Gender differences in primary antiphospholipid syndrome with vascular manifestations in 433 patients from four European centres

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ABSTRACT

Objective. *Gender can influence incidence and clinical course of autoimmune diseases (ADs). Antiphospholipid syndrome (APS) is a rare AD characterised by thromboses and/or pregnancy morbidities and antiphospholipid antibodies (aPL) positivity. Our aim is to conduct a gender-oriented analysis of primary thrombotic APS (t-APS).*

Methods. *Consecutive patients diagnosed with primary t-APS, followed from 1967 to 2019 in four European Centres, were enrolled.*

Results. *The cohort included 296 women and 137 men. Median age at onset [31 (24-46) vs. 41 (29-53) years, p<0.001] was lower in females. In women, venous thromboses were more frequent while, among males, arterial events prevailed. During follow-up, 14% of patients suffered at least two relapses and this occurred especially among males (22% vs. 10%, p=0.001). No gender differences were found in the aPL profile (33% single, 24% double and 43% triple aPL positivity). Most patients had concomitant risk factors (RFs) for thrombosis: established cardiovascular RFs were represented especially among men while estrogenic exposure was the main RF in women.*

Conclusion. *Women presented mostly with venous thromboses at a younger age, while men with arterial events, later in life and suffered more recurrent events. This different frequency of arterial and venous thromboses could be attributed mainly to the presence of additional RFs rather than to biological gender-specific issues. However, some RFs are exclusive or more represented in one gender rather than the other, so assessing the link of causality between gender and manifestations of t-APS remains difficult.*

Introduction

In many rheumatic diseases, the female-to-male incidence ratio is much higher than 1.0 (up to 10.0), and menarche, menses, pregnancy, postpartum period, menopause and hormone replacement therapies can all influence the disease susceptibility (1). In general, women have stronger innate and adaptive immune responses in comparison to men (2). Females and males differ in three major biological points: the number of X chromosomes per cell, the type and quantities of sex hormones present and the occurrence of pregnancy, all of which have immunological consequences (3). X chromosome inactivation and several other genetic and epigenetic differences (4), together with microchimerism, have been used to explain the links between sex and rheumatic diseases (5). However, sex steroids are probably the most important explanatory factors for understanding sexual dimorphism in rheumatic diseases: generally, oestrogens have roles in both enhancing and inhibiting immune reactions, whereas androgens and progesterone exert suppressive effects on many immune reactions (1, 2).

In addition to the difference in disease susceptibility, also the clinical manifestations of autoimmune diseases can differ between the sexes. More severe forms of disease have been suggested to develop in males than in females for several autoimmune disorders. For instance, men with systemic lupus erythematosus had more frequently serositis, renal disease and cardiovascular events compared to women, that characterise a greater disease severity (6, 7). In male patients with systemic sclerosis, a more severe expression of disease and higher mortality has been

described (8): they presented more often diffuse cutaneous involvement, a more sever peripheral vascular involvement and interstitial lung disease (9). Finally, among patients with primary Sjögren's syndrome, males have a more severe disease with more extraglandular manifestations (10).

Antiphospholipid syndrome (APS) is a rare autoimmune disorder mediated by autoantibodies directed against phospholipid-binding proteins, that can be detected by anticardiolipin (aCL) and anti-β2 glycoprotein I (aβ2GPI) immune-assays and by the lupus anticoagulant (LA) coagulation test (11). When not associated with other diseases, APS is defined as primary (12). There are two main clinical variants of the syndrome: vascular or thrombotic (t-APS), characterised by the occurrence of thrombosis, both venous and arterial, and obstetric APS (o-APS), characterised by pregnancy morbidity (11). A rarer variant of APS, known as catastrophic APS (CAPS), is characterised by systemic microangiopathy and is observed in less than 1% of cases of APS (13). Other additional symptoms are attributed to the syndrome and include thrombocytopenia, cutaneous manifestations, APS nephropathy and central nervous system symptoms such as epilepsy and cognitive abnormalities (14, 15). Most patients display both the main manifestations (t-APS and o-APS), but some others can have purely vascular or purely obstetric manifestations.

Generally, a female predominance is observed (female:male ratio 5:1), but predominance was greater in patients with SLE (7:1) than in patients with primary APS (3.5:1) (16). Furthermore, it is to notice that these estimates consider both t-APS and o-APS, that can certainly, at least in part, explain the female predominance.

In the last decades, just few studies have investigated the clinical differences between female and male patients with t-APS, finding a female prevalence in pulmonary embolism (17) and in central nervous system involvement, especially stroke/TIA (18), and a male prevalence in mesenteric thrombosis and Budd-Chiari syndrome (18). However, these studies, due to the rarity of the condition, evaluated small cohorts of patients (49 and 68 patients respectively). In the European cohort of 1000 patients with APS, women had more frequently arthritis, livedo reticularis and migraine, all connected with the higher prevalence of SLE-related APS in females' group, whereas men had a higher incidence of myocardial infarctions, epilepsy episodes, and arterial thromboses in the lower limbs (16).

The aim of our study was, therefore, to evaluate the influence of gender on the laboratory and clinical manifestations, at diagnosis and during follow-up, of primary t-APS. We also evaluated the different prevalence of concomitant risk factors (RFs) for thrombotic events, both venous and arterial. Finally, through the help of Patients' Representatives, we tried to highlight the gender differences in subjective perception of living with APS and its impact on daily life.

Patients and methods

Patient selection

The study has a multicentre, observational and retrospective design. Four tertiary referral centres in France and Italy (Cochin Hospital, Paris, CHU Lille, CHU Charles Nicolle, Rouen and Spedali Civili, Brescia) took part in the study. The final cohort included unselected patients with a formal diagnosis of primary APS, according to Sydney criteria (19), with vascular thrombosis at onset. Women who presented with obstetric events as first aPL-related manifestation were excluded. Equivocal cases or cases that did not fulfill the classification criteria were not included as well as patients with diagnosis of a concomitant systemic autoimmune disease. The patients have been attending the referral centres between the years 1985 and 2019. The study was performed according to the principles of the Declaration of Helsinki.

Clinical and laboratory APS features

Demographic data, clinical features and laboratory findings were obtained from clinical records. For each patient, the retrieved information included "criteria" manifestations (deep vein and peripheral arterial thromboses, pulmonary embolisms, intraabdominal and myocardial infarctions and cerebrovascular accidents) as well as "extra-criteria" clinical features (heart valve lesions, aPL-associated nephropathy, livedo reticularis, neurological manifestations including seizures, cognitive dysfunction and migraine). Patients were considered to have CAPS if they presented with multiple organ involvement, simultaneously or in less than one week with thromboses in small vessels (20). Regarding laboratory tests, thrombocytopenia was defined with a platelet count lower than 100,000/μL (14); aCL and aβ_2GPI antibodies (IgG and IgM isotypes), LA, antinuclear (ANA), anti-double-stranded DNA (anti-ds DNA) and anti-extractable nuclear antigens (ENA: Ro/SSA, La/SSB, U1 small nuclear RNP, and Sm) antibodies as well as levels of C3 and C4 fractions of complement were derived from clinical charts.

Concomitant risk factors

Additional RFs, evaluated at APS diagnosis, included classic cardiovascular RFs (arterial hypertension, obesity, diabetes, smoke, dyslipidemia and hyperhomocysteinemia) and other thrombophilic RFs such as hormonal therapy, pregnancy and post-partum, trauma, surgery and immobilisation and congenital thrombophilia (factor II mutation, factor V mutation, antithrombin III deficiency and protein C and S deficiency, MTHFR mutation).

Treatment

Therapies evaluated included antithrombotic treatments, both anticoagulant and antiaggregant agents and additional treatments including steroids, hydroxychloroquine and immunosuppressants.

Statistical analysis

Continuous variables were presented as arithmetic means ± SD if normally distributed. If not, medians and interquartile range (IQR) were used. Categorical variables were presented as percentages. Continuous variables were compared using the Student t-test if normally distributed. If not, the non-parametric Mann-Whitney test was used.

Categorical variables were compared using contingency tables, *p* value was calculated with Chi-Squared or Fisher exact test, when appropriated. To identify if any concomitant risk factor for thrombotic events was associated independently to male sex, a multivariable logistic regression was performed. A *p*-value less than 0.05 was considered statistically significant. Odds ratios (ORs) were calculated to assess the risk of the presence of each variable. Statistical analyses were performed by means of the GraphPad program (GraphPad Prism 5), using the information stored in the database program.

The point of view of patients' representatives

Two patients' representatives, one male and one female, both with a diagnosis of primary t-APS, were involved in the study, in order to evaluate whether they could express a gender-related perception of the disease. They prepared and answered a self-questionnaire including demographic and lifestyle data, details regarding the pathological events including adherence to anticoagulant therapy and their subjective feelings of having a chronic pathological condition and related-organ damage.

Table I. Clinical manifestations.

	Tot n=433	Males n=137, 32%	Females n=296, 68%	*p*-value OR [IC 95%]
Criteria manifestations				
First event: venous	259 (60)	70 (51)	189 (64)	***0.012*** **1.7 [1.1–2.5]**
DVT (legs, arms)	153 (35)	44 (32)	109 (37)	*0.341*
Pulmonary embolism	75 (17)	19 (14)	56 (19)	*0.197*
Cerebral thrombosis	14 (3)	2 (2)	12 (4)	*0.242*
Portal and IVC thrombosis	10 (2)	3 (2)	7 (2)	*1.000*
Retinal thrombosis	5 (1)	2 (2)	3 (1)	*0.654*
Other*	2 (0.4)	0 (0)	2 (0.6)	*1.000*
First event: arterial	158 (37)	59 (43)	99 (34)	*0.053*
Stroke	115 (27)	39 (29)	76 (26)	*0,541*
Myocardial infarction	17 (4)	6 (4)	11 (4)	*0.741*
Limbs' ischaemia	11 (3)	6 (4)	5 (2)	*0.110*
Retinal ischaemia	8 (2)	1 (1)	7 (2)	*0.445*
Visceral** ischaemia	5 (1)	5 (4)	0 (0)	***0.003***
Other***	2 (0.4)	2 (2)	0 (0)	*0.100*
First event: CAPS	15 (3)	7 (5)	8 (3)	*0.258*
First event: venous + arterial	1 (0.2)	1 (1)	0 (0)	*0.316*
Extra-criteria manifestations				
Heart valve lesions	50 (12)	12 (9)	38 (13)	*0.217*
Livedo reticularis	64 (15)	17 (12)	47 (16)	*0.344*
aPL-associated nephropathy	23 (5)	10 (7)	13 (4)	*0.210*
Seizures	21 (5)	6 (4)	15 (5)	*0.656*
Cognitive dysfunction	22 (5)	7 (5)	15 (5)	*0.985*
Migraine	43 (10)	8 (6)	35 (12)	*0.053*
Thrombocytopenia	76 (18)	23 (17)	53 (18)	*0.776*

Results are presented as number (%). Categorical variables were compared with Chi Squared or exact Fisher test, when appropriate.
OR: odds ratio; CI: confidence interval; DVT: deep vein thrombosis; IVC: inferior vena cava; APS: antiphospholipid syndrome; CAPS: catastrophic APS, aPL: antiphospholipid antibodies.
*concomitant pulmonary embolism and portal thrombosis, concomitant pulmonary embolism and cerebral thrombosis; **visceral: gastrointestinal organs or kidneys; ***simultaneous arterial involvement in more than one side.

Results

The final cohort included 433 unselected patients. Two thirds of the patients were females (n=296, 68%) ad one third males (n=137, 32%) with a female:male ratio of 2.2:1. The majority of patients were Caucasians (94%) followed by Arabian (4%), Hispanic (1%), African (0.7%) and Asian (0.3%) subjects. Median age at APS onset was significantly lower in females compared to males [31 (24–46) *vs*. 41 (29–53) years, $p<0.001$] as well as at APS diagnosis [34 (27–50) *vs*. 46 (34–57) years, $p<0.001$].

In the whole cohort, the most common presenting manifestations were venous thromboses (n=259, 60%) followed by arterial events (n=158, 37%); CAPS occurred in 15 cases (3%); one patient presented with concomitant venous cerebral thrombosis and ischaemic stroke. Venous events were significantly more frequent in women as compared to men (n=189, 64% *vs*. n=70, 51%, $p=0.012$, OR: 1.7 [1.1–2.5]) while the arterial events were more frequent among men (n=59, 43% *vs*. n= 99, 34%, $p=0.053$), although this difference was not statistically significant.

Sites of thrombosis are reported in Table I. Deep vein thrombosis (DVT) and pulmonary embolism account for 88% of the venous events, followed by cerebral, portal, inferior cava and retinal veins involvement. In one case there was simultaneous pulmonary embolism and portal thrombosis, and, in another case, there was a concomitant involvement of cerebral and pulmonary veins. Regarding arterial events, strokes and myocardial infarctions were the most frequent manifestations, followed by ischaemic thrombosis of limbs, retina and abdominal organs. In two cases there was an arterial involvement of more than one site. No gender differences were found in location of venous and arterial events. Noteworthy, only men presented with visceral ischaemia of gastrointestinal organs or kidneys.

Complete aPL profile was available in 357 subjects (82%) and reported in Table II: 116 (33%) patients had single aPL positivity; 87 (24%) patients presented double positivity; 154 (43%) patients had triple positivity. No gender differences were observed in aPL profile, except for a more frequent combination of LA and aCL positivity in males compared to females (n=9, 35% *vs*. n=9, 15%, $p=0.036$).

Data regarding additional immunologic tests were only partially available: women were tested more frequently for ANA and for complement, and ANA prevalence was higher in females than in males (Table II). Despite the presence of ANA in about half of the patients and of anti-ds DNA positivity

Table II. Laboratory tests.

	Tot n=433	Males n=137, 32%	Females n=296, 68%	*p*-value OR [IC 95%]
Antiphospholipid antibodies profile				
Complete aPL profile tested	357 (82)	114 (83)	243 (82)	*0.776*
Single positivity	**116** (33)	**41** (36)	**75** (31)	*0.337*
LA +	56 (48)	21 (51)	35 (47)	*0.639*
aCL +	22 (19)	6 (15)	16 (21)	*0.379*
anti-β_2GPI +	38 (33)	14 (34)	24 (32)	*0.814*
Double positivity	**87** (24)	**26** (23)	**61** (25)	*0.638*
LA + aCL +	18 (21)	9 (35)	9 (15)	***0.036*** **3.1 [1.0–8.9]**
LA + anti-β_2GPI +	22 (25)	5 (19)	17 (28)	*0.396*
anti-β_2GPI + aCL +	47 (54)	12 (46)	35 (57)	*0.336*
Triple positivity	**154** (43)	**47** (41)	**107** (44)	*0.618*
Other laboratory features				
ANA tested	357 (82)	103 (75)	254 (86)	***0.007*** **1.9 [1.2–3.3]**
ANA +	207 (58)	51 (50)	156 (61)	***0.039*** **1.6 [1.1–2.6]**
Anti-ENA tested	219 (51)	62 (45)	157 (53)	*0.148*
Anti-ENA +	25 (11)	8 (13)	17 (11)	*0.664*
Anti-Ro/SSA +	22 (88)	7 (88)	15 (88)	*1.000*
Anti-La/SSB +	1 (4)	0 (0)	1 (6)	*1.000*
Anti-Sm +	2 (8)	1 (13)	1 (6)	*1.000*
Anti-U1RNP +	2 (8)	0 (0)	2 (12)	*1.000*
Anti-dsDNA tested	249 (58)	70 (51)	179 (60)	*0.066*
Anti-dsDNA +	52 (21)	13 (19)	39 (22)	*0.575*
Complement measured	224 (52)	60 (44)	164 (55)	***0.025*** **1.6 [1.1–2.4]**
Low C3 and/or C4	40 (18)	10 (17)	30 (18)	*0.778*
Platelet count measured	433 (100)	137 (100)	296 (100)	*1.000*
Thrombocytopenia	76 (18)	23 (17)	53 (18)	*0.776*

Results are presented as number (%). Categorical variables were compared with Chi Squared or exact Fisher test, when appropriate.
OR: odds ratio; CI: confidence interval; aPL: antiphospholipid antibodies; LA: lupus anticoagulant; aCL: anticardiolipin antibodies; anti-β_2GPI: antibeta2glycoproteinI antibodies; ANA: antinuclear antibodies; anti-ENA: antibodies to extractable nuclear antigens; anti-dsDNA: antibodies to double-stranded DNA.
Thrombocytopenia was defined as a platelet count <100,000/μL.

with low complement levels in 1/5 of them, none of the patients developed symptoms or signs of definite connective tissue disease after a median follow-up time of 7 (3–13) years.

Thrombocytopenia was the most common "extra-criteria" manifestation, followed by livedo reticularis and heart valve lesions, while the neurological manifestations and aPL-associated nephropathy were rare. No differences were observed between males and females in the frequency of these manifestations (Table I).

About 80% of the whole cohort had a concomitant risk factor (RF) for thrombotic events, at APS diagnosis (Table III). All established cardiovascular RFs evaluated, with exception for obesity, were significantly more represented among men, and included smoke, arterial hypertension, dyslipidemia, hyperhomocysteinemia and diabetes. However, none of them was independently associated to male sex. Estrogenic stimuli, including hormonal therapy, pregnancy and post-partum, were present in 116/296 (39%) female patients. Other thrombophilic factors, distributed without gender differences, such as trauma, surgery and immobilisation were present in 53 (13%) cases. Congenital thrombophilia (factor II mutation, factor V mutation, antithrombin III deficiency and protein C and S deficiency) was tested in 298 (69%) patients and at least one alteration was found in 41 (14%) of them. The most common alteration was Leiden factor V mutation, present in 15/41 (37%) patients, followed by protein S deficiency noticed in 12/41 (29%) patients, more frequently in the females' group.

During the follow-up, new thrombotic events occurred in 179/433 (41%) patients. New events were mostly of the same type (n=124/179, 69%), but one third of patients (n=55/179, 31%) presented a switch. The switch was from venous to arterial side in 29/55 patients (53%) and vice versa in 12/55 (22%), while 14/55 patients developed a CAPS (25%). No gender differences were observed regarding the first relapse but, among the 179 patients who suffered recurrencies, 59 (33%) had at least two additional episodes and this occurred especially among males (n= 30, 46% *vs*. n= 29, 25%, *p*=0.008, OR: 2.5 [1.3–4.8]).

Data regarding treatment are available for 415 (96%) patients. Data on cumulative treatment are reported in Table IV: no gender differences in the use of anticoagulant or antiplatelets agents were observed, neither in the use of other therapies such as steroids, hydroxychloroquine and immunosuppressants.

The point of view of Patients' Representatives

Information about demographic data and treatment/disease perception extrapolated from the patients' self-questionnaire is reported in Table V.

Discussion

In the present study, we analysed the gender differences in the prevalence and the most relevant clinical and immunologic features in a wide cohort of primary APS patients with preliminary thrombotic events. To our knowledge, indeed, this represents by far, the largest series of patients with primary APS with vascular events at onset, that have been studied in this respect. Overall, the sites distribution and the prevalence of the vascular events in our cohort is comparable to that reported in previous studies (16). Deep venous thrombosis (35%), stroke (27%) and pulmonary embolism (17%) were the most common manifestations. However, several other "extra-criteria" manifestations were also frequently found, including

Table III. Additional risk factors.

	Tot n= 433	Males n= 137, 32%	Females n= 296, 68%	*p* value OR [IC 95%]	*p* value° OR [IC 95%]
Traditional cardiovascular RFs					
Smoke	147 (34)	66 (48)	81 (27)	***<0.001*** **2.5 [1.6 – 3.8]**	*0.153*
Arterial hypertension	134 (31)	59 (43)	75 (25)	***<0.001*** **2.2 [1.5 – 3.4]**	*0.413*
Dyslipidemia	124 (29)	52 (38)	72 (24)	***0.004*** **1.9 [1.2 - 2.9]**	*0.573*
Hyperhomocysteinemia	40 (9)	23 (17)	17 (6)	***<0.001*** **3.3 [1.7 – 6.4]**	*0.237*
Diabetes	31 (7)	16 (12)	15 (5)	***0.014*** **2.5 [1.8 – 5.1]**	*0.662*
Obesity	51 (12)	13 (10)	38 (13)	*0.315*	*0.662*
Other thrombophilic factors					
Estrogenic stimuli*	116 (27)	0 (0)	116 (39)	***<0.001***	***<0.001*** ***-3.8 [0.1-0.2]***
Trauma / surgery / immobilisation	53 (13)	21 (15)	32 (11)	*0.182*	*0.480*
Congenital thrombophilia tested	298 (69)	94 (69)	204 (69)	*0.949*	*0.052*
Congenital thrombophilia	41 (14)	9 (10)	32 (16)	*0.155*	-
Factor II mutation	10 (24)	1 (11)	9 (28)	*0.410*	-
Factor V Leiden mutation	15 (37)	6 (67)	9 (28)	*0.053*	-
AT deficiency	4 (10)	0 (0)	4 (13)	*0.559*	-
Protein C deficiency	6 (15)	2 (22)	4 (13)	*0.597*	-
Protein S deficiency	12 (29)	0 (0)	12 (38)	***0.039***	-

Results are presented as number (%). Categorical variables were compared with Chi Squared or exact Fisher test, when appropriate.
°Multivariable logistic regression was performed to identify if any additional risk factor for thrombotic events was associated independently to male sex.
*hormone therapy, pregnancy, post-partum.
OR: odds ratio; CI: confidence interval; RF: risk factor; AT: antithrombin III.

thrombocytopenia (18%), livedo reticularis (15%) and heart valve lesions (12%) among others. Some differences in the expression of the disease were observed in relation to the patients' sex and age. In our cohort, females had a median age of 31 years at disease onset, significantly lower compared to males, that had a median age of 41 years at disease onset; in addition, females presented more frequently with venous events while males had a higher number of arterial thromboses. In previous studies that compared genders, a higher prevalence of pulmonary embolism was found in females (17) as well as strokes (18) while more frequent events of gastrointestinal complications were noticed in males (18). Furthermore, other arterial events including myocardial infarctions and peripheral thromboses of lower limbs have been described more frequently in men (16). In our cohort, in contrast, when stratifying for the different sites of events, the previously reported differences are not maintained, apart from abdominal ischaemia that occurred only in the males' patient group. We did not find gender differences in the "non-criteria" manifestations neither in aPL profile. Our cohort, however, is only partially comparable with the cohorts previously studied, composed of a limited number of patients (17, 18) and that included also patients with SLE-related APS (16). In addition, our study included the measurement of aβ_2GPI and the evaluation of "extra-criteria" manifestations, that were not considered in previous reports (17, 18). We observed gender differences in the prevalence of concomitant RFs for thrombotic events, both venous and arterial. In particular, we found that the classical established RFs for cardiovascular diseases (CVDs) (smoke, arterial hypertension, dyslipidemia, hyperhomocysteinemia and diabetes) and therefore for arterial ischaemic events, were more represented in the males' group. This is in agreement

Table IV. Treatment (current or previous).

	Tot n=415	Males n=129, 31%	Females n=286, 69%	*p*-value OR [IC 95%]
Anticoagulant therapy	285 (69)	93 (72)	192 (67)	*0.313*
Antiaggregant therapy	235 (57)	69 (53)	166 (58)	*0.386*
Combination of both treatments	151 (36)	50 (39)	101 (35)	*0.500*
Anticoagulant alone	124 (30)	42 (33)	82 (29)	*0.423*
Antiaggregant alone	84 (20)	19 (15)	65 (23)	*0.061*
No antithrombotic therapy	7 (1.7)	1 (0.7)	6 (2)	*0.443*
Corticosteroids	71 (17)	22 (17)	49 (17)	*0.984*
Hydroxychloroquine	92 (22)	26 (20)	66 (23)	*0.507*
Immunosuppressants	13 (3)	5 (4)	8 (3)	*0.553*

Results are presented as number (%). Categorical variables were compared with Chi Squared or exact Fisher test, when appropriate.
OR: odds ratio; CI: confidence interval.

Table V. The point of view of patients' representatives.

	Male patients	Female patients
Age, years	40	38
Marital status	married	married
Children	yes, 2	yes, 1
Educational qualification	university degree	master degree
Job	ex-engineer	housewife, mother; active in several APS patient associations and initiatives
Age at APS onset, years	22	28
Type of thrombosis	arterial	venous
Site of thrombosis	ischaemia of left popliteal artery	DVT in right ilio-femoral vein
Concomitant RFs	no	estrogenic contraceptives
Recurrencies	yes: distal DVT in right leg silent myocardial infarction stroke	no
Physical sequelae	residual arterial obstruction in the left leg; the silent infarction produced damage to the heart and a LVEF of 30%; sequelae from the stroke like sensory impairment with left hemi-paresthesia, cognitive impairment with severe learning and memory deficits and significant loss of visual field in both eye	mild post-thrombotic syndrome with peripheral oedema of the right leg and the need of graduated compression stockings
Therapy	VKA + LDA	VKA
Therapy adherence	"I was put on oral anticoagulants, and even though I have been taking them for years, I still have not gotten used to it. I have my INR checked in-vein every time and I have a lot of problems both with the people who usually carry out my controls and with the laboratories. It is already hard for me to travel 25 km every 15 days and for them to lose my sample regularly and not being in range most of the time. I have changed my diet, but it is true that I try to adapt the dose to the diet rather than the other way around."	"Being on oral anticoagulants was very hard at the beginning. I felt like it was another burden I had to carry forever. After getting information about how to check and control INR from my doctors, I qualified to get a home machine to self-check my INR. I adapted my dose to the diet I wanted to have. The easiest solution for me was to consume the same amount of vitamin K every day, learning how much vitamin K my usual foods had. I have gotten used to the frequent tests (every 10 days or so) and has become part of my care routine, although the learning and adaptation curve was not easy."
Subjective perception	"My life has changed completely, I have had a life conditioned by the after-effects on a practical level, from not being able to play sports (I used to play a lot) to not being able to work. I have retired early already due to the state of my health. On an emotional level, I am equally conditioned in all types of socialisation and relationships with people."	"Becoming a patient with a chronic illness at 28 years old was hard. I passed from being a healthy, normal, sportive person to a chronically ill patient with a disease I had never heard from, with physical sequelae. My relationship with some people changed as they started treating me like a sick person and I didn't feel that way."

APS: antiphospholipi antibodies syndrome; RF: risk factors; DVT: deep vein thrombosis; LVEF: left ventricular ejection fraction; VKA: vitamin K antagonists; LDA: low dose aspirin.

with the prevalence of these RFs in the general population. Globally, more than 80% of smokers are men (21); the prevalence of high total cholesterol is significantly greater in women but men are more likely to have low high-density lipoprotein and higher levels of low-density lipoprotein (22); men are estimated to have higher rates of cases of diabetes mellitus, with a 30% excess compared to women; finally, men have a higher prevalence of hypertension than women (23) especially in the premenopausal period. Thus, in general, men tend to have a worse arterial vascular risk factor profile than women (24). Regarding the venous side, estrogenic stimuli, including hormonal therapy, pregnancy and post-partum period, were the most represented triggers (27%) for thrombotic events and, since no male patient was under hormonal treatment, they were present only in the females' group. This may also explain the younger age of female patients at APS diagnosis. Trauma, surgery and immobilisation were found in almost 15% of the cohort as well as congenital thrombophilia, without gender differences in prevalence. These triggers correspond to the RFs for venous thrombosis in the general population. Indeed, at least one RF can be identified in over 80 percent of patients with venous thrombosis (25). Furthermore, there is often more than one RF in each patient. Considering estrogenic stimuli, oral contraceptives are the most important cause of thrombosis in young women, because of their widespread use (26). Pregnancy is associated with an increased risk of thrombosis

that may be due in part to obstruction of venous return by the enlarged uterus, as well as the hypercoagulable state associated with pregnancy.

In the pathogenesis of t-APS, differently from o-APS, a "two-hit hypothesis" has been suggested. This was formulated to explain the clinical observation that thrombotic events occur only occasionally, despite the persistent presence of aPL. According to this principle, well demonstrated in animal models (27), the antibody (representing the first hit) induces a thrombophilic state, but clotting takes place only in the presence of another thrombophilic condition (the second hit) (28). Other thrombophilic conditions include the presence of traditional cardiovascular risk factors (smoking, arterial hypertension, dyslipidaemia, diabetes) and thrombosis high risk situations (surgery, prolonged immobilisation, puerperium) and should always carefully evaluated (29).

According to this theory, all the additional RFs evaluated in our study can be considered second hits and seem to take part in determining the venous or arterial side of the thrombotic event.

During the follow-up, despite the standard therapy (anticoagulant in 69%, antiaggregant in 57%, combination in 36%), new events occurred in 179/433 (41%) patients and 59/179 (14%) out of them suffered for at least two relapses; this occurred significantly more frequently in the males' group. The risk of recurrent thrombosis can influence to a large extent the long-term prognosis for patients with APS. In addition, certain manifestations of APS carry a worse prognosis, and permanent damage may occur in various organs (30-32). It has been observed that functional damage is significantly associated with a thrombotic history, in particular to an arterial event (30, 32, 33). Another important consideration is that organ damage following a thrombotic event led to an impaired Health-Related Quality of Life (HRQoL) (33, 34). Although it is not possible to generalise the information derived from the two Patients' Representatives involved in our study, what they report is in line with our findings and with the literature reports: the male patient reported a severe pathological history caused by recurrent arterial events. He also reported an important impact on HRQoL, principally impaired by organ damage and by prolonged anticoagulation therapy. The female reported a venous thrombosis that occurred while on hormonal therapy. These preliminary self-reported data need to be confirmed on a wider scale. A larger survey could be stimulated by the different voice of women and men, here expressed by the two APS Patients' Representatives. Patients' Associations within ERN ReCONNET could be interested to run it on European scale in order to understand gender-related differences in the disease perception.

Conclusions

The gender-oriented analysis of a large cohort of well-defined patients with primary t-APS showed that women had the first vascular event at a younger age and mostly on the venous side, while men presented mainly with arterial events, later in life and suffered from more recurrent events. No significant differences were observed in the distribution of the aPL profile. The different frequency of arterial and venous events in the two groups could be attributed mainly to the presence of additional RFs rather than to biological gender-specific issues. However, it should be underlined that some RFs, such as the use of oestrogens or traditional cardiovascular RFs, are exclusive or more represented in one gender rather than the other, making it difficult to assess the link of causality between gender and manifestations of t-APS. Recurrencies along with the more frequent involvement of the arterial versant, that is associated with an important functional damage and, consecutively with an impaired HRQoL, could define a more severe phenotype of disease in the males' group.

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Competing interests

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