thromb Haemost* 2016;2:1200–8.
- 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;42:1309–11.
- Petri M, Orbai A-M, Alarcón GS *et al.* Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–86.
- Asherson RA. The catastrophic antiphospholipid syndrome. *J Rheumatol* 1992;19:508–12.
- Pengo V, Tripodi A, Reber G *et al.*; Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost* 2009;7:1737–40.
- Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb Haemost* 1995;74:1185–90.
- Sciascia S, Sanna G, Murru V *et al.* GAPSS: the global anti-phospholipid syndrome score. *Rheumatology (Oxford)* 2013;52:1397–403.
- Duarte-García A, Pham MM, Crowson CS *et al.* The epidemiology of antiphospholipid syndrome: a population-based study. *Arthritis Rheumatol* 2019;71:1545–52.
- Erkan D, Yazici Y, Sobel R, Lockshin MD. Primary antiphospholipid syndrome: functional outcome after 10 years. *J Rheumatol* 2000;27:2817–21.
- Griksa EP, Ziakas PD, Zintzaras E, Moutsopoulos HM, Vlachoyiannopoulos PG. Morbidity, mortality, and organ

- damage in patients with antiphospholipid syndrome. *J Rheumatol* 2012;39:516–23.
- 23 Ruiz Irastorza G, Egurbide MV, Martinez-Berriotxo A, Ugalde JA, Aguirre C. Antiphospholipid antibodies predict early damage in patients with systemic lupus erythematosus. *Lupus* 2004;13:900–5.
- 24 Bayraktar UD, Erkan D, Bucciarelli S, Espinosa G, Asherson R; Catastrophic Antiphospholipid Syndrome Project Group. The clinical spectrum of catastrophic antiphospholipid syndrome in the absence and presence of lupus. *J Rheumatol* 2007;34:346–52.
- 25 Reveille JD, Bartolucci A, Alarcón GS. Prognosis in systemic lupus erythematosus. Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. *Arthritis Rheum* 1990;33:37–48.
- 26 Pistiner M, Wallace DJ, Nessim S, Metzger AL, Klinenberg JR. Lupus erythematosus in the 1980s: a survey of 570 patients. *Semin Arthritis Rheum* 1991;21:55–64.
- 27 Drenkard C, Villa AR, Alarcon-Segovia D, Perez-Vazquez ME. Influence of the antiphospholipid syndrome in the survival of patients with systemic lupus erythematosus. *J Rheumatology (Oxford)* 1994;21:1067–72.
- 28 To CH, Petri M. Is autoantibody clustering predictive of clinical subsets and damage in systemic lupus erythematosus? *Arthritis Rheum* 2005;52:4003–10.
- 29 Espinola RG, Pierangeli SS, Gharavi AE, Harris EN, Ghara AE. Hydroxychloroquine reverses platelet activation induced by human IgG antiphospholipid antibodies. *Thromb Haemost* 2002;87:518–22.
- 30 Martinuzzo ME, Maclouf J, Carreras LO, Lévy-Toledano S. Antiphospholipid antibodies enhance thrombin-induced platelet activation and thromboxane formation. *Thromb Haemostasis* 1993;70:667–71.
- 31 Lutters BC, Meijers JC, Derksen RH, Arnout J, de Groot PG. Dimers of beta 2-glycoprotein I mimic the in vitro effects of beta 2-glycoprotein I anti-beta 2-glycoprotein I antibody complexes. *J Biol Chem* 2001;276:3060–7.
- 32 Proulle V, Furie RA, Merrill-Skoloff G, Furie BC, Furie B. Platelets are required for enhanced activation of endothelium and fibrinogen in a mouse thrombosis model of APS. *Blood* 2014;124:611–22.
- 33 Galli M, Daldossi M, Barbui T. Anti-glycoprotein Ib/IX and IIb/IIIa antibodies in patients with antiphospholipid antibodies. *Thromb Haemost* 1994;71:571–5.
- 34 Godeau B, Piette J-C, Fromont P *et al.* Specific antiplatelet glycoprotein autoantibodies are associated with the thrombocytopenia of primary antiphospholipid syndrome. *Br J Haematol* 1997;98:873–9.
- 35 Macchi L, Rispoli P, Ciofani-Sanchez G *et al.* Anti-platelet antibodies in patients with systemic lupus erythematosus and the primary antiphospholipid antibody syndrome: their relationship with the observed thrombocytopenia. *Br J Haematol* 1997;98:336–41.