UNIVERSITAT DE BARCELONA

The role of antiphospholipid antibody pattern in the recurrence of thrombosis in patients with Antiphospholipid Syndrome

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Joint Research Doctoral Thesis

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Joint research doctoral thesis

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A doctoral thesis represents a path filled with obstacles, that drives you on the edge of giving up more than once. Along the way, anytime you add another brick in the wall you are building, you keep learning things about yourself, your resources, your strength, your limits, and your ability to overcome them. But more important, along this journey you experiment that there are amazing people around you that can provide the support you need in many different ways. Inspiring mentors to drive you, special friends to address your anxieties, great colleagues to share discussions and develop ideas. To them, I express my most profound gratitude.

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Abbreviations

aCL: anticardiolipin antibodies

AID: autoimmune diseases

Anti-β2GPI: anti-β2-glycoprotein-I antibodies

Anti-dsDNA: anti-double stranded DNA antibodies

Anti-RNP: anti-ribonucleoprotein antibodies

Anti-Sm: anti-Smith antibodies

aPL: antiphospholipid antibodies

APS: antiphospholipid syndrome

aPTT: activated partial thromboplastin time

AUC: area under the curve

BLyS: B-lymphocyte stimulator

DVT: deep vein thrombosis

EULAR: European Alliance of Associations for Rheumatology

FU: follow-up

HELLP: Hemolysis, Elevated liver enzymes, Low Platelet count

HR: hazard ratio

ICAM-1: intercellular adhesion molecule-1

INR: international normalized ratio

IVIG: intravenous immunoglobulins

LA: lupus anticoagulant

LDA: low-dose aspirin

LMWH: low molecular weight heparin

PAPS: primary APS

Pex: plasma exchange

RR: relative risk

SLE: systemic lupus erythematosus

UFH: unfractioned heparin

VCAM-1: vascular cell adhesion molecule-1

VTE: venous thromboembolism

VKA: vitamin K antagonists

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INTRODUCTION

The antiphospholipid syndrome (APS) is a hypercoagulability condition of autoimmune origin clinically characterized by the development of arterial, venous and/or microvascular thrombosis, pregnancy complications (recurrent early miscarriages, fetal deaths after the 10th week of gestation and/or premature births) and, frequently, hematologic alterations (such as hemolytic anemia and thrombocytopenia), associated to the presence of antiphospholipid antibodies (aPL)[1]. aPL are antibodies that bind to negatively charged phospholipids directly or via proteins, such as β2-glycoprotein I (β2GPI), that act as cofactors. APS can occur either as an isolated condition (primary APS), or in the context of an underlying autoimmune disease, most commonly systemic lupus erythematosus (SLE) [2]. Less frequently, it can be associated with other autoimmune disorders, infections, drugs, and malignancies.

Epidemiology

The aPL are not specific of APS and can be detected in healthy individuals, with an estimated prevalence of 0.1-5% [3]. The frequency of APS in the general population has not been extensively analyzed, and only three epidemiological population-based studies have been performed so far, with an incidence of APS ranging from 0.75 to 2.1 per 100.000 population and a prevalence of 6.2 to 50 cases per 100.000 population [4–6]. There seems to be a female predominance with a female to male ratio around 3 to 5:1 [7], even though a similar prevalence in both sexes has been reported in one of the two population-based studies [4]. The mean age of onset is between the 4th and the 6th decade even though childhood and older onsets have been described [2,8].

The prevalence of aPL has been estimated about 11% among patients with myocardial infarction, 9.5% of patients with deep vein thrombosis and 17% among patients with stroke younger than 50 years of age [9,10]. Moreover, aPL are positive in around 6% of women with pregnancy morbidity, representing the most frequent acquired risk factor for a treatable cause of recurrent pregnancy loss and for pregnancy complications (early and severe pre-eclampsia) [9]. aPL are present in 30-40% of SLE patients and up to a third of these patients (10-15% of all SLE patients)

have a clinically evident APS [11]. On the contrary, only few patients with primary APS tend to evolve into full-blown SLE and, usually, this takes place only after a long period of time [7,8,12].

History

The first stone towards the discovery of APS was probably laid at the start of 20th century, in 1906, with the development of a test for the diagnosis of syphilis by August von Wasserman, Julius Citron and Albert Neisser, based on a reaction between an antibody, called "reagin", and an antigen from lipoid tissue (obtained by alcohol extracts of liver from a fetus with congenital syphilis), which was later purified from bovine hearts by Mary C Pangborn and named cardiolipin [1,13]. During World War II, a mass screening for syphilis showed that more than half of subjects with positive serologic test results had no clinical evidence of infection, while some of them developed an autoimmune disorder. The concept of "biological false-positive serological test for syphilis" (BFP-STS) was introduced [14]. In 1952 Conley and Hartmann described two patients with SLE and a false positive Wasserman test that curiously presented a prolonged coagulation and prothrombin time [15]. Laurell and Nilson later demonstrated that both the Wasserman reagent and this in vitro coagulation inhibitor located in the same gamma-globulin region in electrophoresis [16]. In 1972 Feinstein and Rapaport coined the definition lupus anticoagulant (LA), with the erroneous assumption that such antibody was cause of bleeding, and discovered that it consisted of an immunoglobulin, isotype G or M [17]. It was later disclosed that LA in vitro anticoagulant activity was the result of specific immunological interactions with negatively charged phospholipids [18,19]. Nevertheless, the expression LA turned out to be misleading, since several groups already in the early 60's had shown that although this antibody acts as an anticoagulant in vitro, it is clearly associated with thrombotic events in vivo [20,21]. Moreover, it was noted that most patients with this serologic abnormality did not have SLE [22,23]. Therefore, both the expression "Lupus" and "Anticoagulant" were inappropriate.

A group that played a fundamental role towards the discovery of APS was the one led by Graham R.V. Hughes at London's Hammersmith Hospital. In 1983 Dr Hughes described for the first time the correlation between major cerebrovascular events, abortions, thrombocytopenia, and presence of LA in a famous editorial published in the British Medical Journal [24]. Studies conducted by

Drs Azzudin Gharawi and Nigel Harris in Hughes' laboratory in the early 1980s, prompted the development of solid-phase immunoassays to detect anticardiolipin antibodies (aCL). The aCL showed high correlation with thrombosis, thrombocytopenia, fetal loss, and LA positivity [25]. Moreover, aCL titers correlated with the probability of clinical manifestations. These findings led to the definition of the so-called "anticardiolipin syndrome", which was quickly replaced by APS: it was indeed evident that aCL bind also to negatively charged phospholipids other than cardiolipin (such as phosphatidylserine or phosphatidylinositol) in ELISA plates. By the mid 80's, through various reports of patients with clinical manifestations of APS who did not have SLE, it was evident that this syndrome was a separate entity from SLE [26].

After the formal description of APS, a major advance came in the early 1990s with the identification of β2GPI, a plasma protein "cofactor" that increased the binding of aCL to cardiolipin on ELISA plates [27–29]. β2GPI is a polypeptide part of the superfamily of complement-cascade control proteins, that inhibits the intrinsic coagulation pathway, prothrombinase activity and ADP-mediated platelets aggregation acting as a natural anticoagulant. Since then, a number of "cofactors", such as prothrombin, tissue plasminogen activator (tPA), phosphatidylserine (PS), plasmin, annexin A2, activated protein C (APC), thrombin, antithrombin (AT) and annexin A5 have been discovered [30].

Pathophysiology

The exact etiology of APS is not known, but genetic and environmental factors have been implicated. In the first group, the association between HLA-DR4 and DRw53 and APS has been described [31]. Infections are perhaps the most prominent environmental trigger and there is evidence that molecular mimicry can induce production of pathogenic aPL, presumably because of a breakdown in normal peripheral tolerance mechanisms [32]. Syphilis was the first infectious disease recognized to be linked to aPL production, although it mostly triggers the production of non-pathogenic aPL [33]. Afterwards, many other infections have been linked to the onset of APS, for instance cytomegalovirus, parvovirus B19, human immunodeficiency virus, hepatitis B and C viruses, varicella zoster virus, and human T-cell lymphotropic virus [34].

The aPL are heterogeneous antibodies and more than one mechanism may be involved in causing thrombosis. β2GPI represents the main antigenic target and along with prothrombin accounts for

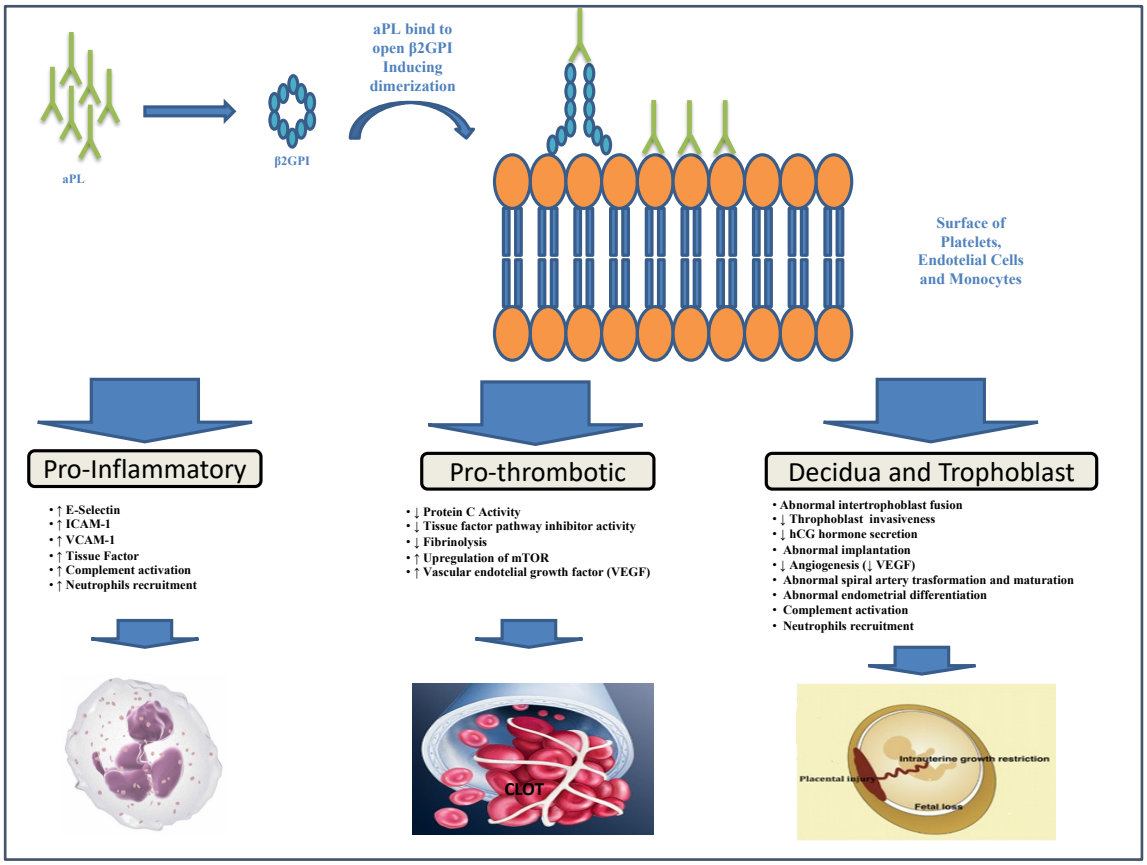
more than 90% of aPL binding activity in APS patients [30]. Interestingly, β2GPI does not bind to unstimulated endothelium in vivo [35], and the initiating stimulus that primes endothelium, when not identifiable as in the case of surgery or infections, might be represented by disturbance of the redox balance in the vascular milieu [36].

Anti-β2GPI autoantibodies can target any of the 5 domains that constitute β2GPI. However, the ones directed against domain I confer LA activity and are associated with the highest risk of thrombosis [37]. The binding of aPL to the phospholipids-bound β2GPI leads to its conformational change and dimerization (the immunogenic form of β2GPI); dimerization of β2GPI on the surfaces of endothelial cells, platelets and monocytes, up-regulates the expression of prothrombotic cellular adhesion molecules (such as E-selectin, intercellular adhesion molecule-1 [ICAM-1] and vascular cell adhesion molecule-1 [VCAM-1]) [38] and tissue factor [39], suppressing the response to the tissue factor pathway inhibitor [40], reducing activated protein C activity [41], and activating complement [42]. Furthermore, it antagonizes the activity of endothelial nitric oxide synthase, leading to impaired endothelial nitric oxide-dependent vascular relaxation [43]. Another putative mechanism of action of aPL is the disruption of the annexin A5 anticoagulant shield from endothelial cells [44]. Annexin A2 [45], a tissue plasminogen activator receptor, toll like receptor-2 and 4 [46,47], and apoE-receptor-2 [48] may serve as intermediary. Finally, a possible explanation for microvascular thrombosis in APS is the aPL–induced up-regulation of the mechanistic target of rapamycin (mTOR) complex on endothelial cells [49].

Pregnancy morbidity was initially related to the impairment of maternal-fetal blood exchange as a result of thrombus formation in the uteroplacental vasculature, a hypothesis supported by findings of placental thrombosis in patients with obstetric APS [50]. However, such a histologic finding is not specific for APS, being also present in other conditions (such as thrombotic microangiopathies), and histologic evidence of thrombosis in the uteroplacental circulation cannot be shown in many placentas from patients with APS [51]. Other theories have thus been put forward to explain APS-related pregnancy morbidity such as defective trophoblast invasion and decidual transformation in early pregnancy or placental injury due to local inflammatory events, particularly complement activation and neutrophils recruitment [50,52]. Congruently

with this hypothesis, it has been shown that heparin prevents pregnancy loss in mice injected with aPL due to the complement inhibitory properties of the drug and not to its anticoagulant effects [53]. Figure 1 provides a summary of the pathophysiological mechanisms leading to thrombosis and pregnancy morbidity in APS.

Figure 1



Antiphospholipid antibodies (aPL) produced by B cells bind to closed β2GPI inducing its conformational change into the open dimeric (immunogenic) form. Annexine A2, toll like receptor-4 and apoE-receptor-2 may function as receptor for β2GPI on cell surfaces. This binding results in endothelial-cell, monocyte, platelet and neutrophil activation and trophoblast and decidua modification leading to inflammation, thrombosis, and pregnancy complications.

Clinical manifestations of APS

As a general rule, APS must be suspected in a young patient presenting with unprovoked thrombosis, especially if at unusual sites and recurrent, or in case of thrombotic or pregnancy complications associated to other autoimmune diseases. Unexplained prolongation of the activated partial thromboplastin time (aPTT), *livedo reticularis* or *racemosa*, and mild thrombocytopenia are clues for suspecting APS. The clinical picture is not constant and ranges from asymptomatic aPL carriers to recurrent arterial and/or venous thrombosis, recurrent pregnancy loss or isolated "non-

criteria" manifestations (which stands for manifestations not included in the current classification criteria). Single vessel involvement or multiple vascular occlusions may give rise to a wide variety of presentations in APS. Any combination of vascular occlusive events may occur in the same individual and the time interval between them also varies considerably from weeks to months or even years.

The "Euro-Phospholipid" project, a study of 1000 European APS patients that started in 1999 as an attempt to describe the epidemiology and the course of APS in real life, has provided accurate information on the prevalence of the majority of clinical manifestations of APS, which is now recognized as a major cause of venous thromboembolism (VTE), new strokes in individuals below the age of 50 and recurrent fetal loss [2]. Patients with VTE most commonly present with lower-extremity deep vein thrombosis (DVT), with or without pulmonary embolism. Stroke and transient ischemic attack are the most frequent arterial events, followed by myocardial infarction. The main obstetric manifestations are pre-eclampsia, eclampsia, HELLP syndrome (Hemolysis, Elevated liver enzymes, Low Platelet count) and abruptio placentae, while fetal involvement includes early (< 10 weeks of gestation) and late fetal losses and premature births. Even though early miscarriages are the most common fetal manifestations, typical pregnancy complications generally develop after 10 weeks of gestation and losses before 10 weeks, especially if not recurrent, would more commonly be attributed to chromosomal defects (which must always be ruled out to make a diagnosis). Reduced blood flow in the uterine arteries measured by Doppler velocimetry is an indirect indicator of the development of placental insufficiency, intrauterine growth restriction and/or preeclampsia [54,55]. Thenceforth, the European Alliance of Associations for Rheumatology (EULAR) guidelines recommend periodic uterine artery Doppler ultrasonography during pregnancy monitoring [56].

The major "non-criteria" manifestations include *livedo reticularis* (a reddish-blue to purple, uniform, reversible, unbroken "net-like" pattern of the skin), *livedo racemosa* (nonuniform, irreversible, fractured, asymmetric pattern), livedoid vasculopathy (painful papules and erythematous-violaceous purpuric plaques, which rapidly evolve into hemorrhagic vesicles or painful small ulcers), valvular heart disease, pulmonary hypertension, diffuse alveolar hemorrhage, APS-related nephropathy (acute or chronic thrombotic microangiopathy) and neurologic manifestations such as chorea, epilepsy, migraine, myelopathy, memory loss and cognitive disfunction (due to aPL related vasculopathy or direct aPL interactions with brain parenchyma following blood-brain

barrier abrogation). Hematologic alterations, mainly thrombocytopenia but also hemolytic anemia, are quite common. Thrombocytopenia is usually mild, and, when severe (platelet count, <20,000 per cubic millimeter), should prompt the clinician to consider an alternative diagnosis [57].

In a small subset of patients (about 1%), thrombosis can involve simultaneously multiple organs, configuring the so-called "catastrophic antiphospholipid syndrome" (CAPS) [58]. It is usually triggered by a precipitating factor such as infection (in almost half of cases), anticoagulation withdrawal, neoplasm, surgery, or pregnancy. Histological confirmation of small vessel occlusion is requested to make a diagnosis of definite CAPS as per classification criteria [59].

Rates of clinical manifestations in APS patients according to the "Euro-Phospholipid" project are reported in Table 1.

Table 1. Main clinical manifestations of APS.

Clinical Manifestation	%
Deep vein thrombosis	38.9
Thrombocytopenia (100,000 platelets/μl)	29.6
Livedo reticularis	24.1
Stroke	19.8
Migraine	20.2
Pulmonary embolism	14.1
Superficial thrombophlebitis	11.7
Cardiac valve thickening/disfunction	11.6
Transient Ischemic Attack	11.1
Hemolytic anemia	9.7
Epilepsy	7.0
Skin ulcers	5.5
Myocardial infarction	5.5
Amaurosis fugax	5.4
Pseudovasculitic skin lesions	3.9
Digital gangrene	3.3
Obstetric Manifestations	
Preeclampsia	9.5
Eclampsia	4.4
Abruptio Placentae	2.0
Fetal Manifestations	
Early fetal loss (before 10^{th} week)	35.4
Late fetal loss (after 10^{th} week)	16.9
Premature birth	10.6

Modified from Cervera, Piette et al. Arthritis and Rheumatism (2002) 46(4): 1019–1027.

Laboratory features of APS

Three aPL are included in the current classification criteria: the aCL, the anti-β2GPI and the LA. The first two are detected via solid-phase immunoassays (usually ELISAs) [60], while LA test is performed following the Scientific and Standardization Subcommittee on Antiphospholipid Antibodies of the International Society of Thrombosis and Hemostasis (SSC-ISTH) recommendations [61,62]. For instance, LA is detected through a three-step procedure that involves prolongation of phospholipid-dependent clotting time such as diluted Russell viper venom time (dRVVT) and the aPTT not reversed by mixing patient's plasma with normal pooled plasma but corrected by the addition of excess phospholipids. One of the major drawbacks of the LA coagulation assays is that they can be altered by anticoagulant therapy, giving false-positive results. On the other hand, aCL and anti-β2GPI antibodies assays show interassay variability owing to differences in calibration and assay characteristics [63]. Apart from aPL, several immunologic abnormalities can be detected in APS. The most common immunological features according to the "Euro-Phospholipid" project are presented in Table 2.

Table 2. Most common immunological features of APS (according to the "Euro-Phospholipid" project).

Immunological feature	%
aCL	87.9
IgG and IgM	32.1
IgG alone	43.6
IgM alone	12.2
LA	53.6
LA alone	12.1
LA and aCL	41.5
Antinuclear antibodies	59.7
Anti-dsDNA	29.2
Anti-Ro/SS-A	14
Anti-La/SS-B	5.7
Anti-RNP	5.9
Anti-Sm	5.5
Rheumatoid Factor	7.8
Cryoglobulins	3.6

Modified from Cervera, Piette et al. Arthritis and Rheumatism (2002) 46(4):1019–1027.

Diagnosis of APS

Since diagnostic criteria for APS are lacking, diagnosis is made through the combination of clinical and laboratory findings. With the aim of selecting homogenous cohorts for research purposes, several classification criteria have been developed over the years. The first classification criteria for the syndrome were proposed by Nigel Harris from Hughes' team in 1987, in a famous editorial published in the British Journal of Rheumatology named "Syndrome of the Black Swan" [64]. He proposed to classify patients if they had at least two clinical criteria among venous or arterial thrombosis, recurrent fetal losses, or thrombocytopenia along with at least one laboratory criterion (LA and/or aCL medium-high titer positivity). Laboratory tests had to be positive in at least two occasions at least 8 weeks apart (to rule out condition that could prompt transient positivity such as infections). Later on, various groups proposed candidate sets of classification criteria, but the first consensus was reached only in 1998, during the 8th International Symposium on aPL held in Sapporo. Such criteria did not include the anti-β2GPI antibodies, and aPL had to be positive in at least two occasions at least 6 weeks apart [65]. In 2005, during the 11th International Symposium in Sydney, Australia, an update of these criteria was proposed, with the inclusion of anti-β2GPI antibodies. The interval between the two aPL determinations was raised to 12 weeks to increase specificity. Moreover, no less than 12 weeks and no more than 5 years could separate the clinical manifestation and the positive aPL test. Patients were stratified according to the presence of risk factors for thrombosis (e.g. arterial hypertension, dyslipidemia, malignancies, oral contraceptives, congenital thrombophilia…) to provide a more uniform basis in selecting patients for research purpose. Some clinical features, such as cardiac valve involvement, *livedo reticularis*, thrombocytopenia, APS nephropathy, and non-thrombotic central nervous system manifestations (i.e. migraine, myelopathy, cognitive dysfunction) were remarked on, but not included among clinical criteria, that remained unchanged. Finally, patients were classified in 4 subcategories according to the possible combinations of aPL positivity [66] (Table 3).

Table 3. Revised classification criteria for APS.

Clinical Criteria

1. Vascular Thrombosis [1]
   - One or more clinical episodes of arterial, venous, or small-vessel thrombosis, in any tissue or organ. Thrombosis should be supported by objective validated criteria (ie, unequivocal findings of appropriate imaging studies or histopathology). For histopathologic support, thrombosis should be present without substantial evidence of inflammation in the vessel wall.

2. Pregnancy morbidity (defined by one of the following)
   - One or more unexplained deaths of a morphologically healthy fetus at or beyond the 10th week of gestation, with healthy fetal morphology documented by ultrasound or by direct examination of the fetus, or
   - One or more premature births of a morphologically healthy newborn baby before the 34th week of gestation because of: eclampsia or severe preeclampsia defined according to standard definitions or recognized features of placental insufficiency [2], or
   - 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.

In studies of populations of patients who have more than 1 type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of patients according to 1 of the 3 criteria.

Laboratory Criteria [3]

1. Lupus anticoagulant (LA) present in plasma, on 2 or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis (Scientific Subcommittee on LA/phospholipid-dependent antibodies).
2. Anticardiolipin (aCL) antibody of IgG or IgM isotype, or both, in serum or plasma, present in medium or high titers (i.e. >40 GPL or MPL, or greater than the 99th percentile) on 2 or more occasions, at least 12 weeks apart, measured by a standardized enzyme-linked immunoassay (ELISA).
3. Anti-β2-glycoprotein-I (anti-β2GPI) antibody of IgG or IgM isotype, or both, in serum or plasma (in titers greater than the 99th percentile), present on 2 or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures.

Definite APS is present if at least one of the clinical criteria and one of the laboratory criteria are met, with the first measurement of the laboratory test performed at least 12 weeks from the clinical manifestation [4].

[1] Coexisting inherited or acquired factors for thrombosis are not reason for excluding patients from APS trials. However, two subgroups of APS patients should be recognized, according to: (a) the presence, and (b) the absence of additional risk factors for thrombosis. Indicative (but not exhaustive) such cases include: age (>55 in men, and >65 in women), and the presence of any of the established risk factors for cardiovascular disease (hypertension, diabetes mellitus, elevated LDL or low HD cholesterol, cigarette smoking, family history of premature cardiovascular disease, body mass index ≥ 30 kg/m^2, microalbuminuria, estimated GFR <60 mL/min), inherited thrombophilia, oral contraceptives, nephrotic syndrome, malignancy, immobilization, surgery. Thus, patients who fulfil criteria should be stratified according to contributing causes of thrombosis.
[2] Generally accepted features of placental insufficiency include: (1) abnormal or non-reassuring fetal surveillance test(s), e.g. a non-reactive non-stress test, suggestive of fetal hypoxemia, (2) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, eg, absent end-diastolic flow in the umbilical artery, (3) oligohydramnios, eg, an amniotic fluid index of 5 centimeters or less, or (4) a post-natal birth weight less than the 10th percentile for the gestational age.
[3] Investigators are strongly advised to classify APS patients in studies into one of the following categories:
I: More than one Laboratory criteria present (any combination)
lIa: Anti-cardiolipin antibody present alone
IIb: Lupus Anticoagulant present alone
lIc: Anti-ß2-GPI- antibody present alone
[4] Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive aPL test and the clinical manifestation.

Adapted from Miyakis et al. Journal of Thrombosis and Haemostasis. 2006;4(2):295-306

Over the past decade, substantial evidence has accumulated on different clinical and laboratory issues related to APS. Moreover, new methodologically rigorous and data-driven approaches to address biases and develop a robust set of classification criteria have been published [67]. Starting from these premises, an international effort to develop new classification criteria, which

is jointly supported by the American College of Rheumatology (ACR) and the EULAR, is ongoing and reached its last stage.

Sometimes a high clinical suspicion of APS is not supported by concomitant positivity of aPL assays included in the serological criteria for APS, which are persistently negative. This is the framework of the so-called seronegative APS which has been described by Hughes and Khamashta in 2003 [68]. Thenceforth, numerous investigators looked for the presence in these patients of aPL not included in the serological criteria for APS. For instance, these non-criteria antibodies include aCL and anti-β2GPI IgA, antibodies specific to phospholipid-binding plasma (cofactor) proteins (such as phosphatidylethanolamine, prothrombin, protein C, protein S, annexin II and V, and domain I of β2GPI), phospholipid–protein complexes (particularly vimentin–cardiolipin complexes), and anionic phospholipids other than cardiolipin (including phosphatidylserine, phosphatidylinositol, and phosphatidic acid). In case of highly suspected APS with persistently negative LA, aCL and anti-β2GPI IgG and IgM, after ruling out other causes of thrombophilia, looking for these non-criteria antibodies can hint the diagnosis.

Treatment

The treatment of APS varies depending on the clinical manifestations, aPL profile, and concurrent cardiovascular risk factors. Treatment options in different clinical scenarios, following current EULAR treatment guidelines [69], are reported in Table 4 (for thrombotic APS) and Table 5 (for obstetric APS).

The mainstay of treatment of thrombotic APS is oral anticoagulation with vitamin K antagonists (VKA) with a target INR between 2 and 3. Indefinite anticoagulation in patients with unprovoked arterial or venous thrombosis is highly warranted, due to the high risk of thrombosis recurrence in case of VKA discontinuation [70]. High-quality evidence to support any management strategy when warfarin therapy fails despite a target INR is lacking. Viable options include higher intensity warfarin therapy (target INR, 3-4), switch to LMWH, the addition of LDA, antimalarials, statins, or a combination of these approaches.

Direct oral anticoagulants (DOAC) are an intriguing option due to the advantages in terms of quality of life for patients who must follow a long-term, often lifetime, VKA treatment and have to come every 2-3 weeks to the clinic to get an INR determination. Nevertheless, they are not recommended in APS, especially for high-risk patients with triple positivity and previous arterial

thrombosis, due to results of three randomized trials, two with rivaroxaban [71,72] and one with apixaban [73], that showed higher rate of thrombosis recurrence in such patients in comparison to VKA [74].

A prompt and aggressive treatment is critical in case of catastrophic APS, and the current standard of care is the so-called triple therapy, a combination of anticoagulants, glucocorticoids, and plasma exchange. Intravenous immunoglobulins (1–2 g/kg, given over a period of 2–5 days) are often associated to the triple therapy and, as well as rituximab [75]. Eculizumab, a humanized monoclonal antibody directed against the C5 protein of complement cascade, preventing the generation of the terminal complement complex C5b-9, has also been used with success in refractory cases [76].

Table 4. Treatment of thrombotic APS according to different clinical scenarios.

Primary Thromboprophylaxis 1. *Asymptomatic aPL carriers (not fulfilling any vascular or obstetric APS classification criteria) with a high-risk aPL profile with or without traditional risk factors.* 2. *SLE with aPL (especially those with a high-risk aPL profile) and no history of thrombosis* 3. *History of obstetric APS outside pregnancy*	*LDA* *(75-100 mg per day)*
Secondary Thromboprophylaxis 4. *Definite APS and first venous thrombosis* *a. Unprovoked: indefinite anticoagulation.* *b. Provoked: short-term anticoagulation (3 to 6 months).*	*VKA* *with a target INR 2–3*
5. *Definite APS and first arterial thrombosis*	*VKA* *with a target INR 2–3* *(INR 3-4 in selected cases)*
6. *Definite APS and recurrent venous thrombosis despite treatment with VKA with target INR 2-3*	*VKA* *with a target INR 3–4* *Or* *LMWH (therapeutic dose)* *Or* *VKA + LDA ± HCQ*
Catastrophic APS	*Glucocorticoids, UFH, Pex, IVIG, Rituximab, Eculizumab (refractory)*

High risk profile: the presence of LA, or of double (any combination of LA, aCL or anti-β2-GPI antibodies) or triple (all three subtypes) aPL positivity in 2 or more occasions at least 12 weeks apart, or the presence of persistently high aPL titers.

Low risk profile: isolated aCL or anti-β2-GPI antibodies at low-medium titers, particularly if transiently positive.

Table 5. Treatment of obstetric APS according to different clinical scenarios.

Asymptomatic carriers of aPL	*LDA (75-100 mg per day)*
Obstetric APS 1. *More than three miscarriages (before 10th week of gestation) or at least one fetal loss (after 10th week)*	*LDA + prophylactic LMWH*
2. *Delivery before 34th week because of preeclampsia, eclampsia, placental insufficiency*	*LDA ± prophylactic LMWH*
3. *History of thrombotic APS*	*LDA + Therapeutic LMWH*
Recurrent obstetric APS despite treatment	*LDA + therapeutic LMWH ± HCQ ± low dose prednisolone (fist trimester)*

LDA must be started before conception and LMWH must be continued up to 6 weeks after delivery in case of pure obstetric APS. If history of thrombosis, switch VKA to LMWH as soon as pregnancy is confirmed and switch back to VKA after delivery.

The cornerstone of treatment to prevent pregnancy complications in women with previous obstetric APS is the combination of LDA and a prophylactic dose of unfractionated heparin (UFH) or LMWH [77]. LDA should be preferably started prior to conception, and heparin should be added as soon as pregnancy is confirmed and continued up to 6 weeks after delivery to prevent maternal thrombosis, given the increased thromboembolic risk in puerperium. Oral anticoagulants should be discontinued at conception because of teratogenicity between 6 and 14 weeks of gestation.

In case of recurrent pregnancy morbidity despite combination therapy, increasing LMWH dose to therapeutic dose, addition of hydroxychloroquine [78] or low-dose prednisolone in the first trimester [79] may be considered. Intravenous immunoglobulins are an option in refractory cases, although with controversial results [80,81]. Even though statins are not typically used in pregnancy, a case-control study which analyzed the use of pravastatin with standard of care in APS patients with pre-eclampsia and/or intrauterine growth restriction showed no progression compared to LDA and LMWH [82]. The putative mechanism of action seems to be increased nitric oxide synthesis [83].

aPL persistence and risk stratification in APS

Recurrence of clinical manifestations is a main concern in APS, a condition that is usually diagnosed in young people [2] with a long life-expectancy. In fact, despite correct anticoagulation, thrombosis can reoccur in APS patients, with a recurrence rate that varies between 0.04 and 0.23 patients/year [84]. Likewise, despite current treatment in obstetric APS, the rate of successful pregnancies does not exceed 75% [77,85]. The high rate of recurrence of clinical manifestations, in spite of adequate treatment with anticoagulation and/or antiplatelet therapy, leads to an increasing cumulative prevalence of the initial clinical features as the disease progresses over time [7]. Known risk factors for thrombotic recurrence are a previous arterial thrombosis [86], high risk aPL profiles such as triple aPL positivity [87] and LA positivity [88,89], association with SLE [11,90] and the presence of concomitant genetic and acquired risk factors for thrombosis (such as arterial hypertension, dyslipidemia, smoking, diabetes mellitus, oral contraceptives, menopause, etc.) [1,91].

Besides the aforementioned drivers, clinical events are triggered by aPL persistent presence over time, as reported by two studies that correlated aPL persistence with thrombotic manifestations in SLE patients [92,93]. In the first study 144 SLE patients with aPL were compared with 144 aPL negative patients. Thrombosis rate was higher in aPL positive patients (20.1% vs 7.6%), and predictor of thrombosis were identified in male sex (HR=6.25), LA positivity (HR=3.48), and persistent aCL positivity over time (defined as positivity in at least 2/3 of total measurements, HR=5.87). In the second study 237 SLE patients were divided in 4 groups: group A = LA positive, group B = LA negative but aCL persistently positive (> 2/3 of total determinations positive), group C = LA negative, aCL transiently positive (< 2/3 of total determinations positive) and aPL negative. The adjusted risk for thrombosis resulted increased in group A and B, but not group C, compared to group D. Moreover, in another study, presented as abstract at the American college of Rheumatology Congress in 2007, our group analyzed factors associated to increased risk of thrombosis recurrence in APS patients, finding that persistently positive aPL (defined as positivity in > 75% of total determinations) increased the risk, being aCL IgG plus LA the profile with the strongest association with recurrence.

In recent years, two score systems have been proposed as risk stratification tool for clinical manifestations in aPL positive patients. The aPL-score (aPL-S) a complex score based solely on the aPL profile, was developed in 2011 by Otomo and colleagues [94]. It includes the aCL, anti-β2GPI and anti-phosphatidylserine/prothrombin (aPS/PT) IgG and IgM plus three different LA mixing tests and two confirmation tests. It has shown to be a good predictor of thrombosis in patients with AID. In 2013 Sciascia and colleagues devised the Global APS Score, a risk tool for APS clinical manifestations that takes into account the four aforementioned aPL (only one LA test is required) plus cardiovascular risk factors, namely arterial hypertension and dyslipidemia [95]. The two scores have been compared, the aPL-S showing superiority in predicting thrombosis, while the GAPSS better ability for diagnosing APS [96,97]. However, the aPL-S, that includes a total of 16 items, is much more difficult to be computed and implemented in routine use than the GAPSS, making the latter more suitable for clinical practice. Another point to consider, that concerns both scores, is that the solid-phase assays for aPS/PT are not yet well standardized, have a limited commercial availability and need additional research to define their clinical significance. For such reasons, they have not been included in the candidate items for the new classification criteria for APS [98]. An adjusted version of the GAPSS, called aGAPSS, which does not include aPS/PT, has been validated both in APS and SLE [95,99,100].

Damage in APS

While a disease activity index in APS is still lacking, a damage score system for thrombotic APS, named Damage Index for APS (DIAPS), has been recently developed, which includes 38 items/APS-specific features from ten different organ and system domains [101]. Each item scores 1 point if present without sequelae and 2 points if present with sequelae. DIAPS showed content, criterion and construct validity and a good correlation with quality of life in thrombotic APS patients from Latin-America.

In summary, when addressing a patient with aPL, the current evidence suggests that low titer aPL positivity (especially if transiently positive) bears a low risk of clinical manifestations, and is usually associated to infections, drugs, or malignancies [102]. On the contrary, persistently medium-high titer positive aPL are more likely to have important clinical implications [69].

Therefore, the assessment of aPL profile and persistence upon evaluation of aPL-positive patients is crucial in determining the risk of clinical manifestations. Moreover, the aGAPSS, a risk score for clinical manifestations in APS that takes into account cardiovascular risk factors and the aPL profile, has been shown to be a good predictor of clinical events in APS. Nevertheless, the aGAPSS is by its very nature dynamic since both cardiovascular risk factors and aPL can change over time, so it needs to be monitored longitudinally at repeated time points. Furthermore, even though a disease activity index for APS is lacking, the aGAPSS, as a dynamic score that predisposes to clinical manifestations, could be used a surrogate of disease activity and predictor of damage.

With all these premises, in the present thesis the pattern of aPL persistence over time and its association with clinical manifestations has been analyzed, along with the role of the aGAPSS as a predictor of clinical recurrence and a surrogate of disease activity predicting damage accrual, in a cohort of patients with APS.

In the following section, we perform a bibliographic research of the current literature that investigated which aPL profiles are associated with aPL persistence, and the role of GAPSS/aGAPPS as predictor of clinical manifestations of APS. Subsequently the hypothesis and objectives are presented, along with the published studies that form the results of the thesis, with a brief discussion concerning each of them.

aPL persistence

Since aPL persistent positivity at medium-high titer predisposes to clinical recurrence, identifying which factors are associated with aPL persistence over time is crucial to optimize the management of APS patients. Therefore, most relevant research on this topic has been reviewed. It should be emphasized that different studies presented various follow-up times and controversial results about persistence.

One of the first reports, published in 2005 by Erkan et al [103], that included 204 patients, analyzed aPL stability over time in different laboratories across United States. The authors found that 87% of initially positive LA, 75% of initially moderate to high positive (> 40 U) aCL and 76% of initially moderate to high positive (> 40 U) anti-β2GPI tests remained persistently positive in the follow-up. Aspirin, VKA or HCQ use did not differ among patients whose aPL title change or not. Mean follow-up time was between 1 (for anti- β2GPI) and 3.5 years.

In a study by Pengo et al. published in 2013 [104], that assessed the confirmation of aPL profile after 3 months in 161 patients who originally tested positive for one or more aPL, the authors found that among subjects with triple positivity at initial testing, 98% (53 of 54) had their aPL profile confirmed after 12 weeks, while the double-positive and single-positive groups had aPL confirmed in 42 of 50 (84%) and 23 of 57 (40%) subjects, respectively, therefore configuring triple positivity as highly predisposing to aPL confirmation on repeated test with single positivity far less commonly confirmed.

In a more recent study, Devignes et al. analyzed the persistence (defined as positivity in all determinations) of aPL beyond 12 weeks (median follow-up of 56 weeks) in 124 patients with baseline positive aPL in 2 determinations at least 12 weeks apart. They found that aPL remained positive in > 93% of cases, having double and triple positive patients the highest rate of persistence (96.8% and 97.9% respectively) [105] and that the extended persistence group had more frequent LA positivity and higher aCL-IgG titers.

Recently, authors from the Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION) published the results of their prospective and retrospective analysis of aPL-positive patients with or without systemic autoimmune diseases

(AID) [106]. They found that 78% of patients presented a so-called stable aPL profile, defined as LA and/or IgG or IgM aCL and/or IgG or IgM anti-β2GPI ≥40 U in at least two-thirds of follow-up measurements. Patients with stable aPL had higher aCL and anti-β2GPI titers at baseline. In the univariate analysis, baseline triple positivity decreased the likelihood of transient positivity (OR 0.25, 95% CI 0.10-0.64), while, curiously, isolated LA positivity (OR 3.3, 95% CI 1.53-7.13) increased it. They speculated that this last finding could be related to the high number of patients under VKA therapy. Mean follow-up time was 5.1 years.

In contrast with such high rate of aPL persistence, a previous retrospective study evaluating the serological course of aPL in 105 fertile women with positive aPL (49 PAPS, 42 aPL positive in at least two occasions 12 weeks apart but not fulfilling criteria for APS and 14 SLE patients), characterized by a longer follow-up (mean 114.4 months), found that only 25.7% of patients were persistently positive while 59% of patients resulted persistently negative. Persistent positivity was defined as positive aPL in at least 2/3 of total determinations and persistent negativity as negative aPL in at least the last 2 determinations drawn more than 6 months apart. Smoking was an independent risk factor for persistent positivity in the multivariate analysis. Moreover, there was a linear association between the number of positive antibodies and persistent positivity. Finally, patients with persistent aPL and higher aPL levels presented higher risk of pregnancy morbidity [107].

In summary, the present evidence indicates that baseline aPL results tend to remain stable over a short period of time (< 5 years), while aPL might tend to become negative during longer follow-up. Baseline multiple positivity, especially triple, predisposes to aPL persistence, as do higher aPL titers, while the role of single positivity is less clear, with some studies reporting association with transient aPL profile [104], while others with extended positivity [105].

GAPSS, aGAPSS and clinical recurrence

The GAPSS was introduced in 2013 by Sciascia et al. as a risk tool for predicting clinical manifestations of APS in SLE patients [95]. The authors divided their cohort of 211 SLE patients in a development (106 pts) and a validation (105 pts) cohort. To set up the score, they performed a multivariate logistic regression analysis in the development cohort and assigned to each risk factor for thrombosis and/or pregnancy loss, identified by multivariate analysis, weighted points

proportional to the β regression values: the coefficient of each variable was divided by the lowest value (corresponding to arterial hypertension) and rounded to nearest integer (Table 6).

Table 6. GAPSS score calculation.

	β-coefficient	GAPSS
Hyperlipidemia	1.73	3
Arterial Hypertension	0.54	1
aCL IgG/IgM	2.63	5
anti-β2GPI IgG/IgM	2.02	4
aPS/PT IgG/IgM	1.78	3
LA	2.35	4

Adapted from Sciascia et al. GAPSS the Global Anti-Phospholipid Syndrome Score. Rheumatology 2013;52:1397-1403. Assignment of points to each factor based on the formula $GAPSS = \beta x / \beta min$ where βx is the β coefficient of the x variable and βmin is the lowest β value among the significant ones identified by the multivariate analysis.

Both in the development and validation cohort the authors found higher GAPSS values in patients who experienced thrombosis and/or pregnancy loss in comparison to patients who did not (9.3 vs 5.3 for the development cohort and 9.5 vs 3.9 for the validation cohort). Higher values were also seen when subclassifying patients according to the clinical manifestation (thrombosis or pregnancy loss). GAPSS values ≥ 10 had the best diagnostic accuracy (AUC 0.736).

The same group prospectively validated the score in a study including 51 SLE patients enrolled in the ALIWAPAS trial, a randomized controlled trial that investigated the efficacy of LDA vs LDA plus low-intensity warfarin for primary thrombosis prevention in aPL-positive patients with SLE [108]. The authors found an increase in the GAPSS from baseline to last visit in patients who experienced thrombosis (GAPSS mean 7.5 vs 10.0), that was absent in patient without vascular events. Even when GAPSS was analyzed yearly, an increase was only seen in patients who experienced thrombosis. An increase of more than 3 points of the GAPSS showed the best risk accuracy for vascular events (HR 48, 95% CI 6.9-333.9). Finally, an increase of both the GAPSS and the aGAPSS was associated with higher risk of vascular events (RR 12.3 and 8.6 respectively). When the same group analyzed GAPSS/aGAPSS performance in 62 patients with primary APS, the authors found that patients with thrombosis presented higher GAPSS (11.5 vs 8.7) and

aGAPSS (10.7 vs 7.1) in comparison to patients with pregnancy loss alone, and that patients with thrombosis recurrence had higher GAPSS (13.7 vs 9.4) and aGAPSS (12.1 vs 7.8) compared to patients without recurrence. GAPSS values ≥ 11 were associated with the highest risk of recurrence (OR 18.27, 95% CI 3.74-114.5) [109].

The role of aGAPSS as predictor of thrombosis recurrence was then retrospectively analyzed by the APS-ACTION group. In this study, that included 379 patients with APS and history of thrombosis, Radin et al. found that patients with thrombotic recurrence had higher aGAPSS (measured at registry entry) than patients without recurrence (7.8 vs 6). When making a separate analysis based on the site of recurrence, arterial but not venous recurrent thrombosis was associated with higher aGAPSS compared to no recurrence [110].

HYPOTHESIS

Data on the course of aPL tests over time are limited and mostly with a short follow-up (far below 5 years except in one case), and most studies involved different centers, each testing aPL in its own laboratory, often with different techniques and cut-off values. Furthermore, most literature describes aPL course in aPL-positive patients, with or without APS and SLE. Performing aPL testing in the same reference laboratory increases the validity of the results, while the presence of clinical manifestations of APS reduces the likelihood of transient aPL positivity.

Various authors associated the one-time GAPSS/aGAPSS with the recurrence of thrombosis in patients with APS [109–112]. However, aPL levels fluctuate over time [106,113,114], ranging from medium-high positivity to negativity while cardiovascular risk factors can be modified by lifestyle and treatment. Moreover, the only prospective study performed so far, has shown that the score tends to increase in patients who undergo vascular events [108]. Therefore, a single assessment of the GAPSS/aGAPSS might not reflect the average risk state of a patient, and the GAPSS/aGAPSS value immediately close to a clinical manifestation, i.e. thrombosis, can be different from another time-point. Conversely, a longitudinal evaluation of the score likely gives a more reliable picture of patient's risk.

In a disease that has a chronic and recurrent nature such as APS, it is crucial to have access to risk stratification and damage assessment tools. While disease activity tools have been successfully implemented in several AID such as SLE [115,116], Sjögren's Syndrome [117] and inflammatory myopathies [118], a disease activity index specific for APS is still lacking. This is due to the pathophysiology of APS, in which inflammation does not play a major role as in other AID, and thrombosis is the main cause of damage. In the absence of a disease activity index, a dynamic score such as the GAPSS/aGAPSS that is related to clinical manifestations, could be used as surrogate of disease activity and predictor of damage.

In the present thesis we aim to test, in a cohort of patients with diagnosis of APS, the hypothesis that aPL persistence, measured as rate of persistent positive aPL patients during follow-up and

as part of the mean aGAPSS score over time, predisposes to thrombosis recurrence. In detail, the thesis aims to test the following hypotheses:

1. aPL remain persistently positive over time even in a follow-up longer than 5 years and aPL persistence is associated with the recurrence of thrombosis and pregnancy morbidity.
2. Patients with a higher average aGAPSS over time, measured as a mean of repeated annual aGAPSS values, present a higher rate of clinical recurrence and average aGAPSS shows different values compared to one-time aGAPSS and different associations with the outcomes.
3. Mean aGAPSS over time, used a surrogate of disease activity, correlates with damage accrual measured as DIAPS change in the follow-up.

OBJECTIVES

Overall objectives

1. Describe the course of aPL positivity over time, assess factors associated with aPL persistence and association between aPL persistence and recurrence of clinical manifestations in a cohort of APS patients.
2. Assess if a higher mean aGAPSS over time is associated with the recurrence of thrombosis and pregnancy morbidity in APS.
3. Test if a higher mean aGAPSS correlates with higher damage accrual during follow-up.

Specific objectives

First study

- Analyze the rate of aPL persistence, defined as positivity in at least two thirds of aCL IgG/IgM, anti-β2GPI IgG/IgM and LA annual determinations, in a cohort of APS patients with a median follow-up longer than 14 years.
- Assess which baseline aPL profiles predispose to aPL persistence over time.
- Check if time influences the odds of aPL persistence.
- Assess if aPL persistence is associated with the recurrence of thrombosis and pregnancy morbidity in APS.

Second study

- Assess if a higher mean aGAPSS over time, measured as a mean of at least 3 annual aGAPSS values, predisposes to recurrence of thrombosis and/or pregnancy morbidity in APS, despite proper treatment (assessed as percentage of time spent within the therapeutic range in patients under VKA therapy).
- Check if the delta of the aGAPSS, measured as the difference between the aGAPSS before clinical recurrence (if present) or at the end of follow-up (in case of no clinical recurrence) and the basal aGAPSS, correlates with clinical recurrence.

- Assess if the mean and delta aGAPSS differ from baseline aGAPSS in terms of association with clinical manifestations, to confirm its evolving nature and the need for longitudinal monitoring.
- Check if the mean and delta aGAPSS values differ between patients with thrombotic and obstetric recurrence, and, within the thrombotic group, between patients with arterial recurrence and patients with venous recurrence.
- Identify the mean aGAPSS cut-off value that best predicts clinical recurrence.

Third study

- Assess if the mean aGAPSS over time correlates with damage accrual, measured as the difference between the DIAPS at the end of follow-up and the basal DIAPS, and therefore can be used a surrogate of disease activity.
- Identify other factors that correlate with damage accrual.
- Describe the rate of involvement of different DIAPS domains in our cohort of APS patient at APS diagnosis and last visit and investigate if there are differences between thrombotic and non-thrombotic APS.
- Compare the mean aGAPSS value in patients with high vs low damage accrual (setting a cut-off for DIAPS change of one) and identify predictors of high damage accrual.
- Analyze the association between DIAPS and mortality.

RESULTS (PUBLISHED STUDIES)

First study

Barilaro G, Coloma-Bazan E, Chacur A, Della Rocca C, Perez-Isidro A, Ruiz-Ortiz E, Viñas O, Tàssies-Panella D, Reverter JC, Molina-Andujar A, Cervera R, Espinosa G

Persistence of antiphospholipid antibodies over time and its association with recurrence of clinical manifestations: A longitudinal study from a single centre

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Persistence of antiphospholipid antibodies over time and its association with recurrence of clinical manifestations: A longitudinal study from a single centre

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ABSTRACT

Purpose: To analyze the antiphospholipid antibody (aPL) persistence over time in patients with antiphospholipid syndrome (APS) and its association with clinical recurrence and to identify predictors of aPL persistence over time.

Patients and methods: 200 patients with a diagnosis of APS and at least three follow-up aPL determinations were included. Persistent aPL profile was defined as the presence of lupus anticoagulant (LAC) and/or IgG/IgM anticardiolipin (aCL) and/or IgG/IgM anti-β2 glycoprotein-I (aβ2GPI) (> 99th percentile) antibodies in at least 66% of follow-up measurements. Multilevel mixed-effect generalized linear models with logit link were used.

Results: 112 (56%) patients maintained persistent aPL profiles over time, while 88 (44%) were transient. Median follow-up time was 172.5 months. Follow-up time did not affect the odds of aPL persistence in multivariate analysis ($p = 1.00$). Baseline triple aPL positivity [OR 78 (95%CI 16.9–359.7, $p < 0.001$)] and double aPL positivity [OR = 7.6 (95%CI 3.7–15.7, $p < 0.001$)] correlated with persistent aPLs over time, while isolated LAC [OR = 0.26 (95% CI 0.08–0.49, $p = 0.002$)] or isolated IgG/IgM aCL [OR = 0.20 (95% CI 0.11–0.59, $p = 0.004$)] positivity, were predictors of transient aPL profile. Patients with persistent aPLs had higher rate of clinical recurrence in comparison to patients with transient aPLs [OR = 2.48 (95%CI 1.34–4.58, $p = 0.003$)].

Conclusions: More than half of patients with baseline medium-high titer aPL positivity had persistent positive aPLs over time. Patients with persistent aPLs were more prone to present recurrence of clinical manifestations. Multiple aPL positivity increased the odds of a persistent aPL profile over time, while isolated LAC and aCL positivity decreased it.

1. Introduction

Antiphospholipid syndrome (APS) is a hypercoagulability state characterized by the development of arterial, venous and/or microvascular thrombosis, pregnancy morbidity (recurrent early miscarriages, fetal deaths after the 10th week of gestation and/or premature births) and, frequently, hematologic complications (such as hemolytic anemia and thrombocytopenia) associated to the presence of antiphospholipid antibodies (aPLs) [1,2]. Three aPLs are included in the current classification criteria [3], namely lupus anticoagulant (LAC), IgG and IgM anticardiolipin (aCL), and IgG and IgM anti-β2-glycoprotein-I antibodies (aβ2GPI) [4].

It is a shared opinion that the association with other systemic autoimmune diseases increases the risk of thrombosis in APS [5,6], while

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other conventional risk factors such as smoking, diabetes mellitus, oral contraceptives, congenital thrombophilias, arterial hypertension and dyslipidemia can act as "second hit" triggering thrombosis [1,7]. Moreover, specific aPL profiles, such as presence of LAC [8,9] or triple aPL positivity [10,11], and the rate of aPL persistence over time [12], are associated with APS related clinical manifestations. In general, low titer aPL positivity (especially if transiently positive) bears a low risk of clinical manifestations, and is usually associated to infections, drugs or malignancies [13]. On the contrary, persistently medium-high titer positive aPLs are more likely to have important clinical implications [14]. Therefore, the assessment of aPL profile and persistence upon evaluation of aPL-positive patients is crucial.

Data on the course of aPL positivity over time are limited. Recently, authors from the Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION) published the results of their prospective and retrospective analysis of patients with aPLs with or without systemic autoimmune diseases [15]. They found that baseline triple positivity increased the likelihood of a so-called stable aPL profile (defined as LAC and/or IgG or IgM aCL and/or IgG or IgM aβ2GPI ≥40 U in at least two-thirds of follow-up measurements), while, curiously, isolated LAC positivity decreased it. In their cohort they included patients with no definite diagnosis of APS, who are more likely to present transient aPL positivity. Moreover, since the study was multicentric, different laboratories with different techniques and cut-off values for aPLs were involved.

Performing aPL testing in the same reference laboratory increases the validity of the results, while the presence of clinical manifestations typical of APS reduces the likelihood of transient aPL positivity. Having in mind these premises, we decided to perform a single centre longitudinal study with the primary objective of assessing the trend of aPL positivity over time in APS patients. Secondary objectives were to identify clinical and laboratory features associated with persistent aPL positivity and predictors of persistent aPL profile over time.

2. Material and methods

2.1. Patients

The study cohort was selected among the 347 individuals with a diagnosis of APS, followed at our reference centre (Department of Autoimmune Diseases at the Hospital Clínic of Barcelona) between February 1985 and February 2022. Inclusion criteria were diagnosis of APS as per current classification criteria [3], and at least three annual determinations of the three "criteria" aPLs.

Clinical data, including demographics, APS-related manifestations (including non-criteria manifestations, such as chorea, epilepsy, headache, myelitis, cardiac valve disease, livedo reticularis/racemosa, skin ulcers, thrombocytopenia, autoimmune hemolytic anemia, and aPL nephropathy) and cardiovascular risk factors (smoking, diabetes mellitus, arterial hypertension, dyslipidemia, surgery, nephrotic syndrome, oral contraceptive use, hypothyroidism and congenital thrombophilias), as well as laboratory data, associated systemic autoimmune diseases, ongoing and previous treatment such as low-dose aspirin (LDA), low-molecular-weight heparin (LMWH), vitamin K antagonists (VKA), hydroxychloroquine and corticosteroids were collected at baseline and annually.

The study was conducted in accordance with the declaration of Helsinki [16] and received approval from the Hospital Clínic Ethics Committee (HCB/2016/0401). All patients gave their informed consent to participate and publish study results.

2.2. Antiphospholipid antibodies and autoimmunity laboratory markers

The IgG and IgM aCL and aβ2GPI autoantibodies were measured using solid-phase standardized immunoassays: enzyme-linked immunosorbent assay (ELISA) (Aeskulisa, Aesku-Diagnostics) or chemiluminescence immunoassay (CLIA) (QUANTA Flash®, Inova Diagnostics, CA). The cut-off recommended by the manufacturer were 15 GPL-MPL/ml and 20 chemiluminescent units (CU), respectively. LAC test was performed following the guidelines of the Subcommittee on Lupus Anticoagulant/Phospholipid-dependent Antibodies of the International Society of Thrombosis and Hemostasis (SSC-ISTH) recommendations [17,18]. aPL persistence was defined as a positive result of LAC and/or aCL IgG or IgM and/or aβ2GPI IgG or IgM at medium-high titer (> 99th percentile as per our reference laboratory) in at least two-thirds (66%) of total follow-up aPL determinations.

Antinuclear antibodies (ANA) were measured by indirect immunofluorescence (IIF) on rodent liver cells and/or HEp-2 cells (titers above 1:80 being considered positive), anti-double stranded DNA (dsDNA) autoantibodies were measured by ELISA or CLIA and/or IIF on Crithidia Luciliae and autoantibodies against extractable nuclear antigen (ENA: Ro60/SSA, La/SSB, Sm and U1-RNP) by ELISA or CLIA.

2.3. Statistical analysis

Characteristics of patients with persistent and transient aPL profile were compared. Categorical variables are presented as numbers and percentages and were compared using the chi-square test or Fisher's exact test whenever appropriate. Continuous variables are presented as means ± standard deviation (SD) or medians with interquartile range (IQR) and were compared by Student's unpaired 2-tailed *t*-test or Wilcoxon rank-sum (for non-normally distributed variables). Levene's test for equality of variances was used to test for variance homogeneity between groups. Multilevel mixed-effect generalized linear model (MEGLM) with logit link was used to assess the effect of time on stable aPL profile over time, introducing random effects to account for within-subject correlation due to repeated measures of aPL profile across follow-up. Univariate and multivariate logistic regression analysis were used to examine predictors of stable aPL profile over time taking into account possible confounding factors and were expressed as Odds Ratio (OR) with 95% confidence interval (95% CI). A two-tailed p value <0.05 was considered statistically significant. Data were analyzed using STATA 17 (StataCorp, College Station, Texas 77845 USA).

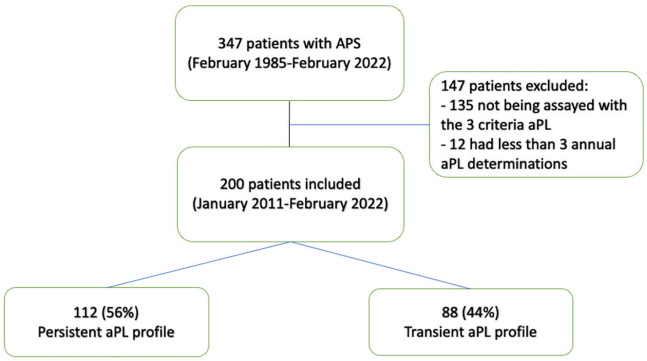
3. Results

3.1. Patients' characteristics

While aCL and LAC tests were available throughout the entire study period, the aβ2GPI test was implemented in our laboratory only since January 2011. Therefore, after excluding 147 patients, 135 for not having been assayed with all the three tests and 12 with less than three aPLs determinations, the final cohort included two hundred patients followed between January 2011 and February 2022 (Fig. 1). Among them, 138 (69%) patients had primary APS and 62 (31%) patients with APS associated to other autoimmune diseases: 41 patients with systemic lupus erythematosus (SLE), 17 with features of SLE not fulfilling current classification criteria [19] (classified as SLE-like), two with systemic sclerosis, one with Sjögren's syndrome and one with Behçet's disease. Mean age at diagnosis was 41.7 (SD 13.3) years, 72% of patients were female. The clinical manifestation that led to APS diagnosis was thrombosis in 133 (66.5%) patients, pregnancy morbidity in 42 (21%) patients, both in 19 patients (9.5%), and others (thrombocytopenia, false positive syphilis test, and prolonged activated partial thromboplastin time) in 6 (3%) patients. Median follow-up duration was 172.5 months (IQR 120–240). Median number of aPL tests was 6 (IQR 4–9). The main characteristics of the APS patients according to aPL persistence are described in Table 1.

3.2. aPL profile persistence over time

112 (56%) patients presented a persistent aPL positivity during



Abbreviations: APS: antiphospholipid syndrome; aPL: antiphospholipid antibodies.

Fig. 1. Flow-chart of the patients included in the study.

follow-up while 88 (44%) had transient aPL positivity (Fig. 1). When comparing patients with primary APS to patients with APS associated to other autoimmune conditions, the latter presented a higher rate of persistent aPLs (50% vs 69.3%; $p = 0.01$). No specific thrombotic or obstetric manifestations at the APS diagnosis were associated with aPL persistence. Among non-criteria APS manifestations, cardiac valve disease (16.1% vs 3.4%, $p = 0.004$) was more frequent in patients with persistent aPL profile.

Multivariate MEGLM adjusted for sex, age at diagnosis, smoking, concomitant autoimmune disease, and corticosteroids use at baseline, indicated that time across follow-up did not increase or decrease the odds of aPL persistence over time ($p = 1.00$).

When assessing the association between different aPL profiles and persistent vs transient aPL positivity at follow-up, we found that aCL IgM (37.5% vs 17.1%, $p = 0.001$), aβ2GPI IgG (51.8% vs 14.7%, $p < 0.001$) and IgM (25.6% vs 11.3%, $p = 0.02$), LAC (78.6% vs 55.7%, $p = 0.01$), double positivity (79.5% vs 36%, $p < 0.001$), and triple positivity (57.1% vs 2.3% $p < 0.001$) at baseline, were significantly more frequent in patients with aPL persistence over time. Conversely, isolated aCL IgG/IgM (9.8% vs 30.7%, $p < 0.001$) and isolated LAC (8% vs 25%, $p < 0.001$) positivity were more frequent in patients with transient aPL profiles at follow-up (Table 1). Moreover, when analyzing the correlation between mean aCL and aβ2GPI IgG or IgM titers over time, measured by both ELISA and CLIA, and aPL persistence, we found that both aCL IgG and IgM, and aβ2GPI IgG and IgM titers were significantly higher in patients who presented a persistent aPL profile (Table 1).

3.3. Predictors of aPL profile persistence over time

In a univariate unadjusted logistic model with stable aPL profile as the outcome, baseline aCL IgM ($p = 0.002$), aβ2GPI IgG ($p < 0.001$), aβ2GPI IgM ($p = 0.02$), LAC ($p = 0.001$), double ($p < 0.001$) and triple ($p < 0.001$) aPL positivity at baseline increased the odds of persistent aPL profile at follow-up. Conversely, isolated aCL IgG/IgM ($p < 0.001$) and isolated LAC ($p = 0.002$) decreased the odds of stable aPL profile (Table 2).

In a multivariate logistic model adjusted for age at diagnosis, disease duration, gender, active smoking, concomitant autoimmune disease, corticosteroid treatment and baseline aPL tests, aCL IgM (OR 2.79, 95% CI 1.21–6.47, $p = 0.02$), aβ2GPI IgG (OR 4.14, 95%CI 1.86–9.23, $p = 0.001$), LAC (OR 3.58, 95% CI 1.59–8.06, $p = 0.001$), double (OR 7.61, 95% CI 3.67–15.74, $p < 0.001$), and triple (OR 78, 95% CI 16.93–359.65, $p < 0.001$) aPL positivity at baseline correlated with persistent aPL profile while isolated aCL IgG/IgM (OR 0.26, 95% CI 0.11–0.59, $p = 0.001$) and isolated LAC (OR 0.20, 95% CI 0.08–0.49, $p = 0.001$) decreased the odds of aPL persistence (Table 2).

3.4. Clinical recurrence

Patients with persistent aPL profile presented a higher rate of recurrence of clinical manifestations (thrombosis and/or pregnancy morbidity) in comparison to patients with transient aPL profile (43.8% vs 23.9%) with an OR = 2.48 (95% CI 1.34–4.58, $p = 0.003$). The rate of thrombotic recurrence was also higher in patients with persistent aPL profile (40.2% vs 18.2%) with an OR = 3.02 (95% CI 1.57–5.81, $p < 0.001$). No significant difference was found in the rate of smoking, cardiovascular risk factors, diabetes mellitus, previous surgery, oral contraceptives, and congenital thrombophilia between patients who had clinical recurrence and patients who had not.

4. Discussion

The pattern of aPL persistence over time is crucial in assessing the risk of clinical manifestations in APS. However, data on the course of aPL tests over time are limited and with a follow-up of 5 years or shorter [15,20,21]. Moreover, all the literature published so far included either patients with positive aPLs (with or without a diagnosis of APS) [15,20,21] or patients with SLE [12,22]. To our knowledge this is the first study analyzing the trend of aPL positivity over time in a cohort of APS patients.

In our study population, we found that aPLs were classifiable as persistent (positive in >2/3 of total determinations) in 56% of APS patients, a rate slightly lower when compared to that of the APS-ACTION cohort [15], where patients were followed up for a median of 5 years. Interestingly, APS associated to other autoimmune conditions showed a higher rate of aPL persistence over time, close to the one of APS-ACTION cohort. Devignes et al. retrospectively analyzed their database of aPL measurements finding a very high rate of extended persistence of aPLs, ranging from 89.6% to 97.9% (for baseline triple positivity) of patients. However, the median follow-up duration was about 1 year (56 weeks) [20]. Furthermore, Erkan et al. [21]in a previous study, found that baseline aPLs remained stable for at least three quarter of subsequent tests, with no influence of treatment on persistence. Nevertheless, the mean follow-up time ranged between 1 and 3.5 years. A possible

Table 1
Demographic characteristics, clinical manifestations, immunological features, and treatment of patients with persistent/transient aPL profile at follow-up.

	Entire series (N = 200)	Persistent aPL profile (N = 112)	Transient aPL profile (N = 88)	p value
Female Sex, n (%)	144 (72)	77 (68.8)	67 (76.1)	ns
Age at diagnosis, mean (SD), years	41.7 (13.3)	41.4 (13.1)	42.1 (13.5)	ns
Disease duration median (IQR), months	172.5 (120–240)	169.7 (96.7)	183.9 (86.5)	ns
APS Type, n (%)				
Primary	138 (69)	69 (61.6)	69 (78.4)	**0.01**
APS associated to other AID [1]	62 (31)	43 (38.4)	19 (21.6)	**0.01**
Thrombosis, n (%)				
Arterial (total)	83 (41.5)	52 (46.4)	31 (35.2)	ns
Venous (total)	99 (49.5)	58 (51.2)	41 (46.6)	ns
Both	19 (9.5)	13 (11.6)	6 (6.8)	ns
Recurrent thrombosis	61 (30.5)	45 (40.2)	16 (18.2)	**0.001**
Pregnancy losses, n (%)				
First trimester miscarriages [2]	56 (29.8)	27 (24.1)	29 (33)	ns
Fetal losses [3]	44 (23.4)	24 (21.4)	20 (22.7)	ns
Non criteria manifestations, n (%)				
Chorea	1 (0.5)	1 (0.9)	0 (0)	ns
Epilepsy	11 (5.5)	7 (6.3)	4 (4.6)	ns
Migraine	20 (10)	13 (11.6)	7 (8)	ns
Myelitis	2 (1)	2 (1.8)	0 (0)	ns
Cardiac valve disease	21 (10.5)	18 (16.1)	3 (3.4)	**0.004**
Livedo reticularis/ racemosa	11 (5.5)	7 (6.3)	4 (4.6)	ns
Skin ulcers	4 (2)	2 (1.8)	2 (2.3)	ns
Thrombocytopenia	41 (20.5)	26 (23.2)	15 (17)	ns
Haemolytic anemia	5 (2.5)	4 (3.6)	1 (1.1)	ns
aPL nephropathy	6 (3)	5 (4.5)	1 (1.1)	ns
Immunological features, n (%)				
ANA	138 (69)	89 (80.2)	49 (55.7)	**<0.001**
Anti-dsDNA antibody	50 (25)	37 (33.6)	13 (14.9)	**0.003**
Anti-Ro/SSA antibody	13 (6.5)	9 (8.4)	4 (4.7)	ns
Anti-La/SSB antibody	6 (3)	5 (4.7)	1 (1.1)	ns
Anti-RNP antibody	12 (9)	9 (8.5)	3 (3.7)	ns
Baseline aPLs, n (%)				
aCL IgG	119 (59.5)	73 (65.2)	46 (52.3)	0.07
aCL IgM	57 (28.5)	42 (37.5)	15 (17.1)	**0.001**
aβ2GPI IgG	71 (35.5)	58 (51.8)	13 (14.7)	**<0.001**
aβ2GPI IgM	40 (20)	29 (25.6)	11(11.3)	**0.02**
LAC	137 (68.5)	88 (78.6)	49 (55.7)	**0.01**
Isolated aCL positivity (IgG/IgM)	38 (19)	11 (9.8)	27 (30.7)	**<0.001**
Isolated aβ2GPI positivity (IgG/IgM)	6 (3)	3 (2.7)	3 (3.4)	ns
Isolated LAC positivity	31 (15.5)	9 (8)	22 (25)	**0.001**
Double positivity [4]	125 (62.5)	89 (79.5)	36 (40.9)	**<0.001**
Triple positivity	66 (33)	64 (57.1)	2 (2.3)	**<0.001**
aPL titers mean (SD)				
aCL IgG ELISA	127	89.4 (94.2)	6.1 (12.7)	**<0.001**
aCL IgG CLIA	157	324.5 (489.3)	7.8 (9.6)	**<0.001**
aCL IgM ELISA	127	28.7 (41.9)	4 (4.9	**<0.001**
aCL IgM CLIA	155	34.8 (45.9)	5.6 (7.9)	**<0.001**
aβ2GPI IgG ELISA	137	84.2 (89.9)	5.6 (7.9)	**<0.001**
aβ2GPI IgG CLIA	158	652.2 (1105.7)	57.3 (237.4)	**<0.001**
aβ2GPI IgM ELISA	137	64 (81.5)	11.1 (26.8)	**<0.001**
aβ2GPI IgM CLIA	156	51.8 (73.6)	8.3 (23.1)	**<0.001**
Treatment, n (%)				
LDA	66 (33)	32 (28.6)	34 (38.6)	ns
VKA	131 (65.5)	81 (72.3)	50 (56.8)	**0.02**
LMWH	18 (9)	12 (10.7)	6 (6.8)	ns
Corticosteroids	11 (5.5)	7 (6.3)	4 (4.6)	ns

Statistically significant p values are bold. Abbreviations: aβ2GPI: anti-beta2-glycoprotein I antibodies; aCL: anticardiolipin antibodies; AID: autoimmune diseases; ANA: antinuclear antibodies; aPL: antiphospholipid antibodies; APS: antiphospholipid syndrome; CLIA: chemiluminescence immunoassay; ds-DNA: double stranded DNA; ELISA: enzyme-linked immunosorbent assay; LAC: lupus anticoagulant; LDA: low dose aspirin; LMWH: low-molecular-weight-heparin; RNP: ribonucleoprotein; VKA: vitamin K antagonist.

[1] Other AID include: 41 patients with SLE, 17 patients with "SLE-like", two patients with systemic sclerosis, one patient with Sjögren syndrome and one with Behçet's disease.

[2] Three consecutive unexplained spontaneous abortions before 10th week.

[3] Unexplained fetal death at or beyond 10th week.

[4] Any combination of two positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria.

Table 2
Univariate and multivariate analysis of baseline predictors of persistent aPL profile at follow-up.

	Odds Ratio (95% CI)	p value
UNIVARIATE ANALYSIS		
Gender (female)	0.69 (0.37–1.30)	0.25
Age at diagnosis	1.00 (0.98–1.02)	0.72
Disease duration	1.00 (0.99–1.01)	0.28
Presence of AID[1]	2.26 (1.20–4.27)	**0.01**
Corticosteroid treatment	1.40 (0.40–4.94)	0.60
Active smoking	1.20 (0.64–2.26)	0.56
Baseline aPLs		
aCL IgG	1.71 (0.97–3.03)	0.07
aCL IgM	2.92 (1.49–5.73)	**0.002**
aβ2GPI IgG	6.11 (3.05–12.3)	**<0.001**
aβ2GPI IgM	2.41 (1.13–5.16)	**0.02**
LAC	2.92 (1.57–5.41)	**0.001**
Isolated aCL positivity (IgG/IgM)	0.25 (0.11–0.53)	**<0.001**
Isolated aβ2GPI positivity (IgG/IgM)	0.78 (0.15–3.96)	0.764
Isolated LAC positivity	0.26 (0.11–0.60)	**0.002**
Double positivity [2]	5.59 (2.99–10.4)	**<0.001**
Triple positivity	57.3 (13.4–244.7)	**<0.001**
MULTIVARIATE ANALYSIS		
aCL IgM[3]	2.79 (1.21–6.47)	**0.02**
aβ2GPI IgG[4]	4.14 (1.86–9.23)	**0.001**
aβ2GPI IgM[5]	2.24 (0.79–6.36)	0.13
LAC[6]	3.58 (1.59–8.06)	**0.001**
Isolated aCL positivity (IgG/IgM)[7]	0.26 (0.11–0.59)	**0.001**
Isolated LAC positivity[7]	0.20 (0.08–0.49)	**0.001**
Double positivity[7]	7.61 (3.67–15.74)	**0.002**
Triple positivity[7]	78 (16.93–359.65)	**<0.001**

Statistically significant p values are bold. Abbreviations: aβ2GPI: anti-beta2-glycoprotein I antibodies; aCL: anticardiolipin antibodies; AID: autoimmune diseases; aPL: antiphospholipid antibodies; LAC: lupus anticoagulant.

[1] AID include: 17 patients with "SLE-like", two patients with Systemic Sclerosis, one patient with Sjögren syndrome and one with Behçet's disease.

[2] Any combination of two positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria.

[3] Adjusted for age at diagnosis, disease duration, gender, active smoking, concomitant autoimmune disease corticosteroid treatment, LAC, aCL IgG and aβ2GPI IgG and IgM presence at baseline.

[4] Adjusted for age at diagnosis, disease duration, gender, active smoking, concomitant autoimmune disease, corticosteroid treatment, LAC, aCL IgG and IgM and aβ2GPI IgM presence at baseline.

[5] Adjusted for age at diagnosis, disease duration, gender, active smoking, concomitant autoimmune disease, corticosteroid treatment, LAC, aCL IgG and IgM and aβ2GPI IgG presence at baseline.

[6] Adjusted for age at diagnosis, disease duration, gender, active smoking, concomitant autoimmune disease, corticosteroid treatment, aCL IgG and IgM and aβ2GPI IgG and IgM presence at baseline.

[7] Adjusted for age at diagnosis, disease duration, gender, active smoking, concomitant autoimmune disease, and corticosteroid treatment.

explanation for the lower rate of persistence in our population is that aPLs could tend to become negative during follow-up, as has been shown in up to 59% of women with positive aPLs [23]. This negativization seems to affect more frequently aCLs [10]. These results need

confirmation in prospective studies with long follow-up time.

Double and triple aPL positivity correlated with aPL persistence, as did aCL IgM, aβ2GPI IgG/IgM, and LAC positivity in the setting of multiple positive aPLs. These results, that partially confirm the ones of the APS-ACTION cohort, are not surprising, since multiple (double and triple) positivity has been shown to be more frequently confirmed after 12 weeks (84 to 98%) than single test positivity (around 40%) [24]. Conversely, when analyzing single positivity, isolated aCL IgG/IgM and LAC presence were associated with transient aPL profile. As in the APS-ACTION cohort, the latter finding might be related to the high number of patients under VKA therapy, which increases the likelihood of false positive results in the LAC test.

Our results confirm the hypothesis that higher aPL titers are associated with a higher rate of aPL persistence over time, while low aPL titers are usually transient, as found in the study from the APS-ACTION group [15]. In fact, regardless the laboratory technique used to measure aCL and aβ2GPI (ELISA or CLIA), patients with persistent aPL profile over time had significantly higher titer in comparison to patients with transient profile.

When assessing predictors of persistent aPL profile in the multivariate analysis, we adjusted for several confounding factors known to influence aPL persistence, such as association with other autoimmune diseases, smoking and corticosteroids, finding an increase of the odds aPL persistence of >7 times for double positivity and 78 times for triple. Conversely, isolated aCL IgG/M and LAC increased the odds of transient aPL positivity. In the study from the APS-ACTION group, the authors found a similar correlation with triple aPL positivity, while isolated LAC and aβ2GPI, but not aCL, increased the odds of "unstable" aPL profile [15]. As already mentioned, in the case of LAC such finding might be related to the high rate of patients under anticoagulation and deserves to be clarified in future studies.

Recurrence of clinical manifestations is a main concern in APS. In fact, this condition is usually diagnosed in young people [25] and both thrombosis and pregnancy morbidity tend to recur despite treatment. For instance, despite anticoagulation, the 5-year rate of thrombosis recurrence can be as high as 16.6% [26]. Identifying markers of increased risk of recurrence is therefore of crucial importance in APS patients. LAC is considered the main risk factor for thrombosis recurrence among aPLs [8], while triple positivity has been associated with higher risk of thrombosis in APS [27,28]. Both were associated to aPL persistence in our population, and aPL persistence correlated to recurrence of cumulative (thrombotic and obstetric) and thrombotic manifestations in APS. A similar result was observed in the APS-ACTION study, where, among the 30 patients with thrombotic events at follow-up, 29 (97%) had a "stable" aPL profile. This might explain why the rate of VKA therapy was higher among patients with "stable" aPL profile.

One limitation of our study is its retrospective design, which implicates that APS clinical manifestations have been assessed "a posteriori". However, aPLs were assessed routinely at each visit; therefore, in case of recurrence, the laboratory evaluation preceded the clinical manifestation in most cases. For sure, a prospective design would be more suitable for assessing risk factors associated to clinical recurrence. Nevertheless, APS is a low prevalence condition, making a prospective study including a consistent number of patients hard to perform. Another limitation is that LAC test was performed under VKA treatment in approximately 65% of patients, a factor that increases the likelihood of false positive results. This can be a possible explanation of the association between isolated LAC positivity and transient aPLs at follow-up.

A main strength of our study is the long duration of follow-up (median of >10 years) allowing to observe the evolution of aPL positivity over a long time and its association with clinical manifestations. Moreover, we included a homogenous cohort of 200 patients from a single centre, all classified as having APS by fulfilment of classification criteria. Finally, all aPLs were tested in the same reference laboratory, with the same cut-off values, allowing a homogenous evaluation of positivity and therefore increasing the robustness of the results.

In summary, we performed a longitudinal assessment of aPLs finding that aCL IgM, aβ2GPI IgG, LAC, double, and triple aPL positivity at baseline increased the odds of aPL persistence over time and that aPL persistence is associated with both thrombotic and obstetric recurrence. In patients with persistent aPLs an intensive treatment regimen to prevent clinical recurrence should be considered. Besides classical therapies used in APS, several treatment strategies can be explored to reduce aPL titers. Hydroxychloroquine (HCQ) prevented thrombosis recurrence by significantly reducing aPL titers over an average 2.6-year follow-up in a randomized open label study [29]. Belimumab an inhibitor of the binding of soluble circulating B lymphocyte stimulator to its target receptors on B cells, has shown to reduce aPL titers in SLE patients independently from antimalarial therapy [30]. Rituximab, an anti-CD20 antibody, has shown efficacy in APS-associated thrombocytopenia, refractory to anticoagulation [31] and represents a treatment option in case of catastrophic APS [32]. These drugs, among others, may represent an effective adjuvant treatment for different clinical manifestations in APS.

5. Conclusions

We found that more than half of patients with APS maintained persistent aPL positivity over >10 years of follow-up. Multiple positivity at baseline increased the likelihood of aPL persistence in the follow-up. Persistent aPLs increased the odds of cumulative (thrombotic and obstetric) and thrombotic recurrence. Such results highlight the need of periodic monitoring of aPLs during follow-up.

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Ethics

The study complies with the declaration of Helsinki and received approval from the Hospital Clínic Ethics Committee (HCB/2016/0401). All subjects gave their informed consent to participate and publish study results.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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The objectives of the first study were to assess the rate of aPL persistence in a cohort of APS patients, to identify aPL profiles associated with persistence and to analyze the relationship between aPL persistence and recurrence of clinical manifestations. aPL persistence was defined as positivity in at least two thirds of aCL IgG/IgM, anti-β2GPI IgG/IgM and LA annual determinations. Among 347 individuals with a diagnosis of APS followed at our reference center, after excluding 147 subjects for not having at least three annual determinations of the three "criteria" aPL (aCL IgG/IgM, anti-β2GPI IgG/IgM and LA) tested, two hundred patients have been included. Median follow-up duration was 172.5 months (IQR 120-240).

Fifty-six percent of patients presented persistent aPL positivity, defined as positivity at medium-high titer in at least two thirds of total determinations. APS associated to other AID presented a higher rate of persistence compared to PAPS (69.3 vs 50%). When assessing the association between aPL persistence over time and specific aPL profiles at baseline we found that aCL IgM (37.5% vs 17.1%), anti-β2GPI IgG (51.8% vs 14.7%) and IgM (25.6% vs 11.3%), LA (78.6% vs 55.7%), double positivity (79.5% vs 36%), and triple positivity (57.1% vs 2.3%) were significantly more frequent in patients with persistent vs transient aPL over time. Conversely, isolated aCL IgG/IgM (9.8% vs 30.7%) and isolated LA (8% vs 25%) positivity were more frequent in patients with transient aPL profiles at follow-up. When comparing the mean aCL and anti-β2GPI IgG or IgM titers over time in patients with persistent vs transient aPL, we found that all were significantly higher in patients who presented a persistent aPL profile. Multilevel mixed-effect generalized linear model with logit link excluded the influence of time on the odds of aPL persistence over time.

In the multivariate logistic model adjusted for age at diagnosis, disease duration, gender, active smoking, concomitant AID, corticosteroid treatment and baseline aPL tests, aCL IgM (OR 2.79, 95% CI 1.21-6.47), anti-β2GPI IgG (OR 4.14, 95%CI 1.86-9.23), LA (OR 3.58, 95% CI 1.59-8.06), double (OR 7.61, 95% CI 3.67-15.74), and triple (OR 78, 95% CI 16.93-359.65) aPL positivity at baseline correlated with persistent aPL profile while isolated aCL IgG/IgM (OR 0.26, 95% CI 0.11-0.59) and isolated LA (OR 0.20, 95% CI 0.08-0.49) decreased the odds of aPL persistence.

Patients with persistent aPL presented a higher rate of recurrence of clinical manifestations (thrombosis and/or pregnancy morbidity) in comparison to patients with transient aPL (43.8% vs 23.9%) with an OR=2.48 (95% CI 1.34-4.58, p=0.003). The rate of thrombotic recurrence, analyzed separately, was also higher in patients with persistent aPL (40.2% vs 18.2%) with an OR=3.02 (95% CI 1.57-5.81, p<0.001).

We therefore concluded that more than half of patients with APS maintained persistent aPL positivity over more than 14 years of follow-up. Multiple positivity at baseline increased the likelihood of aPL persistence in the follow-up. Persistent aPL increased the odds of thrombotic and thrombotic plus obstetric recurrence.

Second study

Barilaro G, Estevez A, Della Rocca C, Perez-Isidro A, Araujo O, Pires da Rosa G, Ruiz-Ortiz E, Tàssies-Panella D, Viñas O, Reverter JC, Cervera R, Espinosa G

Predictive value of the Adjusted Global Anti-Phospholipid Syndrome Score on clinical recurrence in APS patients: A longitudinal study

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Clinical science

Predictive value of the adjusted Global Anti-Phospholipid Syndrome Score on clinical recurrence in APS patients: a longitudinal study

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Abstract

Objective: To assess the effect of the average adjusted global APS score (aGAPSS) over time on recurrence of clinical manifestations in APS patients through a retrospective longitudinal study.

Material and methods: The study included 200 patients with APS. The aGAPSS was calculated for each patient at baseline and on a yearly basis for either up to 6 years (minimum 3 years) or just before the clinical event in patients who experienced clinical recurrence. The mean score per patient was computed. In patients under vitamin K antagonists (VKA) the percentage of time spent within the therapeutic range (TTR) was calculated. Cox regression analysis was performed to determine the cut-off value of the aGAPSS with the strongest association with clinical recurrence.

Results: Higher average aGAPSS values were found in patients who experienced clinical recurrence in comparison to patients who did not [8.81 (95% CI 7.53, 10.08) *vs* 6.38 (95% CI 5.64, 7.12), $P = 0.001$], patients with thrombotic recurrence compared with patients with obstetric recurrence [9.48 (95% CI 8.14, 10.82) *vs* 4.25 (95% CI 0.85, 7.65), $P = 0.006$] and patients with arterial thrombosis compared with patients with venous thrombosis [10.66 (S.D. 5.48) *vs* 6.63 (S.D. 4.42), $P = 0.01$]. aGAPSS values >13 points were associated with the highest risk of recurrence in multivariate analysis [HR = 3.25 (95% CI 1.93, 5.45), $P < 0.0001$]. TTR was not statistically different between patients who had thrombosis recurrence and patients who had not.

Conclusions: Our data support the role of periodic (annual) monitoring of the aGAPSS score in predicting clinical recurrence in patients with APS.

Keywords: APS, adjusted global APS score, clinical recurrence, time spent within the therapeutic range

Introduction

Recurrence of clinical manifestations is a hallmark of APS, a hypercoagulability condition characterized by the development of arterial, venous and/or microvascular thrombosis, pregnancy complications (recurrent early miscarriages, foetal deaths after the 10th week of gestation and/or premature births) and, frequently, haematologic alterations (such as haemolytic anaemia and thrombocytopenia) associated to the presence of aPL [1]. Thrombosis recurrence can occur despite anticoagulation in APS. Generally, patients with arterial thrombosis [2], those with triple aPL positivity profile [3], with aPL persistence over time, and associated autoimmune diseases, especially SLE [4] have a higher risk of recurrence. In addition, acquired risk factors for thrombosis such as smoking, diabetes, oral contraceptives, menopause, congenital thrombophilias, arterial hypertension and dyslipidaemia can act as 'second hit' triggering thrombosis recurrence [5].

Among different score systems proposed as risk stratification tool for clinical manifestations in aPL-positive patients [6, 7], the Global APS Score (GAPSS) has reached increasing attention in recent years. GAPSS considers the aPL profile, including the three antibodies included in the current classification criteria plus the anti-phosphatidylserine-prothrombin complex (aPS-PT) antibodies, along with cardiovascular risk factors, namely arterial hypertension and dyslipidaemia. It has been suggested as a tool for predicting thrombotic and obstetric manifestations in APS and validated both in patients with APS and SLE [8–13]. An 'adjusted' version (aGAPSS), not including aPS-PT, an aPL that is not routinely tested in most laboratories, has also been validated [14, 15].

Various authors associated the basal GAPSS/aGAPSS with the recurrence of thrombosis in patients with APS [8, 9, 12, 16]. However, aPL levels fluctuate over time, ranging from medium-high positivity to negativity [17–19], while

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Rheumatology key messages

- Adjusted global APS score is a tool to predict clinical recurrence in APS.
- Antiphospholipid antibodies and cardiovascular risk factors can change over time.
- Periodic monitoring of the aGAPSS improves the assessment of risk of clinical recurrence in APS.

cardiovascular risk factors can be modified by lifestyle and treatment. Therefore, basal GAPSS/aGAPSS might not reflect the average risk state of a patient, and the GAPSS/aGAPSS value prior to clinical recurrence can be different from the basal one.

Moreover, patients with thrombotic APS treated with vitamin K antagonists (VKA) can suffer from fluctuations in the anticoagulation intensity, measured by international normalized ratio (INR), because of several factors, such as scarce adherence to therapy, genetic polymorphisms and drug or dietary interactions [20]. If a high INR represents a risk factor for bleeding, an infra-therapeutic INR increases the possibility of thrombosis recurrence.

To overcome these issues, we performed a retrospective longitudinal study to assess the effect of the average aGAPSS over time on recurrence of clinical manifestations despite appropriate treatment in APS patients. In addition, in those APS patients with previous thrombosis under treatment with VKA, the percentage of time spent within the therapeutic range (TTR), which is the most frequently reported metric for assessing the quality of anticoagulation control [20], has been reviewed.

Patients and methods

Patients

The study included 200 patients who attended the Department of Autoimmune Diseases at the Hospital Clinic of Barcelona between February 2011 (when aβ2GPI determination was implemented in our laboratory) and February 2022. All patients fulfilled the Sydney criteria for APS [1]. Follow-up visits were performed at least annually. Data on clinical manifestations, autoimmunity, aPL profile, cardiovascular risk factors (including smoking, diabetes mellitus, surgery, atrial fibrillation, nephrotic syndrome, oral contraceptive use, hypothyroidism, congenital thrombophilias, arterial hypertension and dyslipidaemia) and associated autoimmune diseases, as well as ongoing and previous treatment such as low-dose aspirin (LDA), low-molecular-weight heparin (LMWH), VKA and corticosteroids were collected at any visit.

Arterial hypertension and dyslipidaemia were assessed following National Institute for Health and Care Excellence (NICE) guidelines [21]. Arterial hypertension was defined as a blood pressure >140/90 mmHg detected in at least two occasions or use of oral antihypertensive drugs. Serum total and high-density lipoprotein (HDL) cholesterol levels were determined with standardized enzymic methods and interpreted according to current cut-off values. Physical examination along with blood pressure determination was performed at each visit.

aPL and other autoantibodies

Lupus anticoagulant (LAC) was detected according to the approved recommendations by the Subcommittee on Lupus Anticoagulant/Phospholipid-dependent Antibodies of the International Society on Thrombosis and Haemostasis guidelines, at the time it was determined [22, 23]. The anticardiolipin (aCL) and anti-β2 glycoprotein-I (aβ2GPI) antibodies were measured using solid-phase standardized immunoassays (ELISAs or chemiluminescence immunoassays). Antinuclear antibodies (ANA) were measured by indirect immunofluorescence (IIF) on rodent liver cells and/or HEp-2 cells, anti-double stranded DNA (dsDNA) antibodies were measured by ELISA or chemiluminescence assay and/or IIF on Crithidia Luciliae and antibodies against extractable nuclear antigen (ENA: Ro60/SSA, La/SSB, Sm and U1-RNP) by ELISA or chemiluminescence assay.

aGAPSS

The aGAPSS was calculated for each patient at baseline and on a yearly basis for either up to 6 years (minimum 3 years) or just before the clinical event in patients who experienced thrombosis or pregnancy morbidity. The score was computed, as previously reported, by adding together the points corresponding to the risk factors as following: one for arterial hypertension, three for dyslipidaemia, four for LAC and anti-β2GPI (IgM or IgG) antibodies, and five for aCL (IgM or IgG) antibodies. aCL and anti-β2GPI had to be present at medium-high titre (>99th percentile). The mean total score per patient was computed and considered the reference aGAPSS. Moreover, we calculated the delta of the aGAPSS during the follow-up as the difference between the aGAPSS before clinical recurrence (if present) or at the end of follow-up (in case of no clinical recurrence) and the basal aGAPSS.

Time within the therapeutic range of anticoagulation

The percentage of time spent within the therapeutic range (TTR) was determined by the step method proposed by Rosendaal *et al.* [24]. In patients who had a new thrombosis under anticoagulant treatment, TTR was calculated based on the international normalized ratio (INR) values of the 6 months before the thrombotic episode. In patients without thrombosis TTR was calculated based on the INR values of the last 6 months of follow-up. In both groups, for ITT calculation the first initial 3 months of treatment were not considered.

Outcomes

Clinical recurrence, obstetric and/or thrombotic, was diagnosed following the updated classification criteria for APS [1]. For instance, thrombosis had to be diagnosed by objective tests (imaging or histopathology), while obstetric recurrence consisted in one among early spontaneous miscarriage (before the 10th week of gestation), unexplained foetal death of a morphologically normal foetus at or beyond the 10th week of gestation or premature birth of a morphologically normal neonate before the 34th week of gestation.

The study was conducted in accordance with the declaration of Helsinki [25] and received approval from the Hospital Clinic Ethics Committee (HCB/2016/0401). All patients gave their verbal informed consent to participate and publish study results.

Statistical analysis

Categorical variables are presented as numbers and percentages and were compared using the χ^2 test or Fisher's exact test whenever appropriate. Continuous variables are presented as means (S.D.). Student's unpaired 2-tailed *t* test was used when comparing two groups, while ANOVA or Kruskal–Wallis non-parametric test when comparing >2 groups. Levene's test for equality of variances was used to test for variance homogeneity between groups. A two-tailed *P*-value <0.05 was considered statistically significant.

Time between the first clinical event and the development of a clinical recurrence (thrombotic or obstetric) or the date of the last registered outpatient visit in patients without events (censored observations) were analysed by the Kaplan–Meier 'product-limit estimator' method. Survival curves based on the chosen aGAPSS cut-off value were compared by log-rank test. A Cox proportional hazards regression model was built to identify the mean aGAPSS value and other variables associated with recurrence during follow-up. Univariate analysis identified all variables associated with recurrent events during follow-up. Then, variables significant at $P < 0.25$ were tested as independent variables in multivariate analysis. The risk of recurrence was expressed as hazard ratios (HR) with 95% CI.

Data were analysed using STATA 17 (StataCorp, College Station, TX, USA).

Results

Patients' characteristics

The overall cohort included 138 (69%) patients with primary APS and 62 (31%) patients with APS associated to other autoimmune diseases: for instance, the last group consisted in 41 patients with SLE, 17 patients with features of SLE not fulfilling current classification criteria [26] (classified as SLE-like), two patients with systemic sclerosis, one patient with SS and one with Behçet's disease. Mean age at diagnosis was 41.7 (S.D. = 13.3) years, 72% of patients were female. Age at diagnosis was significantly higher in primary APS (44.3 years, S.D. = 13.8) compared with other groups, while disease duration was higher in APS associated to SLE (215.2 months, S.D. = 102.3) compared with other groups.

The clinical manifestation that led to diagnosis was thrombosis in 133 patients (66.5%), pregnancy morbidity in 42 patients (21%), and both in 19 patients (9.5%). Abnormal laboratory features (thrombopenia, false-positive syphilis test, prolonged activated partial thromboplastin time) had led to APS diagnosis in six patients (3%). ANA ($P < 0.001$), anti-dsDNA ($P < 0.001$), anti-Ro60/SSA ($P < 0.001$), anti-La/SSB ($P = 0.01$), anti-Sm ($P = 0.006$), anti-U1-RNP ($P < 0.001$) and LAC ($P < 0.001$) were significantly more prevalent in patients with an associated condition than in those with primary APS. Patients' characteristics in the whole cohort and depending on APS type are reported in Table 1.

Clinical recurrence

Considering the whole series, 70 (35%) patients presented one or more recurrences despite treatment, 58 (29%) of them in form of thrombotic recurrence, nine (5%) obstetric and three (1.5%) both. The number of recurrence episodes ranged from one to five documented events. Clinical recurrence was significantly more frequent in APS associated to other conditions than primary APS (48.4 *vs* 29.0%, OR = 1.67: 95% CI 1.16, 2.41, $P = 0.008$).

No significant difference was found in the rate of smoking, cardiovascular risk factors, diabetes mellitus, previous surgery, oral contraceptives and congenital thrombophilia between patients who had clinical recurrence and patients who had not. Conversely, when comparing patients with thrombotic to patients with obstetric recurrence, arterial hypertension ($P = 0.02$) and dyslipidaemia ($P = 0.04$) were more frequent in the former group.

Regarding aPLs, anti-β2GPI antibodies (58.6% *vs* 42.3%; $P = 0.03$) and triple aPL positivity (42.8% *vs* 27.7%; $P = 0.03$) were more prevalent in the group with clinical recurrence, while LAC almost reached statistical significance (77.1% *vs* 63.9%; $P = 0.054$). When considering the different isotypes, we found a statistically significant correlation with recurrence only for anti-β2GPI IgG (51.4% *vs* 27.1%, $P = 0.001$). No difference was detected between thrombotic and obstetric recurrence. Among the 61 patients who had an episode of recurrent thrombosis, 19 patients (31.1%) had venous-venous recurrence, 16 (26.2%) had mixed venous-arterial or arterial-venous recurrence and 26 patients (42.6%) experienced arterial-arterial recurrence. Smoking ($P = 0.03$ and $P = 0.001$, respectively) and dyslipidaemia ($P = 0.03$ and $P = 0.02$, respectively) were more frequent in arterial and mixed thrombosis recurrence compared with venous. Among 131 patients under VKA treatment, data for TTR calculation was available in 76 (58%), not resulting statistically different between patients who had thrombosis recurrence and patients who had not (mean TTR rate 54.7% *vs* 63.1%, $P = 0.17$).

Table 2 reports the main characteristics of APS patients depending on the presence or absence of recurrent manifestations (total, thrombotic and obstetric) while Table 3 reports results depending on the site of thrombotic recurrence.

aGAPSS

Mean aGAPSS of the whole cohort was 7.23 (S.D. 4.79), ranging from 0 to 17. When comparing primary APS to APS associated to other conditions, there was no statistically significant difference [6.84 (S.D. 4.85) *vs* 8.08 (S.D. 4.60), $P = 0.09$]. Overall, 164 patients presented a variation of their score during the follow-up, in three (1.5%) cases due to change in cardiovascular risk factors, in 31 (15.5%) cases due to change in aPL positivity and hypertension/dyslipidaemia, while in 130 (65%) cases due to variation of aPL profile only.

Higher mean aGAPSS values were found in patients who experienced clinical recurrence (thrombotic, obstetric or both) in comparison to patients who did not [8.81 (95% CI 7.53, 10.08) *vs* 6.38 (95% CI 5.64, 7.12), $P = 0.001$] (Fig. 1A). Patients presenting thrombotic recurrence (alone and with obstetric recurrence) had higher mean aGAPSS than patients with no thrombotic recurrence [9.31 (95% CI 7.98, 10.65) *vs* 6.32 (95% CI 5.59, 7.03), $P = 0.001$] (Fig. 1B). These results were confirmed when considering the basal value of the aGAPSS ($P = 0.006$ and $P = 0.002$, respectively) (Table 2).

Patients with thrombotic recurrence alone presented higher mean aGAPSS in comparison to patients with only obstetric recurrence [9.48 (95% CI 8.14, 10.82) *vs* 4.25 (95% CI 0.85, 7.65), $P = 0.006$] (Fig. 1C), while the basal aGAPSS difference

Table 1. Basal demographic characteristics, clinical manifestations, immunological features and treatment of the whole cohort and according to the APS type

	Total	Primary	SLE-associated	Other[a]	*P*-value
Overall	200	138	41	21	
Female sex, *n* (%)	144 (72)	96 (69.6)	32 (78)	16 (76.2)	ns
Age at diagnosis, mean (S.D.), years	41.7 (13.3)	44.3 (13.8)	35.1 (8.6)	37.9 (12)	**< 0.001**
Disease duration mean (S.D.), months	180.6 (90.3)	167 (84.1)	215.2 (102.3)	196.9 (85.3)	**0.007**
Cardiovascular risk factors, *n* (%)					
Smoking	54 (27)	39 (28.3)	10 (24.3)	5 (23.8)	ns
Diabetes mellitus	8 (4)	7 (5.1)	1 (2.4)	0 0	ns
Surgery	48 (24)	37 (26.8)	8 (19.5)	3 (14.3)	ns
Oral contraceptives	22 (11.2)	17 (12.5)	4 (10.3)	1 (4.8)	ns
Atrial fibrillation	0 0	0 0	0 0	0 0	ns
Nephrotic syndrome	6 (3)	1 (0.7)	5 (12.2)	0 0	ns
Hypothyroidism	16 (8)	9 (6.5)	4 (9.8)	3 (14.3)	ns
Congenital thrombophilias[b]	7 (3.5)	6 (4.3)	0 0	1 (4.8)	ns
Hypertension	66 (33)	43 (31.2)	18 (43.9)	5 (23.8)	ns
Dyslipidaemia	66 (33)	50 (36.2)	13 (31.7)	3 (14.3)	ns
Thrombosis, *n* (%)	164 (82)	109 (78.9)	38 (92.7)	17 (81)	ns
Arterial	63 (31.5)	43 (31.2)	15 (36.6)	5 (23.8)	ns
Venous	81 (40.5)	55 (39.9)	15 (36.6)	11 (52.4)	ns
Both	20 (10)	11 (8)	8 (19.5)	1 (4.8)	ns
Pregnancy losses, *n* (%)					
First trimester miscarriages	56 (29.8)	42 (32.8)	9 (22)	5 (23.8)	ns
Foetal losses	44 (23.4)	28 (21.9)	11 (26.8)	5 (23.8)	ns
Immunological features, *n* (%)					
ANA	138 (69)	79 (57.2)	40 (97.6)	19 (90.5)	**<0.001**
Anti-dsDNA antibody	50 (25)	8 (5.7)	31 (75.6)	11 (52.4)	**<0.001**
Anti-Ro/SSA antibody	13 (6.5)	4 (2.9)	8 (19.5)	1 (4.8)	**<0.001**
Anti-La/SSB antibody	6 (3)	1 (0.7)	4 (9.8)	1 (4.8)	**0.01**
Anti-U1RNP antibody	12 (6)	0 0	10 (24.3)	2 (9.5)	**<0.001**
Anti-Sm	9 (4.5)	2 (1.4)	6 (14.6)	1 (4.8)	**0.006**
aPLs, *n* (%)					
aCL	153 (76.5)	106 (76.8)	31 (75.6)	16 (76.2)	ns
IgG/IgM	119/57	79/37	25/12	15/8	
aβ2GPI	95 (47.5)	60 (43.4)	21 (51.2)	14 (66.7)	ns
IgG/IgM	70/40	43/28	20/4	7/8	
LAC	137 (68.5)	84 (60.9)	37 (90.2)	16 (76.2)	**<0.001**
Triple positivity	65 (32.5)	40 (29)	15 (36.6)	10 (47.6)	ns
Treatment, *n* (%)					
LDA	66 (33)	49 (35.5)	8 (19.5)	9 (42.9)	ns
VKA	131 (65.5)	88 (63.8)	30 (73.2)	13 (61.9)	ns
LMWH	18 (9)	16 (11.6)	1 (2.4)	1 (4.8)	ns
Corticosteroids	11 (5.5)	3 (2.2)	4 (9.8)	4 (19)	ns

aβ2GPI: anti-beta2glycoprotein I antibodies; aCL: anticardiolipin antibodies; dsDNA: double stranded DNA; LAC: lupus anticoagulant; LDA: low dose aspirin; LMWH: low-molecular-weight-heparin; ns: not significant; RNP: ribonucleoprotein; VKA: vitamin K antagonist. Bold value indicates the significance of *P*-value < 0.05.

[a] The 'Other' group includes: 17 patients with 'SLE-like', two patients with systemic sclerosis, one patient with SS and one with Behçet's disease.

[b] Congenital thrombophilias include factor V Leiden mutation ($n = 1$) and prothrombin G20210A mutation ($n = 6$).

was not statistically significant [9.31 (95% CI 8.06, 10.56) *vs* 7 (95% CI 3.83, 10.17), $P = 0.18$] (see Fig. 1D and Table 2).

Within the recurrent thrombosis group, patients with arterial-arterial thrombosis and patients with venous-arterial/arterial-venous thrombosis showed higher mean aGAPSS than those with venous-venous thrombosis recurrence [10.66 (S.D. 5.48) and 10.94 (S.D. 4.49) *vs* 6.63 (S.D. 4.42), $P = 0.01$ and $P = 0.01$, respectively] (see Table 3 and Fig.1E).

When considering patients with only obstetric recurrence, the aGAPSS value was not statistically different in comparison to patients with no recurrence [4.25 (CI 1.25, 7.17) *vs* 6.38 (CI 5.59, 7.17), $P = 0.17$].

When analysing extra-criteria manifestations, we found a significant difference of the aGAPSS for migraine (9.86 *vs* 6.94, $P = 0.009$), epilepsy (10.19 *vs* 7.06, $P = 0.035$), myelitis (12 *vs* 7.18, $P < 0.001$) and cardiac valve disease (10.92 *vs* 6.80, $P < 0.001$), whereas we did not find any significant difference of the aGAPSS for thrombocytopenia, livedo reticularis/racemosa, cutaneous ulcers, haemolytic anaemia and APS nephropathy.

Finally, we calculated the delta of the aGAPSS during follow-up, finding a delta of 2.19 for patients who developed clinical recurrence *vs* –2.02 for patients who did not ($P < 0.001$). Also, when considering specifically thrombotic or thrombotic plus obstetric recurrence the delta was 2.55 in patients with recurrence *vs* –1.9 in patients with no recurrence ($P < 0.001$). Last, when comparing thrombotic *vs* obstetric recurrence, the delta was 2.62 for patients with only thrombotic recurrence *vs* –0.33 for patients with only obstetric recurrence ($P < 0.001$).

Predictors of clinical recurrence

Univariate Cox regression analysis showed that several cut-off value of the aGAPSS were predictive of clinical recurrence,

Table 2. Comparison of the main characteristics of patients according to the presence of clinical recurrence and type (thrombotic or obstetric) of recurrence

	No recurrence	Any	*P*-value	Thrombotic	Obstetric	*P*-value
Total, *n* (%)	130	70		58	9	
Diagnosis, *n* (%)						
Primary APS	98 (75.3)	40 (57.1)		31 (53.4)	8 (89)	
APS associated to SLE	19 (14.6)	22 (31.5)		19 (32.8)	1 (11)	
APS associated to other AID	13 (10.1)	8 (11.4)		8 (13.8)	0 0	
Cardiovascular risk factors, *n* (%)						
Smoking	33 (25.4)	21 (30)	ns	17 (29.3)	3 (33.3)	ns
Diabetes mellitus	4 (3.1)	4 (5.7)	ns	4 (6.9)	0 0	ns
Surgery	26 (20)	22 (31.4)	ns	19 (32.8)	2 (22.2)	ns
Oral contraceptives	12 (9.2)	7 (10)	ns	6 (10.4)	n.a.	
Atrial fibrillation	0 0	0 0	ns	0 0	0 0	ns
Nephrotic syndrome	4 (3.1)	2 (2.9)	ns	2 (3.5)	0 0	ns
Hypothyroidism	10 (7.7)	6 (8.6)	ns	5 (8.6)	1 (11.1)	ns
Congenital thrombophilia	5 (3.8)	2 (2.8)	ns	2 (3.5)	0 0	ns
Hypertension	39 (30)	27 (38.6)	ns	26 (44.8)	1 (11.1)	**0.02**
Dyslipidaemia	50 (30.8)	26 (37.1)	ns	25 (43.1)	1 (33.3)	**0.04**
Basal aGAPSS, mean (S.D.)	7.12 (3.9)	9 (7.86)	**0.006**	9.31 (4.77)	7 (3.12)	0.18
Mean aGAPSS, mean (S.D.)	6.38 (4.26)	8.81 (5.34)	**0.001**	9.48 (5.13)	4.25 (3.95)	**0.006**
TTR (% of time in range)	63.1			54.7		ns*
aPLs, *n* (%)						
aCL	96 (73.9)	57 (71.4)	ns	47 (81.0)	7 (77.8)	ns
IgG/IgM	71/34	48/23			6/1	
a*β*2GPI	55 (42.3)	41 (58.6)	**0.03**	35 (60.3)	4 (44.4)	ns
IgG/IgM	35/22	36/18			3/2	
LAC	83 (63.9)	54 (77.1)	0.054	46 (79.3)	5 (55.6)	ns
Triple positivity	36 (27.7)	30 (42.8)	**0.03**	27 (46.6)	2 (22.2)	ns
Treatment						
LDA	39 (30)	27 (38.6)	ns	19 (32.8)	7 (77.8)	**0.03**
LMWH	14 (10.8)	4 (5.7)	ns	2 (3.5)	2 (22.2)	ns
VKA	83 (63.9)	48 (68.6)	ns	44 (75.9)	2 (22.2)	**0.01**
Corticosteroids	9 (6.9)	2 (2.9)	ns	2 (3.5)	0 0	ns

a*β*2GPI: anti-beta2glycoprotein I antibodies; aCL: anticardiolipin antibodies; aGAPSS: adjusted Global Antiphospholipid Syndrome Score; AID: autoimmune diseases; LAC: lupus anticoagulant; LDA: low dose aspirin; LMWH: low molecular weight heparin; ns: not significant; TTR: time spent within the therapeutic range; VKA: vitamin K antagonists.

* *P*-value comparing TTR in patients with thrombosis recurrence *vs* patients without thrombosis recurrence. Data available for 76 of the 131 patients under vitamin k antagonists.

among whom an aGAPSS >13 had the highest HR of the event [HR = 3.17 (95% CI 1.92, 5.25), $P < 0.0001$]. When focusing specifically on thrombotic recurrence the association was even stronger [HR = 3.99 (95% CI 2.36, 6.75), $P < 0.0001$] (Table 4). As shown by Kaplan–Meier survival analysis (Fig. 2), the cumulative proportion of recurrence-free individuals was significantly lower in patients with an aGAPSS higher than 13 points ($P < 0.001$ by log-rank test).

When analysing several factors separately in univariate analysis, we did not find an association between clinical recurrence and smoking [HR = 1.28 (95% CI 0.74, 2.21), $P = 0.38$], surgery [HR = 1.15 (95% CI 0.70, 1.89), $P = 0.32$], oral contraceptives [HR = 1.31 (95% CI 0.66, 2.61), $P = 0.45$], congenital thrombophilias [HR = 1.08 (95% CI 0.26, 4.46), $P = 0.91$], arterial hypertension [HR = 1.34 (95% CI 0.32, 2.17), $P = 0.23$], dyslipidaemia [HR = 1.19 (95% CI 0.73, 1.94), $P = 0.48$], SLE [HR = 1.61 (95% CI 0.96, 2.70), $P = 0.07$], aCL antibodies [HR = 1.48 (95% CI 0.81, 2.71), $P = 0.20$], LAC [HR = 1.44 (95% CI 0.82, 2.52), $P = 0.21$], triple positivity [HR = 1.61 (95% CI 0.99, 2.58), $P = 0.053$], LDA [HR = 1.11 (95% CI 0.68, 1.80), $P = 0.69$] and VKA treatment [HR = 1.39 (95% CI 0.84, 2.32), $P = 0.19$]. There was a significant association with anti-β2GPI antibodies [HR = 1.81 (95% CI 1.12, 2.92), $P = 0.01$]. In the multivariate analysis considering SLE and VKA therapy as confounding factor, aGAPSS >13 retained statistical significance [HR = 3.25 (95% CI 1.93, 5.45), $P < 0.0001$].

Discussion

Recurrence of clinical manifestations is a main concern in APS. In fact, this condition is usually diagnosed in young people [27] and both thrombosis and pregnancy morbidity tend to recur despite treatment. For instance, in spite of anticoagulation, the 5-year rate of thrombosis recurrence can be as high as 16.6% [28]. Identifying markers of increased risk of recurrence is therefore of crucial importance in APS patients. The aPL profile (in particular, triple positivity) [29, 30], cardiovascular risk factors and association to SLE are considered the main risk factors for thrombosis recurrence [31]. The aGAPSS combines the first two items in a useful score to stratify patients according to the risk of recurrence.

To the best of our knowledge, this is the first study longitudinally evaluating the association between the mean aGAPSS and the recurrence of clinical manifestations in APS. In addition, we have considered the rate of time spent within the therapeutic range in patients under VKA therapy. Sciascia *et al.* in a former study prospectively evaluated the clinical relevance of the GAPSS in a cohort of 51 SLE patients with aPL without previous thrombotic events [32]. The authors

Table 3. Comparison of the main characteristic of APS patients (right before recurrence) depending on the type of thrombotic recurrence

	Arterial	Venous	*P*-value (arterial *vs* venous)	Mixed	*P*-value (venous *vs* mixed)
Total, *n*	26	19		16	
Diagnosis, *n* (%)					
Primary APS	13 (50)	11 (58)		8 (50)	
APS associated to SLE	10 (38.5)	4 (21)		7 (43.8)	
APS associated to other AID	3 (11.5)	4 (21)		1 (6.2)	
Cardiovascular risk factors, *n* (%)					
Smoking	9 (34.6)	1 (5.2)	**0.03**	8 (53.3)	**0.001**
Diabetes mellitus	3 (11.5)	0 0	ns	1 (6.3)	ns
Surgery	8 (30.8)	5 (26.3)	ns	7 (43.7)	ns
Oral contraceptives	4 (15.4)	2 (10.5)	ns	1 (6.3)	ns
Atrial fibrillation	0	0	ns	0	ns
Nephrotic syndrome	1 (3.8)	0	ns	1 (6.3)	ns
Hypothyroidism	4 (15.4)	1 (5.3)	ns	1 (6.3)	ns
Congenital thrombophilia	0	1 (5.2)	ns	1 (6.3)	ns
Hypertension	13 (50)	6 (31.6)	ns	8 (50)	ns
Dyslipidaemia	14 (53.9)	2 (10.5)	**0.03**	10 (62.5)	**0.02**
Mean aGAPSS, mean (S.D.)	10.7 (5.5)	6.6 (4.4)	**0.01**	10.9 (4.5)	**0.01**
TTR (% of time in range)	55.3 (5.4)	50.1 (9.2)	ns	58.8 (11)	ns
aPLs, *n* (%)					
aCL	21 (80.8)	15 (78.9)	ns	14 (87.5)	ns
aβ2GPI	15 (57.7)	9 (47.4)	ns	13 (81.3)	ns
LAC	22 (84.6)	13 (68.4)	ns	14 (87.5)	ns
Triple positivity	14 (53.9)	6 (31.6)	ns	8 (50)	ns
Treatment					
LDA	7 (26.9)	8 (42.1)	ns	5 (31.3)	ns
LMWH	1 (3.9)	0	ns	1 (6.3)	ns
VKA	21 (80.8)	11 (57.9)	ns	14 (87.5)	Ns
Corticosteroids	1 (3.9)	1 (5.3)	ns	0	Ns

aβ2GPI: anti-beta2glycoprotein I antibodies; aCL: anticardiolipin antibodies; aGAPSS: adjusted Global Antiphospholipid Syndrome Score; AID: autoimmune diseases; aPLs: antiphospholipid antibodies; LAC: lupus anticoagulant; LDA: low dose aspirin; LMWH: low molecular weight heparin; ns: not significant; TTR: time spent within the therapeutic range;VKA: vitamin K antagonists.

performed a longitudinal evaluation of the GAPSS finding that patients with an increase in the GAPSS during follow-up had a higher risk of experiencing thrombotic events. An increase of more than three points seemed to have the best risk accuracy for vascular events. However, in such study the role of correct anticoagulation with VKA treatment could not be assessed because no patient was under full oral anticoagulation. In our population, we found a statistically significant difference in the aGAPSS delta (delta = aGAPSS right before clinical recurrence—baseline aGAPSS) in patients who developed clinical (thrombotic pius obstetric) or thrombotic recurrence in comparison to patients who did not. Moreover, we found a 2.6 points delta in patients with thrombotic recurrence, compared with a negative delta in patients with obstetric recurrence. This result highlights the value of the longitudinal evaluation of the aGAPSS as a risk score for thrombotic recurrence.

In our study, the computed mean aGAPSS of patients with recurrence of clinical manifestations (thrombotic, obstetric or both) resulted higher than the mean aGAPSS of patients with no recurrence. Moreover, patients with thrombotic recurrence (alone and with obstetric recurrence) had a higher mean aGAPSS when compared with patients without recurrence of thrombosis. This association was also confirmed when considering the basal aGAPSS. Conversely, when considering patients with only thrombotic recurrence, the mean aGAPSS resulted higher in comparison to patients with only obstetric recurrence, while the difference in the basal aGAPSS value was not statistically significant. This result highlights the importance of longitudinal monitoring of aPLs and cardiovascular risk factors over time.

When considering patients with thrombotic recurrence among themselves, we detected the highest aGAPSS values in patients experiencing arterial thrombosis (aGAPSS >10 points). These results are in line with the ones of several other authors as reported in a recent systematic review with a pooled analysis [33]. The mean aGAPSS of patients with venous-venous recurrence was lower than mean aGAPSS of patients with arterial or mixed thrombosis recurrence. These results are in concordance with the ones reported in a cross-sectional study from the APS-Action cohort [16].

In our cohort, the aGAPSS of patients with only obstetric recurrence was not statistically different in comparison to patients with no recurrence. Sciascia *et al.* in their original study found a higher GAPSS in patients with a history of pregnancy loss compared with patients with no clinical events [11]. However, the study included only SLE patients, and did not analyse the recurrence of obstetric morbidity under standard of care. In our study the difference might reflect the possibility that obstetric recurrence could not be as related to aPL and cardiovascular risk factors' persistence over time as thrombotic recurrence.

When analysing extra-criteria manifestations, we found a significant higher aGAPSS in patients with migraine, epilepsy, myelitis and cardiac valve disease. Other authors suggested that patients with higher aGAPSS might be at higher risk for developing extra-criteria manifestations of APS [34] and this issue should be further evaluated.

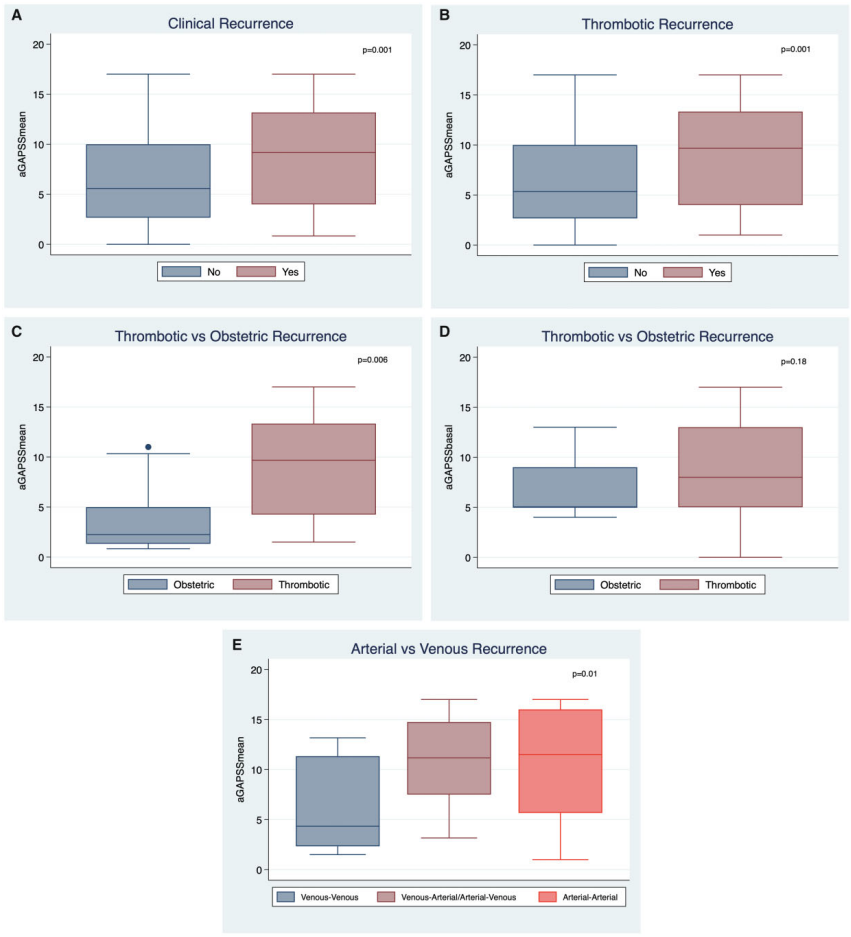

Figure 1. aGAPSS according to different subsets of recurrence Distribution of mean adjusted Global Antiphospholipid Syndrome Score (aGAPSS) according to different subsets of recurrence. Data are shown as box plots, where each box represents the 25th to 75th percentiles and lines inside the box represent the median. The whiskers represent the 95% CI.Higher aGAPSS values were detected in patients with clinical recurrence versus those without recurrence (**A**), thrombotic recurrence (alone or with obstetric recurrence) versus no thrombotic recurrence (**B**), thrombotic recurrence alone versus obstetric recurrence alone (**C**). In the last case, when using the first score available instead of the average score, the difference did not reach statistical significance (**D**). Finally, when comparing the different types of thrombosis recurrences, arterial recurrence and mixed recurrence showed higher aGAPSS values than venous recurrence (**E**)

Table 4. Prediction of clinical and thrombotic recurrence with different aGAPSS cut-offs in univariate survival analysis

	Clinical recurrence		Thrombotic recurrence	
aGAPSS cut-off	HR	*P*	HR	*P*
>8	2.00 (95% CI 1.25, 3.22)	0.004	2.49 (95% CI 1.48, 4.17)	0.0004
>9	2.10 (95% CI 1.31, 3.37)	0.002	2.52 (95% CI 1.52, 4.19)	0.0004
>11	1.93 (95% CI 1.20, 3.14)	0.009	2.31 (95% CI 1.39, 3.85)	0.002
>13	3.17 (95% CI 1.92, 5.25)	<0.0001	3.99 (95% CI 2.36, 6.75)	<0.0001
>14	2.49 (95% CI 1.40, 4.44)	0.005	3.04 (95% CI 1.68, 5.48)	0.0008

aGAPSS: adjusted Global APS Score; HR: hazard ratio.

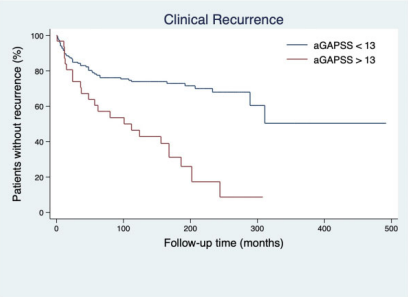

Figure 2. Kaplan–Meier curve of survival probability for an aGAPSS of 13
Kaplan–Meier curve of survival probability (absence of clinical recurrence) of patients with aGAPSS higher and lower than 13 points

We performed a Cox regression analysis to identify the best cut-off value of aGAPSS for predicting recurrence and we found that an aGAPSS >13 points was highly predictive of clinical recurrence, even when adjusting for association with SLE and VKA therapy. Lower cut-off values reached statistical significance, but the cut-off of 13 provided the best discrimination. Our cut-off value is higher than the one found in another study [14] that included also patients without APS. This aspect highlights the evidence that different cohorts can present different cut-off values, being patients with a diagnosis of APS likely prone to have higher aGAPSS, as seen in other studies [12, 31].

When analysing the aPL profile anti-β2GPI antibodies and triple aPL positivity were more frequent in the group with clinical recurrence while LAC almost reached statistical significance. Both LAC and triple positivity are associated with a higher risk of first thrombotic event and thrombosis recurrence in APS [3, 30]. Among the other risk factors considered, arterial hypertension and dyslipidaemia were both more frequent in patients with thrombotic recurrence compared with obstetric recurrence, while smoking and dyslipidaemia were more frequent in thrombotic arterial or mixed recurrence than venous recurrence. Given that these are cardiovascular risk factors, their association with thrombosis is not surprising and confirms the importance of a strict control of modifiable risk factors in APS patients.

The main strength of our study is the longitudinal assessment of the aGAPSS score, throughout at least three years. Because both aPL positivity and cardiovascular risk factors can change over time, a longitudinal evaluation is necessary to provide the highest accuracy. The high rate (above 80%) of patients that presented a variation in their score, in the vast majority due to change in the aPL profile, goes in such direction, as does the discrepancy in our cohort between mean aGAPSS and basal aGAPSS when considering thrombosis *vs* obstetric recurrence. Moreover, our study included a homogeneous cohort of 200 patients from a single centre, all classified as having APS by fulfilment of classification criteria. All aPLs were tested in the same laboratory, with the same cut-off values, allowing a homogeneous evaluation of positivity.

When assessing the risk of thrombotic recurrence in APS patients under VKA therapy, the assessment of the effect of therapy and therapy compliance is essential and cannot be disregarded. There are many factors associated with poor INR control. Among them the most frequently involved are lack of adherence to therapy and polymorphisms of CYP2C9 or vitamin K epoxide reductase (VKORC1). For instance, this last mutation can result in either a heightened (group A haplotype) or reduced (group B haplotype) effect of VKA [20]. In our study, through TTR determination, we could exclude that thrombosis recurrence in patients under VKA therapy was provoked or favoured by worse anticoagulation control. We acknowledge that the fact that we could not assess the TTR in all patients under VKA therapy, because not all of them were being followed in our haemostasis unit, represents a limitation. However, this parameter was available in more than half patients under VKA (58%), making our results consistent.

Another limitation of our study is its retrospective design, which implicates that the score has been computed after the onset of recurrence. However, aPL, dyslipidaemia and arterial hypertension were assessed routinely at each visit so, in case of recurrence, the actual evaluation preceded the clinical manifestation in most cases. A prospective design would definitely be more suitable for assessing risk factors associated to clinical recurrence. Nevertheless, APS is a low prevalence condition, making a prospective study including a consistent number of patients hard to perform.

In summary, we performed a longitudinal assessment of the aGAPSS finding that a higher mean score is associated with both thrombotic and obstetric recurrence, being arterial thrombosis recurrence, the manifestation associated to the highest score. In patients with persistently high aGAPSS (particularly if higher than 13) an intensive treatment regimen to prevent recurrence, especially in case of arterial thrombosis as first clinical manifestation, should be considered. Confirmation of these results in large, prospective multicentric studies is warranted.

Conclusions

aGAPSS is an easy tool to assess the risk of clinical manifestations recurrence in patients with APS. In the light of the results of our study and considering the increasing evidence in the literature, retesting for aPL during follow-up, at least annually, and monitoring cardiovascular risk factors, may be useful. Patients with persistently high aGAPSS during follow-up might benefit from more strict control of all cardiovascular risk factors along with higher intensity treatment in preventing clinical recurrence.

Research agenda

Because aGAPSS is in great part determined by aPL positivity, a therapeutic strategy aiming at reducing aPL titres might be an interesting option. Hydroxychloroquine use was associated with a significant decrease in aPL titres over an average 2.6-year follow-up in a randomized prospective study [35]. Rituximab, a B-cell depletion therapy that has been used for severe and catastrophic APS, has shown variable effects on aPL titres [36]. Belimumab is an inhibitor of the binding of soluble circulating B lymphocyte stimulator to its target receptors on B cells. In several reports [37–39] and in a post-hoc analysis of three big trials [40, 41], it led to reduction of aPL, especially when associated to antimalarial therapy. Well designed, large, long-term prospective studies investigating

the effect of such medications on the aGAPSS and on clinical recurrence could open new perspectives on APS treatment.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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The objective of the second study was to assess the value of a longitudinal monitoring of the mean aGAPSS over time as predictor of clinical recurrence in APS patients. In almost all literature published so far, in fact, the score was assessed in only one time point. However, since both aPL and cardiovascular risk factor are not exempt from fluctuations over time, we postulated that a longitudinal evaluation of the score would give a more reliable picture of a patient's risk.

The aGAPSS was calculated for each patient at baseline and on a yearly basis for up to 6 years in patients who did not present clinical recurrence or right before the clinical event in patients who experienced thrombosis or pregnancy morbidity (as per current classification criteria). The mean total score was computed and considered the reference aGAPSS. Moreover, we computed the delta of the aGAPSS, as the difference between the aGAPSS before clinical recurrence (if present) or at the end of follow-up (in case of no clinical recurrence) and the basal aGAPSS. Only patients with at least three annual determinations of the aGAPSS were included.

Among the 200 patients included, 164 presented a variation of their score during the follow-up, in three (1.5%) cases due to change in cardiovascular risk factors, in 31 (15.5%) cases due to change in aPL positivity and hypertension/dyslipidaemia, while in 130 (65%) cases due to variation of aPL profile only.

Overall, a total of 70 subjects presented clinical recurrence, 58 thrombotic, 9 obstetric, and 3 both. We found a higher mean aGAPSS in patients with recurrence compared to patients without (8.81 vs 6.38), with a similar result when considering the baseline aGAPSS (9 vs 7.12) and the delta aGAPSS (2.19 vs 2.02). Conversely, when comparing patients with thrombotic recurrence to patients with only obstetric recurrence, the baseline aGAPSS was not significantly different, while both the mean aGAPSS (9.48 vs 4.25) and the delta (2.62 vs -0.33) were significantly higher in the first group. Also, within the thrombotic group, the mean aGAPSS of patients with arterial-arterial (10.66) or venous-arterial (10.94) recurrence was higher than the aGAPSS of patients with venous-venous thrombosis (6.63). Finally, the aGAPSS of patients with obstetric recurrence did not differ from the one of patients without recurrence.

In 76 of 131 patients with previous thrombosis under VKA therapy, the percentage of time spent within the therapeutic range (TTR) was determined through the step method proposed by Rosendaal et al [119], finding no difference between patients who experienced thrombosis recurrence and patients who did not (54.7 vs 63.1, p=0.17).

We finally performed a Cox regression analysis to find the best cut-off value that predicted clinical recurrence and we found that a mean aGAPSS > 13 had the highest HR for the event (HR = 3.25, 95% CI 1.93-5.45).

We therefore concluded that periodic, at least annual monitoring of aPL and cardiovascular risk factors is highly recommended to stratify the risk of recurrence in APS patients and predispose preventive strategies.

Third study

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The adjusted Global Anti-Phospholipid Syndrome Score as predictor of damage accrual measured by Damage Index for APS: a longitudinal study

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Original Article

Clinical science

The adjusted Global Anti-Phospholipid Syndrome Score as predictor of damage accrual measured by Damage Index for APS: a longitudinal study

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Abstract

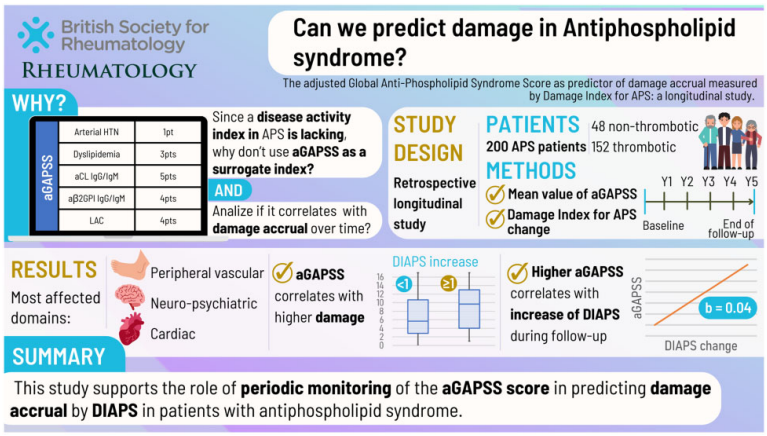
Objective: To analyse the association between the average 'adjusted' Global APS Score (aGAPSS) over time, as a surrogate of disease activity, and change in Damage Index for APS (DIAPS) during follow-up in patients with thrombotic and non-thrombotic APS.

Methods: Two hundred APS patients (138 primary, 62 associated to other autoimmune diseases) were included. DIAPS change was calculated as the difference between basal DIAPS and DIAPS at the end of follow-up. The aGAPSS was calculated for each patient at baseline and on a yearly basis for up to 6 years (minimum 3 years). The average score per patient was computed and considered the reference aGAPSS. Linear regression models were designed to analyse the association between mean aGAPSS and DIAPS change. Moreover, factors associated to high (increase of DIAPS $\geq$1 during follow-up) *vs* low (increase of DIAPS $<$1 during follow-up) damage accrual were assessed.

Results: A higher mean aGAPSS value was associated to a DIAPS increase during follow-up (b = 0.04, $P < 0.001$) in the multivariate analysis. Higher mean aGAPSS values were found in patients with a DIAPS increase $\geq$1 during follow-up compared with patients with an increase of $<$1 point [9.22 (95% CI 7.58, 10.86) *vs* 6.72 (95% CI 6.0, 7.43), $P = 0.003$]. aGAPSS increased the odds a DIAPS increment of $\geq$1 point during follow-up [OR = 1.12 (95% CI 1.04, 1.21), $P = 0.003$].

Conclusions: Our data support the utility of longitudinal assessing of the aGAPSS score in predicting damage accrual, measured by DIAPS, in APS.

Graphical Abstract



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Keywords: APS, adjusted global APS score, damage index for APS, damage accrual, antiphospholipid antibodies

Rheumatology key messages

- The adjusted global APS can be used as a surrogate of disease activity.
- Antiphospholipid antibodies and cardiovascular risk factors fluctuate over time, resulting in a change of the aGAPSS during follow-up.
- Longitudinal assessing of the aGAPSS predicts damage accrual measured by DIAPS change in follow-up.

Introduction

When assessing the course of a systemic autoimmune disease (AID), it is essential to have assessment tools that enable to evaluate from one side disease activity (generally due to ongoing inflammation and potentially reversible) and from the other disease damage (irreversible changes secondary to disease activity itself or treatment). In fact, as in a chain-reaction, persistent disease activity leads to higher damage and damage predicts mortality. Therefore, both contribute to disease severity, increase the economic costs, and reduce the health-related quality of life. Not for nothing, prevention of organ damage is an overarching principle of treat-to-target strategy in AID and a major therapeutic goal in clinical trials [1].

Recurrence of clinical manifestations is the hallmark of APS, an autoimmune thrombophilia characterized by the development of arterial, venous and/or microvascular thrombosis, pregnancy complications (recurrent early miscarriages, foetal deaths after the 10th week of gestation and/or premature births) and, frequently, haematologic alterations associated to the presence of antiphospholipid antibodies (aPLs) [2]. In fact, despite adequate treatment with anticoagulation and/or antiplatelet therapy, the initial clinical features show an increasing cumulative prevalence as the disease progresses over time [3–5]. The chronic and recurrent nature of the disease highlights the need for risk stratification and damage assessment tools.

While disease activity tools have been successfully implemented in several AID such as SLE [6, 7], SS [8] and inflammatory myopathies [9], a disease activity index specific for APS is still lacking. This is due to the pathophysiology of the disease, in which inflammation does not play a major role as in other AID, and thrombosis is the main cause of damage. On the other hand, a damage score system for thrombotic APS, named Damage Index for APS (DIAPS), has been recently developed, which includes 38 items/APS-specific features from ten different organ and system domains. DIAPS showed content, criterion and construct validity and a good correlation with quality of life in thrombotic APS patients from LatinAmerica [10]. It has not been validated in non-thrombotic APS and ethnicities other than Latin-American Mestizo.

In recent years, two score systems have been proposed as a risk stratification tool for clinical manifestations in aPL positive patients. The aPL-score (aPL-S) [11] includes the anticardiolipin (aCL), anti-β2-glycoprotein-I (aβ2GPI) and antiphosphatidylserine/prothrombin (aPS/PT) IgG and IgM plus three different lupus anticoagulant (LAC) mixing tests and two confirmation tests. It has been shown to be a good predictor of thrombosis in patients with AID [11]. The Global APS Score, conversely, takes into account the four aforementioned aPLs (only one LAC test is necessary) plus cardiovascular risk factors, namely arterial hypertension and dyslipidaemia [12]. The two scores have been compared, the aPL-S showing superiority in predicting thrombosis, while the GAPSS better ability for diagnosing APS [13, 14]. However, the aPL-S, that includes a total of 16 items, is much more difficult to be computed and implemented in routine use than the GAPSS, making the latter more suitable for clinical practice.

Another point to consider, that concerns both scores, is that the solid-phase assays for aPS/PT are not yet well standardized, have a limited commercial availability and need additional research to define their clinical significance. For such reasons, they have not been included in the candidate items for the new classification criteria for APS [15].

An adjusted version of the GAPSS, called aGAPSS, which does not include aPS/PT, has been validated both in APS and SLE [12, 16, 17]. Various authors associated the basal aGAPSS with the recurrence of thrombosis in patients with APS [18–21]. However, aPL levels fluctuate over time, ranging from medium-high positivity to negativity [22–24], while cardiovascular risk factors can be modified by lifestyle and treatment. Therefore, a single assessment of the aGAPSS might not reflect the average risk state of a patient, and the aGAPSS value immediately close to a clinical manifestation, i.e. thrombosis, can be different from another time-point.

Starting from these premises, we conducted a retrospective longitudinal study to analyse the association between the average aGAPSS over time, as a surrogate of disease activity, and damage accrual measured by change in DIAPS during follow-up in a cohort of APS patients with or without a history of thrombosis. Secondary objectives were to assess if there is a difference in the mean aGAPSS between patients with high damage accrual (considered as DIAPS increase $\geq$1 point) *vs* low damage accrual (considered as DIAPS increase $<$1 point), check if aGAPSS increases the odds of a DIAPS increment $\geq$1 point in the follow-up and analyse the relationship between DIAPS and mortality.

Patients and methods

Patients

The study included 200 patients who attended the Department of Autoimmune Diseases at the Hospital Clinic of Barcelona between February 2011 (when aβ2GPI determination was implemented in our laboratory) and February 2022. All patients fulfilled the Sydney criteria for APS [2]. Follow-up visits were performed at least annually. Data on clinical manifestations, autoimmunity, aPL profile, cardiovascular risk factors (including smoking, diabetes mellitus, arterial hypertension, dyslipidaemia, surgery, atrial fibrillation, nephrotic syndrome, oral contraceptive use, hypothyroidism and congenital thrombophilias), and associated AID, as well as ongoing and previous treatment such as low-dose aspirin (LDA), low-molecular-weight heparin (LMWH), vitamin K

antagonists (VKA), corticosteroids and HCQ were collected at any visit.

Arterial hypertension and dyslipidaemia were assessed following National Institute for Health and Care Excellence (NICE) guidelines [25]. Arterial hypertension was defined as a blood pressure >140/90 mmHg detected in at least two occasions or use of oral antihypertensive drugs. Serum total and high-density lipoprotein (HDL) cholesterol levels were determined with standardized enzymic methods and interpreted according to current cut-off values. Physical examination along with blood pressure determination was performed at each visit.

The study was conducted in accordance with the declaration of Helsinki [26] and received approval from the Hospital Clinic Ethics Committee (HCB/2016/0401). All patients gave their verbal informed consent to participate and publish study results (Ethics Committee waived requirements for written consent because the study was retrospective).

aPL and other autoantibodies

aCL and aβ2GPI IgG and IgM antibodies were measured using solid-phase standardized immunoassays: ELISA (Aeskulisa, Aesku-Diagnostics, Wendelsheim, Germany) or chemiluminescence immunoassay (CLIA) (QUANTA Flash®, Inova Diagnostics, CA, USA). The cut-off recommended by the manufacturer were 15 GPL-MPL/ml and 20 chemiluminescent units (CU), respectively. LAC test was performed following the guidelines of the Subcommittee on Lupus Anticoagulant/Phospholipid-dependent Antibodies of the International Society of Thrombosis and Haemostasis (SSC-ISTH) recommendations [27, 28]. ANA were measured by indirect immunofluorescence (IIF) on rodent liver cells and/or HEp-2 cells (titres above 1:80 being considered positive), anti-double stranded DNA (dsDNA) antibodies were measured by ELISA or CLIA and/or IIF on Crithidia Luciliae and antibodies against extractable nuclear antigen (ENA: Ro60/SSA, La/SSB, Sm and U1-RNP) by ELISA or CLIA.

aGAPSS & DIAPS

The aGAPSS was calculated for each patient at baseline and on a yearly basis for up to 6 years (minimum 3 years). The score was computed, as previously reported, by adding together the points corresponding to the risk factors as following: 1 for arterial hypertension, 3 for dyslipidaemia, 4 for LAC and anti-β2GPI (IgM or IgG) antibodies, and 5 for aCL (IgM or IgG) antibodies. aCL and anti-β2GPI had to be present at medium-high titre (>99th percentile). The mean total score per patient was computed and considered the reference aGAPSS. DIAPS was computed at baseline and at last visit summing the score for each of the 38 items included in the index [10]. For instance, each item was ranked as 0 if absent, 1 if present without sequelae, and 2 if present with sequelae. To score, an item had to be present for at least six months. Difference between mean DIAPS at last visit and mean DIAPS at APS diagnosis was calculated.

Statistical analysis

Categorical variables are presented as numbers and percentages and were compared using the χ^2 test or Fisher's exact test whenever appropriate. Continuous variables are presented as means (S.D.) or medians (interquartile range) (IQR) if not normally distributed. Student's unpaired 2-tailed *t* test was used when comparing groups. Levene's test for equality of variances was used to test for variance homogeneity between groups. Linear regression models were built to study the association between aGAPSS and DIAPS change, including as possible confounding factors in the final model, all variables that, when excluded from the reference model, caused a change in the b coefficient >10%. If more than one model was eligible, we selected the most precise (the one with the smallest C.I. of b coefficient). A logistic regression model was built to assess factors associated to high damage accrual (DIAPS increase ≥1). A two-tailed *P*-value <0.05 was considered statistically significant. Data were analysed using STATA 17 (StataCorp, College Station, TX, USA).

Results

Patients' characteristics

The overall cohort included 138 (69%) patients with primary APS (PAPS) and 62 (31%) patients with APS associated to other AID: for instance, the last group consisted of 41 patients with SLE, 17 patients with features of SLE not fulfilling current classification criteria [29] (classified as SLE-like), two patients with systemic sclerosis, one patient with Sjögren's syndrome and one with Behçet's disease. The clinical manifestation that led to diagnosis was thrombosis in 133 patients (66.5%), pregnancy morbidity in 42 patients (21%), and both in 19 patients (9.5%). Abnormal laboratory features (thrombocytopenia, false-positive syphilis test, and prolonged activated partial thromboplastin time) had led to APS diagnosis in six patients (3%). Patients were divided into two groups: 152 whose APS diagnosis was secondary to thrombosis (thrombotic APS), alone or with other clinical features, and 48 who were diagnosed because of other clinical manifestations (non-thrombotic APS).

Table 1 reports the baseline characteristics of the whole cohort. Obstetric morbidities ($P < 0.001$) and LDA treatment ($P < 0.001$) were more frequent in the non-thrombotic APS group, whereas arterial hypertension ($P = 0.006$), dyslipidaemia ($P = 0.04$) and VKA therapy ($P < 0.001$) were more frequent in the thrombotic APS group. Incidence rate of thrombosis recurrence in the whole cohort was 0.03 per patient-year, being more frequent in the thrombotic APS group ($P = 0.02$), with a maximum number of four flare-ups in three cases. There were a total of 17 (8.7%) bleeding episodes in the whole cohort, with no significant differences between the two groups. Anti-dsDNA ($P = 0.048$) and LAC ($P = 0.01$) were more prevalent in thrombotic APS. Non-criteria APS manifestations were equally distributed among the two groups, except migraine ($P = 0.005$) that resulted more frequently among thrombotic APS patients.

aGAPSS & DIAPS

Mean aGAPSS of the whole cohort was 7.23 (S.D. 4.79), ranging from 0 to 17. Thrombotic APS patients showed a higher average aGAPSS in comparison non-thrombotic APS [7.6 (S.D. 4.8) *vs* 6.1 (S.D. 4.7), $P = 0.05$]. In the thrombotic APS group, mean aGAPSS was significantly higher in patients with arterial thrombosis *vs* those with venous thrombosis [8.6 (S.D. 4.98) *vs* 6.7 (S.D. 4.3), $P = 0.02$]. Mean DIAPS of the entire cohort increased from 1.03 (S.D. 0.81) at baseline to 1.31 (S.D. 1.05) at last visit, resulting higher in thrombotic APS compared with non-thrombotic APS both at baseline [1.3 (S.D. 0.68) *vs* 0.19 (S.D. 0.58), $P < 0.001$] and last visit [1.59 (S.D. 0.96) *vs* 0.42 (S.D. 0.82),

Table 1. Demographic characteristics, clinical manifestations, immunological features, and treatment of patients with thrombotic *vs* non-thrombotic APS

	Entire series ($n = 200$)	Thrombotic[a] APS ($n = 152$)	Non-thrombotic[a] APS ($n = 48$)	*P*-value
Female Sex, *n* (%)	144 (72)	96 (63.1)	48 (100)	<0.001
Age at diagnosis, mean (S.D.), years	41.7 (13.3)	42.9 (14.2)	36 (8)	<0.001
Disease duration median (IQR), months	172.5 (120–240)	166.5 (111–226)	210.5 (136–260)	0.03
APS Type, *n* (%)				
Primary	138 (69)	100 (65.8)	38 (79.2)	ns
APS associated to other AID[b]	62 (31)	52 (34.1)	10 (20.8)	ns
Thrombosis, *n* (%)				
Arterial	83 (41.5)	74 (48.7)	9 (18.8)	<0.001
Venous	99 (49.5)	96 (63.2)	3 (6.3)	<0.001
Both	19 (9.5)	17 (11.2)	2 (4.2)	ns
Recurrence	61 (30.5)	53 (34.9)	8 (16.7)	0.02
Pregnancy losses, *n* (%)				
First trimester miscarriages[c]	56 (29.8)	31 (20.4)	25 (52.1)	<0.001
Foetal losses[d]	44 (23.4)	16 (10.5)	28 (58.3)	<0.001
Cardiovascular risk factors, *n* (%)				
Smoking	54 (29)	42 (29.6)	12 (27.3)	ns
Diabetes mellitus	8 (4)	8 (5.3)	0 (0)	ns
Surgery	48 (24)	36 (23.7)	12 (25)	ns
Oral contraceptives	22 (11.2)	20 (13.4)	2 (4.2)	ns
Nephrotic syndrome	6 (3)	5 (3.3)	1 (2.1)	ns
Congenital thrombophilias[e]	7 (3.5)	7 (4.6)	0 (0)	ns
Arterial hypertension	66 (33)	58 (38.2)	8 (16.7)	0.006
Dyslipidaemia	66 (33)	56 (36.8)	10 (20.8)	0.04
Non criteria manifestations, *n* (%)				
Chorea	1 (0.5)	1 (0.7)	0 (0)	ns
Epilepsy	11 (5.5)	9 (5.9)	2 (4.2)	ns
Migraine	20 (10)	20 (13.2)	0 (0)	0.005
Myelitis	2 (1)	2 (1.3)	0 (0)	ns
Cardiac valve disease	21 (10.5)	19 (12.5)	2 (4.2)	ns
Livedo reticularis/racemosa	11 (5.5)	9 (5.9)	2 (4.2)	ns
Skin ulcers	4 (2)	3 (2)	1 (2.1)	ns
Thrombocytopenia	41 (20.5)	29 (19.1)	12 (25)	ns
Haemolytic anaemia	5 (2.5)	5 (3.3)	0 (0)	ns
aPL nephropathy	6 (3)	5 (3.3)	1 (2.1)	ns
Immunological features, *n* (%)				
ANA	138 (69)	108 (71.5)	30 (62.5)	ns
Anti-dsDNA antibody	50 (25)	43 (28.9)	7 (14.6)	0.048
Anti-Ro/SSA antibody	13 (6.5)	10 (6.9)	3 (6.4)	ns
Anti-La/SSB antibody	6 (3)	6 (4.1)	0 (0)	ns
Anti-RNP antibody	12 (9)	8 (5.6)	4 (9.1)	ns
Baseline aPLs, *n* (%)				
aCL IgG	119 (59.5)	91 (59.9)	28 (58.3)	ns
aCL IgM	57 (28.5)	42 (27.6)	15 (31.3)	ns
aβ2GPI IgG	71 (35.5)	57 (37.8)	14 (29.2)	ns
aβ2GPI IgM	40 (20)	29 (19.2)	11(22.9)	ns
aPS/PT[f]	5 (2.5)	5 (3.3)	0 (0)	ns
LAC	137 (68.5)	111 (73)	26 (54.2)	0.01
Double positivity[g]	125 (62.5)	96 (63.2)	29 (60.4)	ns
Triple positivity	66 (33)	52 (34.2)	14 (29.2)	ns
Treatment, *n* (%)				
LDA	66 (33)	26 (17.1)	40 (83.3)	<0.001
VKA	131 (65.5)	127 (83.6)	4 (8.3)	<0.001
LMWH	18 (9)	11 (7.2)	7 (14.6)	ns
Corticosteroids	11 (5.5)	11 (7.2)	0 (0)	ns
HCQ	48 (24)	39 (25.7)	9 (18.8)	ns

[a] Thrombotic APS group includes patient that were diagnosed of APS because of thrombosis (alone or with other manifestations); non-thrombotic APS group includes patients who did not have episodes of thrombosis at APS diagnosis and were diagnosed because of other clinical manifestations (i.e. pregnancy morbidity).
[b] Other AID include: 41 patients with SLE, 17 patients with 'SLE-like', two patients with systemic sclerosis, one patient with Sjögren syndrome and one with Behçet's disease.
[c] Three consecutive unexplained spontaneous abortions before 10th week.
[d] Unexplained foetal death at or beyond 10th week.
[e] Congenital thrombophilias include factor V Leiden mutation ($n = 1$) and prothrombin G20210A mutation ($n = 6$).
[f] aPS/PT were testes in only 18 patients in total.
[g] Any combination of two positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria.
aβ2GPI: anti-β2-glycoprotein I antibodies; aCL: anticardiolipin antibodies; AID: autoimmune diseases; aPL: antiphospholipid antibodies; aPS/PT: anti-phosphatidylserine/prothrombin; CLIA: chemiluminescence immunoassay; ds-DNA: double stranded DNA; LAC: lupus anticoagulant; LDA: low dose aspirin; LMWH: low-molecular-weight-heparin; RNP: ribonucleoprotein; VKA: vitamin K antagonist.

Table 2. Distribution of DIAPS domains involvement at baseline (APS diagnosis) and at the end of follow-up for patients with thrombotic and non-thrombotic APS

	Baseline DIAPS			DIAPS at the end of FU		
	Entire series ($n = 200$)	Thrombotic APS ($n = 152$)	Non-thrombotic APS ($n = 48$)	Entire series ($n = 200$)	Thrombotic APS ($n = 152$)	Non-thrombotic APS ($n = 48$)
Peripheral vascular[a]	82 (41)	81 (53.3)	1 (2.1)	87 (43.5)	83 (54.6)	4 (8.3)
Pulmonary[b]	9 (4.5)	8 (5.3)	1 (2.1)	12 (6)	11 (7.2)	1 (2.1)
Cardiovascular[c]	15 (7.5)	14 (9.2)	1 (2.1)	28 (14)	26 (17.1)	2 (4.2)
Neuropsychiatric[d]	43 (21.5)	40 (26.3)	3 (6.3)	50 (25)	44 (28.9)	6 (12.5)
Ophtalmologic[e]	12 (6)	12 (7.2)	0 (0)	15 (7.5)	14 (9.2)	1 (2.1)
Renal[f]	2 (1)	2 (1.3)	0 (0)	8 (4)	7 (4.6)	1 (2.1)
Muscoloskeletal[g]	4 (2)	3 (2)	1 (2.1)	5 (2.5)	4 (2.6)	1 (2.1)
Cutaneous[h]	6 (3)	6 (4)	0 (0)	9 (4.5)	9 (5.9)	0 (0)
Gastrointestinal[i]	11 (5.5)	10 (6.6)	1 (2.1)	12 (6)	11 (7.2)	1 (2.1)
Endocrine[j]	1 (0.5)	1 (0.7)	0 (0)	3 (1.5)	2 (1.3)	1 (2.1)

[a] Consists of deep vein-thrombosis, intermittent claudication, tissue loss (minor/major), and/or vascular venous insufficiency.
[b] Consists of pulmonary infarction, pulmonary arterial hypertension, chronic thromboembolic pulmonary hypertension, and/or respiratory insufficiency.
[c] Consists of coronary artery bypass, myocardial infarction, cardiomyopathy, and/or aPL-associated heart valve disease (with or without valvular replacement).
[d] Consists of cognitive impairment, seizures, ischaemic stroke with hemiparesia/hemiplegia, multi-infarct dementia, cranial neuropathy, sudden sensorineural hearing loss, transverse myelitis, optic neuropathy, and/or abnormal movements.
[e] Consists of retinal vaso-occlusive disease, and/or blindness.
[f] Consists of chronic renal failure, proteinuria 24 h > 3.5 g/vol and/or renal thrombotic microangiopathy.
[g] Consists of avascular necrosis.
[h] Consists of chronic cutaneous ulcers.
[i] Consists of mesenteric thrombosis, Budd–Chiari syndrome and/or cirrhosis of the liver.
[j] Consists of suprarenal insufficiency, hypopituitarism, infertility.
DIAPS: damage index for antiphospholipid syndrome; FU: follow-up.

$P < 0.001$]. In the thrombotic APS group DIAPS increased more in patients with arterial than venous thrombosis [0.33 (S.D. 0.67) *vs* 0.13 (S.D. 0.52), $P < 0.049$].

Table 2 describes the rate of different DIAPS domains' involvement at APS diagnosis and at last visit. Among patients with thrombotic APS, the most frequently affected domains at baseline were peripheral vascular (53.3%), neuropsychiatric (26.3%) and cardiac (9.2%), whereas, among non-thrombotic APS, neuropsychiatric was the most represented domain (6.3%). During follow-up, among thrombotic APS patients, cardiac domain involvement showed the highest increase (from 9.2% to 17.1% of patents) while in non-thrombotic APS patients, the highest increase in the rate of involvement was shown by the neuropsychiatric domain (from 6.3% to 12.5%).

Predictors of DIAPS change

Univariate linear regression analysis showed that the mean aGAPSS over time was associated to DIAPS increase during follow-up (b = 0.04, $P < 0.001$). When we made subgroup analysis, the aGAPSS and DIAPS maintained their positive correlation either when considering only thrombotic (b = 0.03, $P = 0.009$) or only non-thrombotic APS patients (b = 0.04, $P = 0.001$). When analysing several factors separately in univariate analysis, we found a statistically significant association between DIAPS change and age at diagnosis (b = –0.008, $P = 0.02$), disease duration (b = 0.001, $P = 0.047$), arterial hypertension (b = 0.35, $P < 0.001$), presence of SLE (b = 0.31, $P = 0.005$), use of HCQ (b = 0.26, $P = 0.01$), anti-β2GPI antibodies (b = 0.22, $P = 0.01$), LAC (b = 0.27, $P = 0.005$), double aPL positivity (b = 0.23, $P = 0.01$) and triple aPL positivity (b = 0.22, $P = 0.02$). Conversely, there was no association between DIAPS increase and sex (b = –0.13, $P = 0.19$), active smoking (b = 0.33, $P = 0.48$), oral contraceptives (b = –0.21, $P = 0.16$), congenital thrombophilias (b = 0.29, $P = 0.24$), dyslipidaemia (b = 0.17, $P = 0.08$), aCL antibodies (b = 0.09, $P = 0.41$), LDA (b = 0.01, $P = 0.90$) and VKA treatment (b = 0.13, $P = 0.14$). In the multivariate analysis including as confounding factors age at diagnosis, disease duration, SLE, congenital thrombophilia and HCQ, only aGAPSS (b = 0.04, $P < 0.001$) retained statistical significance (Table 3).

When considering the two groups separately in the univariate analysis, in the thrombotic group age at diagnosis (b = –0.011, $P = 0.003$), SLE (b = 0.32, $P = 0.01$), HCQ (b = 0.25, $P = 0.038$), arterial hypertension (b = 0.27, $P = 0.01$), anti-β2GPI antibodies (b = 0.24, $P = 0.027$) and LAC (b = 0.27, $P = 0.026$) were associated with DIAPS change, whereas in the non-thrombotic group we found an association only with arterial hypertension (b = 0.775, $P < 0.001$).

When comparing patients with high (increase of DIAPS ≥1 during follow-up) *vs* low (increase of DIAPS <1 during follow-up) damage accrual, higher mean aGAPSS values were found in patients with a high damage [9.22 (95% CI 7.58, 10.86) *vs* 6.72 (95% CI 6.0, 7.43), $P = 0.003$] (Fig. 1). Moreover, when performing a logistic regression analysis to look for predictors of high damage accrual (DIAPS increase ≥1) we found that aGAPSS [OR = 1.11 (95% CI 1.04, 1.20, $P = 0.004$)], arterial hypertension [OR = 3.03 (95% CI 1.49, 6.13, $P = 0.002$)], presence of SLE [OR = 3.27 (95% CI 1.54, 6.97, $P = 0.002$)], HCQ [OR = 3.37 (95% CI 1.62, 7.01, $P = 0.001$)] and LAC positivity [OR = 4.13 (95% CI 1.54, 11.12, $P = 0.005$)] were associated with high damage, whereas higher age at diagnosis [OR = 0.96 (95% CI 0.93, 0.99, $P = 0.01$)] was slightly protective. aGAPSS association with high damage was confirmed in the multivariate analysis [OR = 1.12 (95% CI 1.04, 1.21, $P = 0.003$)] including SLE, age at diagnosis, disease duration, congenital thrombophilia and HCQ as confounding factors.

Table 3. Univariate and multivariate linear regression analysis of predictors of DIAPS change during follow-up

	Beta coeff (95% CI)	*P*-value
Univariate Analysis		
aGAPSS	0.04 (0.02, 0.05)	**<0.001**
Gender (female)	–0.13 (–0.32, 0.06)	0.188
Age at diagnosis	–0.008 (–0.015, –0.001)	**0.017**
Disease duration	0.001 (0.00001, 0.002)	**0.047**
Active smoking	0.33 (–0.59, 1.27)	0.482
Oral contraceptives	–0.21 (–0.50, 0.081)	0.156
Congenital thrombophilias[a]	–0.3 (–0.81, 0.21)	0.243
Arterial hypertension	0.35 (0.17, 0.53)	**<0.001**
Dyslipidaemia	0.17 (–0.17, 0.36)	0.075
Presence of SLE	0.31 (0.09, 0.52)	**0.005**
aCL	0.09 (–0.12, 0.30)	0.408
aβ2GPI	0.22 (0.05, 0.40)	**0.013**
LAC	0.27 (0.08, 0.46)	**0.005**
Double positivity[b]	0.23 (0.05, 0.42)	**0.011**
Triple positivity	0.22 (0.03, 0.40)	**0.024**
LDA	0.01 (–0.17, 0.20)	0.902
VKA	0.13 (–0.04, 0.33)	0.139
HCQ	0.26 (0.06, 0.47)	**0.012**
Multivariate Analysis[c]		
aGAPSS	0.037 (0.020, 0.055)	**<0.001**
Age at diagnosis	–0.005 (–0.012, 0.002)	0.168
Disease duration	0.0005 (–0.0005, 0.001)	0.307
Presence of SLE	0.155 (–0.095, 0.406)	0.222
Congenital Thrombophilias	–0.27 (–0.734, 0.188)	0.245
HCQ	0.094 (–0.141, 0.329)	0.432

[a] Congenital thrombophilias include factor V Leiden mutation ($n = 1$) and prothrombin G20210A mutation ($n = 6$).
[b] Any combination of two positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria.
[c] Adjusted for age at diagnosis, disease duration, and presence of SLE.
aβ2GPI: anti-β2-glycoprotein I antibodies; aCL: anticardiolipin antibodies; aGAPSS: adjusted global APS score LAC: lupus anticoagulant; LDA: low dose aspirin; VKA: vitamin K antagonists.

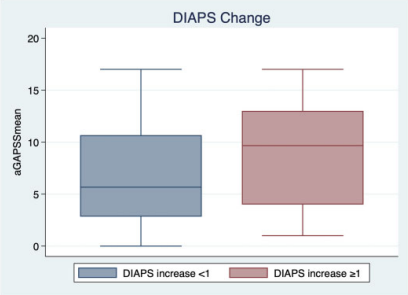

Figure 1. aGAPSS in low *vs* high damage accrual. Distribution of mean adjusted Global Antiphospholipid Syndrome Score (aGAPSS) in patients with patients with high (increase of DIAPS ≥1 during follow-up) vs low (increase of DIAPS <1 during follow-up) damage accrual. Data are shown as box plots, where each box represents the 25th to 75th percentiles and lines inside the box represent the median. The whiskers represent the 95% CI. Higher aGAPSS values were detected in patients with high in comparison to low damage accrual

Finally, when analysing the association between DIAPS and mortality, we found that baseline DIAPS was associated with increased odds of death during follow-up [OR = 3.73 (95% CI 1.43, 9.74, $P = 0.007$)] while DIAPS at the end follow-up was not [OR = 1.89 (95% CI 0.91, 3.92, $P = 0.086$)].

Discussion

The main objective, when addressing an AID, is the control of disease activity, a reversible process usually related to inflammation, in order to prevent damage, a permanent change secondary to disease activity itself. APS is usually diagnosed in young people [30] and both thrombosis and pregnancy morbidity, the main clinical manifestations, tend to recur despite treatment. For instance, in spite of anticoagulation, the 5-year rate of thrombosis recurrence can be as high as 16.6% [31]. Due to this high rate of clinical recurrence, the damage burden tends to increase over time in APS.

Because a disease activity tool specific for APS is still lacking, we decided to use the aGAPSS as a surrogate disease activity instrument, considering its evolving nature: in fact, both cardiovascular risk factors and aPLs can vary over time, reflecting a variable risk of clinical manifestations. To the best of our knowledge, this is the first study evaluating the association between the aGAPSS and the variation of DIAPS in APS patients. Moreover, most DIAPS studies published so far have been realized in Latin-American populations, therefore this is one of the first studies carried out in Caucasians. Finally, there is a lack of information about DIAPS performance in non-thrombotic APS, so we performed a sub-analysis of this group of patients.

Using linear regression models, we found that mean aGAPSS over time was associated to DIAPS increase during follow-up, a result that was confirmed even when assessing thrombotic and non-thrombotic APS patients separately. Interestingly, in a recent small study, Radin *et al.* found a significantly positive correlation between GAPSS and DIAPS in aPL-positive patients [32]. These results support the idea that aGAPSS, as a score that considers the effect of persistent aPL positivity and cardiovascular risk factors, reflects disease activity and, consequently, damage probability in APS. As a clue to that, arterial hypertension, a component of the aGAPSS, was associated to increase in DIAPS both in thrombotic and non-thrombotic APS patients. Furthermore, when considering the thrombotic APS group separately, we found that mean aGAPSS was significantly higher in patients with arterial thrombosis *vs* patient with venous thrombosis, a result that is in line with a previous study from our group [33], and that, alongside, DIAPS increased more in patients with arterial than venous thrombosis, a further proof of the positive correlation between the two scores.

We also compared patients with high *vs* low damage accrual, setting a cut-off value of 1 point of DIAPS increase, finding that mean aGAPSS was higher in patients with high damage accrual. Moreover, in the multivariate logistic regression analysis, we found that aGAPSS was associated with high damage accrual. These results further highlight the utility of the aGAPSS as a disease activity instrument and predictor of severe damage.

When analysing the aPL profile, anti-β2GPI antibodies, LAC, double and triple aPL positivity were associated to an increase of DIAPS over time. This is predictable, as it is well known that LAC and triple positivity are associated with a higher risk of first thrombotic event and thrombosis recurrence in APS [34, 35], and are therefore major risk factors for an increased damage accrual over time.

Arterial hypertension and association with SLE also correlated with an increase of DIAPS over time. This is not surprising as both are well known risk factors for thrombosis in APS

patients [36]. For instance, in a Brazilian study that assessed 100 APS patients (50% PAPS, 50% SLE-associated) over 10 years, the authors found a 35% increase of DIAPS in PAPS (from 1.72 to 2.04), whereas SLE-associated APS reached a 139% increment from baseline (from 0.82 to 2.24) [37], indicating that the presence of SLE increased the damage accrual over time. Moreover, other authors have found a higher DIAPS in AID-associated APS compared with PAPS [32, 38]: the cause of that is most likely to be sought in clinical manifestations secondary to the associated AID, and in immunosuppressive treatment side effects. Interestingly we found that HCQ administration was associated with a DIAPS increase during follow-up. However, this is probably related to the presence of SLE, as all patients taking HCQ had SLE. Finally, a younger age at diagnosis and longer disease duration also showed association with increased damage burden: this is also logical, as an earlier onset of the disease and longer duration naturally lead to a higher damage accrual.

In our population, peripheral vascular was the domain that most frequently contributed to damage, followed by neuropsychiatric and cardiovascular. This result is different from a cohort from Latin America, where the most frequently affected domains were neuropsychiatric, peripheral vascular and pulmonary (in this order) [39], and might reflect a variability in clinical manifestations among different ethnicities.

Finally, baseline DIAPS was associated with an increased death rate in our cohort, reflecting the idea that damage predicts mortality. Conversely, DIAPS at the end of follow-up did not show this correlation: this might reflect the effect of therapy and reduced damage accrual in patients under standard of care treatment.

A main strength of our study is the longitudinal assessment of the aGAPSS score, throughout at least three years. As both aPL positivity and cardiovascular risk factors can change over time, a longitudinal evaluation is necessary to provide the highest accuracy. Moreover, our study included a homogeneous cohort of 200 patients from a single centre, all classified as having APS by fulfilment of classification criteria. All aPLs were tested in the same laboratory, with the same cut-off values, allowing a homogeneous evaluation of positivity. Finally, DIAPS was calculated by the same author (G.B.) and confirmed by two reference experts in APS (R.C. and G.E.).

Our study also has several limitations. The first is related to its retrospective design, which implicates that the aGAPSS has been computed after the onset clinical manifestations. However, aPLs, dyslipidaemia and arterial hypertension were assessed routinely at each visit so, in case of clinical recurrence, the actual evaluation preceded the clinical manifestation in most cases. Surely a prospective design would be more suitable for assessing risk factors associated to increased damage; nevertheless, APS is a low-prevalence condition, making a prospective study including a consistent number of patients hard to perform. Second, the definition of high damage accrual as DIAPS increase $\geq$1 point is arbitrary. However, Medina *et al.* from the same group that originally developed the score considered a DIAPS $\geq$3 as severe damage [39]; therefore, we thought that an increase of $\geq$1 point would represent a significant damage increment. Third, some limitations are related to the nature of DIAPS per se. To start, all items are binary, giving the same relevance to organ damages that result in different clinical and prognostic implications; for instance, pulmonary hypertension secondary to chronic thromboembolic events carries a worse prognosis than, for example, adrenal insufficiency, which is easily treated with steroid replacement therapy. Furthermore, potentially severe non-criteria manifestations such as multiple sclerosis-like disease or diffuse pulmonary haemorrhage are not included. Finally, DIAPS does not take into account treatment-related complications (i.e. haemorrhagic stroke due to anticoagulation) [40]. A new version is under development and planned for completion by the end of 2023.

Controlling disease activity is the best way to prevent damage in AID. However, because APS pathophysiology relies more on thrombotic than inflammatory mechanisms, the development of a disease activity index is more difficult to accomplish than in other conditions such as SLE, SS or inflammatory myopathies, and might require a different approach. Some manifestations, like transient ischaemic attacks, haemolytic anaemia or thrombocytopenia, which are amenable to treatment, do lend themselves more readily to consideration as activity features [41]. Nevertheless, because such manifestations can be related to SLE, they should probably be considered as a sign of activity only in patients with PAPS. Moreover, the role of aPL pattern over time in relation to disease activity needs to be further investigated. A development and validation of a disease activity index in APS is currently ongoing [42]. While such an instrument becomes available, an easy tool such as the aGAPSS, that has shown to be associated with clinical manifestations, can be used as a surrogate of disease activity. Besides classical therapies used in APS, several treatment strategies can be explored to control disease activity and prevent damage. HCQ has shown anti-inflammatory, immunomodulatory, and thromboprophylactic effects [43], and was associated with a significant decrease in aPL titers over an average 2.6-year follow-up in a randomized open-label study [44]. Therefore, it might be a treatment option in patients at high risk of damage accrual, and even though in our cohort we found an increase of DIAPS in patients under HCQ, it would be interesting to assess its effect in primary APS.

In summary, we performed a longitudinal assessment of the aGAPSS finding that a higher mean score is associated with higher damage accrual, measured through DIAPS increase during follow-up. Confirmation of these results in large, prospective multicentric studies is warranted.

Conclusions

Presently, DIAPS is the only instrument available to measure damage in APS. aGAPSS is an easy tool to assess the risk of clinical manifestations in patients with APS and can be used as a surrogate of disease activity while a specific index is developed. Periodic monitoring of aPLs and cardiovascular risk factors during follow-up is warranted to have an up-to-date picture of patients' risk.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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The objective of the third study was to assess if the mean aGAPSS over time linearly correlates with damage accrual, measured as the difference between the DIAPS at the end of follow-up and the basal DIAPS. Since an activity index for APS is lacking, we postulated that aGAPSS, in light of its dynamic nature, could serve a surrogate of disease activity.

aGAPSS was calculated for each patient at baseline and on a yearly basis for up to 6 years (minimum three years). DIAPS was computed at baseline and at last visit summing the score for each of the 38 items included in the index. DIAPS change was computed as the difference between mean DIAPS at last visit and mean DIAPS at APS diagnosis.

We split the entire cohort of 200 patents in two groups: 152 patients whose APS diagnosis was secondary to thrombosis (thrombotic APS), alone or with other clinical features, and 48 who were diagnosed because of other clinical manifestations (non-thrombotic APS).

Among patients with thrombotic APS, the most frequently affected domains at baseline were peripheral vascular (53.3%), neuropsychiatric (26.3%) and cardiac (9.2%), whereas, among non-thrombotic APS, neuropsychiatric was the most represented domain (6.3%).

DIAPS change showed a positive linear correlation with mean aGAPSS over time (b=0.04) which was confirmed when analyzing thrombotic and non-thrombotic group separately. When analyzing the association between different factors and DIAPS change we found a positive correlation with disease duration, arterial hypertension, presence of SLE, anti-β2GPI antibodies, LA, double and triple aPL positivity and a negative correlation with age at diagnosis. However, only aGAPSS retained statistical significance in the multivariate analysis.

We then compared patients with high vs low damage accrual (setting a cut-off of 1 point of DIAPS increase during follow-up) finding that mean aGAPSS over time was higher in patients with high damage (9.22 vs 6.72). Moreover, in the multivariate logistic regression analysis aGAPSS was a predictor of high damage accrual (OR 1.12, 95% CI 1.04-1.21).

Lastly, when analyzing the association between DIAPS and mortality, we found that baseline DIAPS correlated with increased odds of death during follow-up (OR 3.73, 95% CI 1.43-9.74), whereas DIAPS at the end of follow-up did not.

We could therefore conclude that mean aGAPSS over time is a good predictor of damage accrual and, while a disease activity for APS becomes available, can serve surrogate a disease activity in APS patients.

DISCUSSION

Recurrence of clinical manifestations is a main concern in APS, a condition that usually has its onset in the 4th decade of life [2], with the initial clinical features that manifest an increasing cumulative prevalence as the disease progresses over time. In the biggest prospective study realized to date, the "Euro-phospholipid" project, that included 1000 patients, the 5-year rate of thrombosis recurrence was 16.6%[8]. The chronic and recurrent nature of the disease highlights the need for risk stratification and damage assessment tools.

As stated in the treat-to-target recommendations of several AID [120–122], the ultimate aim of treatment in AID is the prevention of irreversible damage through control of disease activity, a reversible process related to inflammation. A similar strategy is hard to implement in APS. In fact, while a damage tool for APS, the DIAPS, has been set up, a disease activity tool is still lacking. This is not surprising, considering the pathophysiology of the disease, in which, in contrast with other AID, inflammation does not play a major role and thrombosis is the main driver of damage. Therefore, risk stratification for clinical recurrence and damage accrual must rely on other instruments.

Since APS discovery, several risk factors for thrombosis recurrence have been identified, such as previous arterial thrombosis, cardiovascular risk factors, association with other AID (mainly SLE) and specific aPL profiles such as presence of LA and triple positivity. Moreover, while low-titer transient aPL positivity seems to be associated to external factors such as infections, drugs, and malignancies, bearing a low risk of clinical manifestations, persistently positive aPL at medium-high titers are more likely to have clinical implications.

The course of aPL positivity over time has been investigated by several groups with controversial results: most authors reported that baseline aPL tend to remain positive over time, but in the study with the longest follow-up (almost 10 years), aPL became persistently negative in almost 60% of pregnant women with initial aPL positivity [107]. Moreover, while baseline multiple (especially triple) positivity and higher aPL titers are unquestionably associated with aPL persistence, the role of single positivity is less clear, with some studies reporting association with transient aPL [104], whereas others with persistent positivity [105].

Among different score systems proposed as risk stratification tool for clinical manifestations in aPL positive patients, the GAPSS/aGAPSS has drawn increasing attention in last years. This score, that takes into account the aPL profile along with cardiovascular risk factors, has been devised in a cohort of aPL positive SLE patients and then validated in primary APS, showing to be a good predictor of recurrence of clinical manifestations. It is, by its very nature, a dynamic score, since aPL might fluctuate over time and cardiovascular risk factors can be modified by lifestyle and treatment. Therefore, a single assessment of the GAPSS/aGAPSS might not reflect the average risk state of a patient, and the GAPSS/aGAPSS value immediately close to a clinical manifestation it is probably different from that of another time-point. However, almost all studies included only one time point determination of the score in their design, and the only one study that monitored the score longitudinally [108] found an increase in the GAPSS from baseline to last visit in patients who experienced thrombosis. Therefore, we postulated that periodic, longitudinal monitoring of the GAPSS, gives a more reliable picture of the current risk of clinical manifestations for each patient.

The first study of the present thesis analyzed the course of aPL positivity over time in a monocentric cohort of aPL patients, with the aim of checking what is the rate of aPL persistence over a long follow-up (median FU of more than 14 years) and which baseline aPL profiles are predictors of persistent aPL positivity over time. Moreover, we assessed the link between aPL persistence over time and clinical recurrence.

aPL were classifiable as persistent (positive in >2/3 of total determinations) in 56% of our patients, a rate slightly lower when compared to the majority of previous studies, but higher than the 25.7% reported in the study with the longest follow-up (more than 10 years) performed so far. Interestingly, APS associated to other AID presented a higher rate of persistence compared to PAPS, close to the one reported by the APS-ACTION group in their study [106].

When we looked for baseline aPL profiles associated to aPL persistence during follow-up, we found that aCL IgM, anti-β2GPI IgG/IgM, and LA positivity in the setting of multiple positive aPL along with double and triple positivity correlated with aPL persistence. Moreover, higher aPL baseline titers correlated with aPL persistent positivity over time. Such results are in concordance with previous literature, in which triple positivity and higher aPL titers were linked to aPL persistence. Conversely, isolated aCL IgG/IgM and LA presence correlated with transient

aPL positivity. The latter finding, that has also been reported in the APS-ACTION cohort, might be related to the high number of patients under VKA therapy, which increases the likelihood of false positive results in the LA test. Nevertheless, the finding that single aPL positivity negatively correlates with persistence is no news.

When assessing the association between aPL persistent positivity and recurrence of clinical manifestations, we found that aPL persistence correlated with recurrence of cumulative (thrombotic plus obstetric) as well as exclusively thrombotic manifestations. This confirms previous literature, including a work from our group, that associated aPL persistence with thrombotic manifestations in SLE and APS.

One limitation of our study is its retrospective design, which implicates that APS clinical manifestations have been assessed "a posteriori". However, aPL were routinely assessed at each visit: therefore, in case of recurrence, the laboratory evaluation preceded the clinical manifestation in most cases. Another limitation is that LA test was performed under VKA treatment in approximately 65% of patients, a factor that increases the likelihood of false positive results. As mentioned above, this can be a possible explanation of the association between isolated LA positivity and transient aPL. On the other hand, a main strength of our study is the long follow-up (median > 14 years) allowing to observe the evolution of aPL positivity over a long time and its association with clinical manifestations. Moreover, the selection of a homogenous cohort of 200 patients from a single centre, all classified as having APS by fulfilment of current classification criteria, and the fact that all aPL were tested in the same reference laboratory, with the same cut-off values, allowing a homogenous evaluation of positivity, increase the robustness of our results.

In summary our study confirms the tendency of baseline aPL to remain persistently positive in APS patients, the association between multiple positivity and aPL persistence and between single positivity and aPL transience, and the fact that aPL persistent positivity predisposes to recurrence of clinical manifestations.

The second study assessed the value of longitudinal monitoring of the aGAPSS as predictor of recurrence of APS clinical manifestations. We decided to use the modified version of the GAPSS, that does not include aPS-PT antibodies, since they are not tested in most laboratories. Moreover, instead of using the baseline value, we calculated the mean aGAPSS score over time and the delta

of the aGAPSS (computed as the difference between the last and the first value), considered both as a more reliable picture of the instant risk of recurrence for each patient.

We found that the mean and delta aGAPSS were higher in patients who experienced clinical recurrence compared to patients who did not, result that was confirmed when considering the baseline aGAPSS. Conversely, when comparing patients that experienced thrombotic recurrence to patients with obstetric recurrence alone, while the mean and the delta aGAPSS were significantly higher in the first group, the baseline aGAPSS was not statistically different. This finding highlights the usefulness of longitudinal monitoring of the score in the follow-up, as depicting a different risk from the one-time assessment.

When considering patients with thrombotic recurrence among themselves, in line with former literature, we detected higher mean aGAPSS values in patients experiencing arterial thrombosis than patients with venous thrombosis. The mean aGAPSS of patients with obstetric recurrence was not significantly different in comparison to patients with no recurrence, a discordant result with respect to what found by Sciascia et al. in their original study [95]. However, such work included only SLE patients, which most likely were not under treatment. This outcome might reflect the possibility that obstetric recurrence could not be as related to aPL and cardiovascular risk factors' persistence over time as thrombotic recurrence.

We then performed a Cox regression analysis to identify the best cut-off value for predicting recurrence of clinical manifestations, finding that a mean aGAPSS cut-off of 13 was the best discriminator with the highest HR. Such value is higher than the one reported by Fernandez-Moisterin et al. in their Spanish cohort that included patients with and without a diagnosis of APS [100], reflecting the hypothesis that different cohorts might present different cut-off values, with patients fulfilling APS criteria prone to have higher aGAPSS.

To increase the validity of our results, we calculated the TTR in patients under oral anticoagulation with VKA, finding no differences between patients that presented thrombotic recurrence and patients who did not. However, we were not able to assess TTR in all patients, which represents a limitation of the study. Anyway, the parameter was available in 58% of patients, a rate that is likely representative of the whole population. Conversely, a main strength of our study is the longitudinal assessment of such a dynamic the score, throughout at least three years. The high rate (above 80%) of patients that presented a change in their score during the follow-up, in most cases due to variation of aPL positivity, and the discrepancy in the results

between the mean and delta aGAPSS and the baseline aGAPSS emphasize this aspect. Further strengths are the inclusion of a homogenous cohort of APS patients from a single center and the use of the same reference laboratory for testing aPL, with unique techniques and cut-off values.
In conclusion, we found that a higher average aGAPSS over time predisposes to both thrombotic and obstetric recurrence, being arterial thrombosis recurrence, the manifestation associated with the highest score. The rate of aGAPSS variation over time was high (above 80%), highlighting the indication for longitudinal monitoring with repetitive measures of the score, as pointed out also by the different results obtained with mean and delta aGAPSS compared to baseline aGAPSS. A mean aGAPSS > 13 points was highly predictive of clinical recurrence.

In the third study we assessed the utility of using the average aGAPSS over time as a predictor of damage accrual, measured through DIAPS change in the follow-up. Since a disease activity index for APS is still lacking, we postulated that a dynamic score such as the aGAPSS could serve as a surrogate of a disease activity instrument.
Mean aGAPSS over time and DIAPS change presented a linear correlation, being an increase in aGAPSS associated to an increase in DIAPS during the follow-up. This correlation was maintained when considering thrombotic and non-thrombotic patients separately. Moreover, when comparing patients with high and low damage accrual, we found higher mean aGAPSS values in the first group. Finally, in the logistic regression analysis, mean aGAPSS increased the odds of high damage accrual during follow-up.
We also analyzed the role of aPL profile, finding that anti-β2GPI antibodies, LA, double and triple aPL positivity were associated to an increase of DIAPS over time. This is not surprising, as LA and triple positivity are associated with a higher risk of first thrombotic event and thrombosis recurrence in APS, therefore predisposing to more damage accrual over time. Association with SLE also correlated with increased damage accrual, as found in a previous cohort from Brazil, that compared 50 patients with primary APS with 50 patients with SLE-associated APS, finding a 35% increase of DIAPS over 10 years in PAPS, and a 139% increase in APS associated to SLE [123].
In our population, peripheral vascular was the domain that most frequently contributed to damage, followed by neuropsychiatric and cardiovascular. This result is different from a cohort from Latin America [124], in which the most frequently affected domains were neuropsychiatric,

peripheral vascular and pulmonary (in this order), and might reflect a variability in clinical manifestations among different ethnicities.

Finally, we analyzed the association between DIAPS and mortality, finding that only baseline DIAPS correlated with increased odds of death during follow-up. This result confirms the idea that early damage predicts mortality, while late damage can be mitigated by the effect of therapy. One limitation of our study is the arbitrary definition of high damage accrual. Nevertheless, Medina et al. from the same group that originally developed the score, considered a DIAPS ≥ 3 as severe damage [124]; hence, we assumed that an increase of 1 point would represent a significant damage increment. Moreover, some limitations are related to the nature of DIAPS per se. To start, all items are binary, giving the same relevance to organ damages that result in different clinical and prognostic implications; for instance, pulmonary hypertension secondary to chronic thromboembolic events carries a worse prognosis than, for example, adrenal insufficiency, which is easily treated with steroid replacement therapy. Furthermore, potentially severe non-criteria manifestations such as multiple sclerosis-like disease or diffuse pulmonary hemorrhage are not included. Finally, DIAPS does not take into account treatment-related complications (i.e. hemorrhagic stroke due to anticoagulation). A new version is under development and planned for completion by the end of 2023.

In summary our data support the usefulness of the longitudinal monitoring of the aGAPSS, as a surrogate of disease activity and predictor of damage accrual in patients with APS.

The original question that gave impulse to the present thesis was if aPL persistence over time increases the probability of clinical recurrence, especially thrombotic recurrence, in APS. Our results confirm such hypothesis. In fact, we found that patients with aPL positivity in at least 2/3 of total determinations, had a higher rate of recurrence of clinical manifestations. Moreover, when considering aPL profile and aPL persistence as part of the average aGAPSS score over time, we found that a higher mean aGAPSS over time increased the probability of clinical recurrence (especially thrombotic recurrence) and was associated to damage accrual, expressed as DIAPS change. Hence, when dealing with APS, periodic monitoring of aPL and cardiovascular risk factors is of great help to assess the risk of recurrences, both thrombotic and obstetric, and can prompt the establishment of a highly intense treatment regimen with multiple targets to prevent them. Besides anticoagulation, possible options can be: hydroxychloroquine, that has been shown

to prevent thrombotic recurrence by significantly reducing aPL titers over an average 2.6-year follow-up in a randomized open label study [125]; belimumab, an inhibitor of the binding of soluble circulating B-lymphocyte stimulator (BLyS) to its target receptors on B cells, that has shown to reduce aPL titers in SLE patients independently from antimalarial therapy [126], but also has effects that go beyond inhibiting aPL production [127]; and rituximab, an anti-CD20 antibody, that has shown efficacy in APS-associated thrombocytopenia, refractory to anticoagulation [128] and is used in catastrophic APS [129].

First study

1. aPL baseline positivity tends to remain stable over time in APS. In our cohort 58% of APS patients maintained a persistent positivity over a median FU of more than 14 years.
2. Multiple aPL positivity and higher aPL titers correlated with aPL persistence, while isolated aCL and LA were predictors of aPL transience in the FU.
3. aPL persistence increased the odds of recurrence of cumulative (thrombotic plus obstetric) as well as exclusively thrombotic manifestations.
4. Periodic monitoring of aPL is warranted in the follow-up of APS patients. In patients with aPL persistence an intensive treatment regimen to prevent recurrence should be considered.

Second study

1. Both mean aGAPSS over time and delta of the aGAPSS correlated with cumulative (thrombotic and obstetric) and exclusively thrombotic recurrence. Arterial thrombotic recurrence was the manifestation with the highest score.
2. aGAPSS periodic monitoring is probably more useful in predicting thrombotic than obstetric recurrence. In fact, when compared to patients without recurrence, subjects with thrombotic recurrence presented a higher mean aGAPSS while patients with only obstetric recurrence did not.
3. The rate of aGAPSS change over time in our cohort was high (above 80%). Moreover, while aGAPSS mean and delta were higher in patients with thrombotic recurrence respect to patients with obstetric recurrence, baseline aGAPSS did not show difference. Such findings highlight the usefulness of longitudinal monitoring of aPL and cardiovascular risk factors in the follow-up of APS patients.
4. A mean aGAPSS > 13 points was the best predictor of clinical recurrence.

Third study

1. A higher mean aGAPSS linearly correlated with damage accrual, measured through DIAPS increase during follow-up.
2. Mean aGAPSS values were higher in patients with high damage accrual and aGAPSS was a predictor of high damage accrual during follow-up.
3. Higher baseline DIAPS increased the odds of death during follow-up, while there was not association with DIAPS at the end of follow-up.

Overall conclusions

1. In a cohort of 200 patients with APS, aPL remained persistently positive in more than half of total population over a long follow-up.
2. aPL persistence is associated with multiple positivity and higher aPL titers at baseline and correlates with recurrence of clinical manifestations.
3. aGAPSS showed to have a dynamic trend in our cohort, with different values at different time-points in more than 80% of measurements.
4. A longitudinal monitoring of the aGAPSS compared to one-time assessment of the score better defined the risk for recurrence of clinical manifestations in our cohort. A persistently high aGAPSS and an aGAPSS increasing over time are predictors of thrombotic recurrence.
5. aGAPSS is a useful instrument for predicting damage accrual in APS patients, especially until a disease activity index becomes available.
6. In light of our results, periodic monitoring of aPL and risk factors for thrombosis as hypertension and dyslipidemia is highly recommended in APS patients.

RESEARCH AGENDA

Well designed, large, long-term prospective studies investigating the course of aPL profile over time along with its association with clinical recurrence and the effect of medications that could reduce aPL titers, would open new perspectives on APS management. An activity index for APS is highly awaited and currently under development. A new version of DIAPS that overcomes the pitfalls of the current one is under development.

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APPENDIX

During my PhD I worked on several other articles and projects which led to various publications that are hereby reported.

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Review Article

The antiphospholipid syndrome

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ABSTRACT

APS is a hypercoagulability condition characterized by the development of thrombosis and pregnancy morbidity (recurrent early miscarriages, fetal deaths after the 10th week of gestation and/or premature births), that occur in patients with antiphospholipid antibodies, namely lupus anticoagulant, anticardiolipin antibodies, and anti-β2-glycoprotein-I antibodies. It is usually isolated but can occur in the setting of another autoimmune disease, mainly systemic lupus erythematosus. Moreover antiphospholipid antibodies can be found in individuals without the disease. Treatment of thrombosis is based on indefinite anticoagulation while low-dose aspirin and low molecular weight heparin are the cornerstone of pregnancy morbidity treatment. Catastrophic antiphospholipid syndrome is treated with anticoagulation, plasma-exchange, and corticosteroids. Standardization of serological assays, inclusion of other antibodies and manifestations in the classification criteria, treatment of non-criteria manifestations and refractory cases are areas of uncertainty.

El síndrome antifosfolipídico

Palabras clave:
Síndrome de anticuerpos antifosfolipídicos
Anticardiolipina Beta2-glicoproteína 1
Anticoagulante lúpico
Anticoagulación oral
Heparina

RESUMEN

El SAF es una condición de hipercoagulabilidad caracterizada por el desarrollo de trombosis y morbilidad obstétrica (abortos recurrentes, muertes fetales antes de la semana 10 de gestación y/o partos prematuros) en pacientes con anticuerpos antifosfolipídicos, específicamente el anticoagulante lúpico, los anticuerpos anticardiolipina y anti-β2-glicoproteína-1. En la mayoría de los casos se presenta de forma aislada, pero puede asociarse a otras enfermedades autoinmunes como el lupus eritematoso sistémico. Además, los anticuerpos antifosfolipídicos se pueden encontrar en individuos sin la enfermedad. El tratamiento de la trombosis se basa en anticoagulación indefinida, mientras que aspirina a dosis bajas y heparina de bajo peso molecular representan la base del tratamiento de la morbilidad obstétrica. El síndrome de anticuerpos antifosfolipídicos catastrófico se trata con una

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combinación de anticoagulación, corticoides y recambios plasmáticos. La estandarización de los ensayos serológicos, la inclusión de otros anticuerpos y otras manifestaciones en los criterios clasificatorios, el tratamiento de las manifestaciones no criterio y de los casos refractarios representan las áreas de incertidumbre del síndrome.

Introduction

When August Von Wasserman developed his test for the diagnosis of syphilis,[1] based on an auto-antibody (called "reagin") directed against an antigen from lipoid tissue, which was later purified and named cardiolipin by Mary C Pangborn,[2] he could never imagine that he was laying the first stone toward the discovery of a syndrome that would have been described, eventually, by Graham Hughes, almost 80 years later.[3] Since its first description, advances in recognition of both the clinical and pathophysiological aspects of the condition have been notable, and even though antiphospholipid syndrome (APS) was originally described as an acquired autoimmune thrombophilia, we know that other mechanisms are involved in several manifestations of the disease.

APS is a hypercoagulability condition characterized by the development of arterial, venous and/or microvascular thrombosis, and pregnancy morbidity (recurrent early miscarriages, fetal deaths after the 10th week of gestation and/or premature births), that occur in patients with persistent antiphospholipid antibodies (aPL) namely lupus anticoagulant (LAC), IgG or IgM anticardiolipin antibodies (aCL), or IgG or IgM anti-β2-glycoprotein-I antibodies (aβ2GPI). APS can occur either as an isolated condition (primary APS), or in the context of an underlying autoimmune disease, most commonly systemic lupus erythematosus (SLE). Less frequently, it can be associated with other autoimmune conditions, infections, drugs and malignancies.

The original description of the syndrome was made by Graham Hughes in 1983,[3] even though the first reports of thrombosis in patients with SLE and LAC date back to late 1950s and early 1960s.[4–6] Single vessel involvement or multiple vascular occlusions may give rise to a wide variety of presentations in the APS. Any combination of vascular occlusive events may occur in the same individual and the time interval between them also varies considerably from weeks to months or even years. The "Euro-Phospholipid" project, a study of 1000 European APS patients,[7] has provided accurate information on the prevalence of the majority of clinical manifestations of this syndrome, which is now recognized as a major cause of deep vein thrombosis (DVT) with or without pulmonary embolism, new strokes in individuals below the age of 50 and recurrent fetal loss. The major nonthrombotic manifestations include livedo reticularis, valvular heart disease, APS-related nephropathy, chorea, epilepsy, memory loss, migraine and myelopathy. Hematologic alterations, such as hemolytic anemia and thrombocytopenia are also very common. In a subset of patients (about 1%), thrombosis can involve simultaneously multiple organs, configuring the so-called "catastrophic antiphospholipid syndrome" (CAPS).[8] This review highlights the epidemiology, pathogenesis and the most common clinical manifestations as well as the management of this autoimmune disease.

Epidemiology

The aPL are not specific of APS and can be found in healthy individuals. Nevertheless, the prevalence of aPL positivity and APS in the general population has not been extensively analyzed and only two epidemiological population-based studies have been performed so far. In the first one, the authors studied the epidemiology of APS between 2000 and 2015 in an inception cohort of Olmsted County, Minesota, through a record linkage system. The annual incidence of APS in adults aged ≥18 years was 2.1 (95% confidence interval 1.4–2.8) per 100,000 population. Incidence rates were similar in both sexes. The estimated prevalence of APS was 50 (95% CI 42–58) per 100.000 population, and was similar in both sexes.[9] In the second study, performed in Korea between 2007 and 2018, with data extracted from the Health Insurance and Review Agency, an incidence of 0.75 per 100,000 person-year (95% confidence interval 0.73–0.78) was found, while the prevalence was 6.19 per 100,000 people.[10]

The prevalence of DVT occurrence in the general population is estimated at 2–5%, 10–20% associated with APS, suggesting that the prevalence of venous thrombosis associated with APS may be as high as 0.3–1% of the general population.[11] Moreover, the prevalence of aPL has been estimated about 11% among patients with myocardial infarction and 17% among patients with stroke younger than 50 years of age.[12] aPL antibodies are present in 30–40% of SLE patients and up to a third of these patients (10–15% of SLE patients) have clinical manifestations of APS, especially venous or arterial thromboses.[13,14] On the contrary, only few patients with primary APS tend to evolve into full-blown SLE and, usually, this takes place only after a long period of time.[15] Among women with pregnancy complications, the prevalence of aPL is about 6%, and aPL are now regarded as the most frequent acquired risk factor for a treatable cause of recurrent pregnancy loss and for pregnancy complications (early and severe pre-eclampsia).[12,13] The prevalence of CAPS has been estimated to be less than 1% of all APS patients.[16]

Pathogenesis

The aPL are heterogeneous antibodies and more than one mechanism may be involved in causing thrombosis. As

demonstrated by various studies, the major target of aPL is β2-glycoprotein I (β2GPI), a plasma protein that binds avidly to phospholipid surfaces, whose binding with aβ2GPI leads to its conformational change and dimerization (the immunogenic form of β2GPI).[17–21] The binding of aPL to β2GPI on the surfaces of platelets, endothelial cells and monocytes upregulates the expression of prothrombotic cellular adhesion molecules such as E-selectin, ICAM-1, VCAM-1,[22] and of tissue factor[23] suppressing the activity of the tissue factor pathway inhibitor,[24] reducing activated protein C activity,[25] and activating complement.[26] Annexin A2,[27] a tissue plasminogen activator receptor, toll like receptor-4[28,29] and apoE-receptor-2[30] may serve as intermediary. A possible explanation for microvascular thrombosis in APS is the aPL-induced upregulation of the mechanistic target of rapamycin (mTOR) complex on endothelial cells.[31]

Pregnancy morbidity was initially related to the impairment of maternal-fetal blood exchange as a result of thrombus formation in the uteroplacental vasculature, an hypothesis supported by findings of placental thrombosis in patients with obstetric APS.[32] However, such a histologic finding is not specific for APS, being also present in other conditions, and histologic evidence of thrombosis in the uteroplacental circulation cannot be shown in many placentas from patients with APS. Other theories have thus been put forward to explain APS-related pregnancy morbidity such as defective trophoblast invasion[33] and decidual transformation in early pregnancy or placental injury as a result of local inflammatory events, particularly complement activation and neutrophils recruitment.[32,34] The function of complement seems particularly interesting in such setting and a prospective, multicenter, observational study entitled PROMISSE (Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and SLE – NCT00198068) to examine the role of complement as a potential surrogate marker that predicts poor pregnancy outcomes in patients with APS is under way and scheduled for completion in 2021. Figure 1 provides a brief summary of the pathophysiological mechanisms leading to thrombosis and pregnancy morbidity in APS.

Clinical manifestations and diagnosis of APS

According to the present classification criteria of APS, stated in 2006 during the 11th International Congress on Antiphospholipid Autoantibodies,[35] diagnosis can be made in the presence of at least one clinical manifestation (either thrombosis or pregnancy morbidity) along with the positivity (at medium-high titer) of one or more aPL in at least two occasions 12 weeks apart (Table 1). The aCL and anti-β2GPI are detected via solid-phase immunoassays (usually ELISAs),[36] while LAC test is performed following the Scientific and Standardization Subcommittee on Antiphospholipid Antibodies of the International Society of Thrombosis and Haemostasis (SSC-ISTH) recommendations.[37] For instance, LAC is detected through a three-step procedure which involves prolongation of phospholipid-dependent clotting time such as diluted Russell viper venom time (dRVVT) and the activated partial thromboplastin time (aPTT) not reversed mixing patient plasma with normal plasma, but reversed by the addition of excess phospholipids. One of the major drawbacks of the LAC coagulation assays is that they can be altered by anticoagulant therapy, giving false-positive results.[37] Furthermore, the aCL and aβ2GPI antibodies assays show interassay variation owing to differences in calibration and differences in assay characteristics.[38]

Since aPL can be present in healthy individuals and in a majority of conditions (such as infections, neoplasms and other autoimmune diseases), a generalized search for aPL in the absence of any relevant condition is strongly discouraged to prevent incidental findings. APS must be suspected in case of a young patient presenting with unprovoked thrombosis, especially if at unusual sites and recurrent, or in thrombotic or pregnancy complications associated to other autoimmune diseases. Venous thromboembolism is the most frequent manifestation in APS, with a frequency of 39% in the Europhospholipid Project cohort.[7] Patients with venous thromboembolism most commonly present with lower-extremity DVT, pulmonary embolism, or both. Stroke and transient ischemic attack are the most common arterial events. Combined, DVT (usually in the legs) and ischemic stroke account for 90% of all complications.[39] The following accompanying clinical findings may be a clue that a patient has APS: unexplained prolongation of the aPTT, livedo reticularis or racemosa, signs or symptoms of another systemic autoimmune disease, and mild thrombocytopenia. Severe thrombocytopenia (platelet count, <20,000 per cubic millimeter) is rare[40] and should prompt the clinician to consider other causes. Thrombosis recurrence is a hallmark of APS; interestingly, patients with arterial thrombosis have a higher risk of recurrence compared with those with venous thrombosis, and a tendency for recurrences in the same vascular (arterial) bed is the rule.[41] Other risk factors for recurrence are triple aPL positivity, LAC persistent positivity, and associated SLE.[42]

Recurrent miscarriages at <10 weeks of gestation are the most frequent obstetric manifestation of APS.[43] However, the most typical complications of pregnancy generally develop after 10 weeks of gestation and losses before 10 weeks, especially if not recurrent, would more commonly be attributed to chromosomal defects (which must always be excluded to make a diagnosis). Late pregnancy loss, with early or severe preeclampsia, or with the HELLP syndrome (hemolysis, elevated liver-enzyme levels, and low platelet counts) are the typical obstetric manifestations. Reduced blood flow in the uterine arteries measured by Doppler velocimetry is an indirect indicator for the development of placental insufficiency, intrauterine growth restriction and/or preeclampsia.[44,45] Thenceforth, the European League Against Rheumatism (EULAR) guidelines recommend the use of uterine artery Doppler ultrasonography during pregnancy monitoring.[46]

The major nonthrombotic manifestations are hemolytic anemia, thrombocytopenia, *livedo reticularis* (a reddish-blue to purple, uniform, reversible, unbroken "net-like" pattern of the skin), *livedo racemosa* (nonuniform, irreversible, fractured, asymmetric pattern), livedoid vasculopathy (painful papules and erythematous-violaceous, purpuric plaques, which rapidly evolve into hemorrhagic vesicles or painful small ulcers), valvular heart disease, pulmonary hypertension, diffuse alveolar hemorrhage, APS-related nephropathy (acute

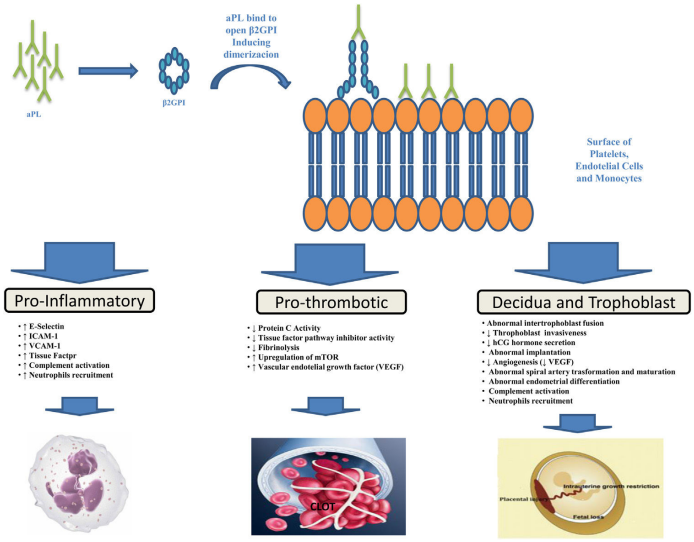

Fig. 1 – Antiphospholypid antibodies (aPL) produced by B cells bind to open, immunogenic, β2-glycoprotein I (β2GPI), leading to conformational change and dimerization. Annexine A2, Toll Like receptor-4 and apoE-receptor-2 may serve as receptor for β2GPI on cell surfaces. This binding results in endothelial-cell, monocyte, platelet and neutropphil activation and trophoblast and decidua modification leading to inflammation, thrombosis and pregnancy complications.

Table 1 – Adapted from 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; with permission.

Classification criteria for APS (2006)

Clinical criteria

1. Vascular thrombosis
   - One or more clinical episodes of arterial, venous, or small-vessel thrombosis, in any tissue or organ.
   - Thrombosis should be supported by objective validated criteria (i.e., unequivocal findings of appropriate imaging studies or histopathology).
   - For histopathologic support, thrombosis should be present without substantial evidence of inflammation in the vessel wall.
2. Pregnancy morbidity (defined by one of the following)
   - One or more unexplained deaths of a morphologically healthy fetus at or beyond the 10th week of gestation, with healthy fetal morphology documented by ultrasound or by direct examination of the fetus.
   - One or more premature births of a morphologically healthy newborn baby before the 34th week of gestation because of: eclampsia or severe preeclampsia defined according to standard definitions or recognized features of placental failure
   - 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
   - In studies of populations of patients who have more than 1 type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of patients according to 1 of the 3 criteria

Laboratory criteria

1. Lupus anticoagulant (LAC) present in plasma, on 2 or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis (Scientific Subcommittee on LAC/phospholipid-dependent antibodies)
2. Anticardiolipin (aCL) antibody of IgG or IgM isotype, or both, in serum or plasma, present in medium or high titers (i.e., >40 GPL or MPL, or greater than the 99th percentile) on 2 or more occasions, at least 12 weeks apart, measured by a standardized enzyme-linked immunoassay (ELISA)
3. Anti-β2-glycoprotein-I (anti-β2GPI) antibody of IgG or IgM isotype, or both, in serum or plasma (in titers greater than the 99th percentile), present on 2 or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

Abbreviations: GPL, G phospholipid units; MPL, M phospholipid units.

or chronic thrombotic microangiopathy), adrenal hemorrage, chorea, epilepsy, memory loss and cognitive disfunction (due to aPL related vasculopathy or direct aPL interactions with brain parenchyma following blood-brain barrier abrogation), migraine, and myelopathy.[7]

Catastrophic APS is a rare, life-threatening form of APS that occurs in less than 1% of patients and is characterized by involvement simultaneously or in less than a week, of multiple organs, tissues or systems. It usually follows a precipitating factor, such as infection (in almost half of cases), anticoagulation withdrawal, neoplasm, surgery or pregnancy. Histological confirmation of small vessel occlusion is necessary to make a diagnosis as per classification criteria.[47]

Sometimes a high clinical suspicion of APS is not supported by concomitant positivity of aPL assays included in the serological criteria for APS (LAC and IgG and IgM isotypes of aCL and aβ2GPI antibodies) which are persistently negative. This is the framework of the so-called seronegative APS which has been described by Hughes and Khamashta in 2003.[48] Thenceforth, numerous investigators looked for the presence in these patients of aPL not included in the serological criteria for APS. For instance, these non-criteria antibodies include aCL and aβ2GPI IgA, antibodies specific to phospholipid-binding plasma (cofactor) proteins (such as phosphatidylethanolamine, prothrombin, protein C, protein S, annexin V and domain I of β2GPI), phospholipid–protein complexes (particularly vimentin–cardiolipin complexes), and anionic phospholipids other than cardiolipin (including phosphatidylserine, phosphatidylinositol and phosphatidic acid).[49–54] In case of highly suspected APS with persistently negative LAC, aCL and aβ2GPI IgG and IgM, after ruling out other causes of thrombophilia, looking for these non-criteria antibodies can suggest the diagnosis.

Treatment

The management of APS has been subject to controversy in recent years. Anticoagulation therapy is considered the cornerstone of treatment, but the optimal agents and the intensity of treatment remain a matter of debate. Recently, updated guidelines on the treatment of APS by the EULAR have been published.[55] However, since APS is a fairly new and rare disease, good-quality data to guide treatment are scarce and treatment decisions rely on expert opinion in many cases. The treatment of APS varies depending of the clinical manifestations, aPL profile, and concurrent cardiovascular risk factors. Treatment options in different clinical scenarios are reported in Tables 2 and 3.

It is not infrequent that a patient is found to be positive for aPL during an evaluation for a systemic autoimmune disease or because of an elevated activated partial-thromboplastin time (aPTT), or a false positive result of syphilis test. In such cases it must be considered that aPL represent a risk factor for thrombosis and pregnancy complications, which are commonly multifactorial. Thus, a risk stratification based on age, aPL profile, concomitant genetic and acquired risk factors for thrombosis (such as dyslipidemia, smoke, hypertension, diabetes, contraceptive use, menopause, etc.) along with the presence of systemic autoimmune diseases must be taken into accountjhh and a strict follow-up is mandatory. A major risk factor is the high-risk aPL profile, including any of the following: the presence of LAC as the aPL subtype most closely related to thrombosis,[56] the presence of double (any combination of LAC, aCL and aβ2GPI antibodies) or triple aPL positivity, or the presence of persistently high (above 40 IgG or IgM phospholipid units or >99th percentile) aCL or aβ2GPI titers.[57] Furthermore, thrombosis is more strongly associated with IgG isotype than with the IgM isotype antibodies.[58] A score that takes into accounts cardiovascular risk factors (namely hypercholesterolemia and hypertension) and the aPL profile, the Global Anti-Phospholipid Syndrome Score (GAPSS), has shown to be related to thrombotic and obstetric events probability.[59,60]

The use of low-dose aspirin (LDA) for primary thrombosis prevention is controversial since the quality of evidence is low.[61] The APLASA trial,[62] that studied primary thromboprophylaxis with LDA in asymptomatic aPL carriers, did not show efficacy, but it was underpowered to detect any difference between LDA and placebo. A meta-analysis of seven observational studies of 460 asymptomatic aPL carriers found the risk of first thrombosis to be reduced by half in those who used LDA versus those who did not use LDA.[63] Therefore, the last EULAR recommendations suggest to treat aPL carriers with a high-risk profile and/or a concomitant SLE or patients with obstetric APS outside pregnancy with LDA.[55] A moderate-to-high-risk aPL profile warrants avoidance of estrogen-based contraceptives when possible and aggressive postoperative prophylaxis with low molecular weight heparin (LMWH) if feasible.

In patients with venous thrombosis related to APS, after an initial therapy with unfractionated or LMWH, a long-term anticoagulant therapy with a vitamin K antagonist such as warfarin (target international normalized ratio [INR] 2–3), is recommended. Higher intensity anticoagulation, with a target INR 3 to 4, did not further reduce the risk of recurrent thrombosis, in two randomized clinical trials.[64,65] Indefinite anticoagulation in patients with unprovoked venous thromboembolism is highly warranted, due to the high risk of thrombosis recurrence in case of VKA discontinuation.[66] Nevertheless, in case of provoked first venous thrombosis (as after surgery, prolonged immobility, long-distance travel, etc.), the benefit of long-term anticoagulation is less clear, and therapy should be discontinued – especially in cases with transient positivity and low-risk aPL profile – as in patients without APS, according to international guidelines.[67]

In case of arterial thrombosis, treatment with VKA with a target INR of 2–3 has showed no difference in thrombosis recurrence compared to a target of 3–4 in two clinical trials.[64,65] Nevertheless, the higher intensity INR approach is preferred by some centers, due to the low number of patients with arterial thrombosis included in the aforementioned trials. The association of VKA and LDA is often reserved to patients with clinically significant risk factors for cardiovascular disease or patients in whom a single antithrombotic agent has failed to prevent recurrence.[57] In decision-making, physicians should take into account the individual's risk of recurrent thrombosis and major bleeding, as well as the patient's preferences after discussion.

In case of thrombosis recurrence, high-quality evidence to support any particular management strategy when warfarin

Table 2

Treatment of thrombotic APS following different clinical scenarios

Primary thromboprophylaxis	LDA (75–100 mg per day)
1. Asymptomatic aPL carriers (not fulfilling any vascular or obstetric APS classification criteria) with a high-risk aPL profile with or without traditional risk factors.	
2. SLE with aPL (especially those with a high-risk aPL profile) and no history of thrombosis	
3. History of obstetric APS outside pregnancy	
Secondary thromboprophylaxis	
1. Definite APS and first venous thrombosis	VKA with a target INR 2–3
a. Unprovoked: indefinite anticoagulation.	
b. Provoked: short-anticoagulation.	
2. Definite APS and first arterial thrombosis	VKA with a target INR 2–3 (3–4 in selected cases)
3. Definite APS and recurrent venous thrombosis despite treatment with VKA with target INR 2–3	VKA with a target INR 3–4 Or LMWH Or VKA + LDA ± HCQ
Catastrophic APS	Glucocorticoids, UFH, Pex, IVIG, Rituximab, Eculizumab (refractory)

Abbreviations: aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; LDA, low dose aspirin; SLE, Systemic Lupus Erithematosus; VKA, Vitamin K antagonist (e.g. Warfarin); INR, international normalized ratio; LMWH, low molecular weight heparin (at therapeutic dose); UFH, unfractioned heparin; Pex, plasma exchange; IVIG, intravenous immunoglobulin.
High risk profile: the presence (in 2 or more occasions at least 12 weeks apart) of lupus anticoagulant (measured according to ISTH guidelines), or of double (any combination of lupus anticoagulant, anticardiolipin (aCL) antibodies or anti-β2-glycoprotein-I antibodies) or triple (all three subtypes) aPL positivity, or the presence of persistently high aPL titers.
Low risk profile: isolated aCL or anti-β2-glycoprotein-I antibodies at low-medium titers, particularly if transiently positive.

Table 3

Treatment of obstetric APS following different clinical scenarios

Asymptomatic carriers of aPL	LDA (75–100 mg per day)
Obstetric APS	
1. More than three miscarriages (before 10th week of gestation) or at least one fetal loss (after 10th week)	LDA + prophylactic LMWH
2. Delivery before 34th week because of preeclampsia, eclampsia, placental insufficiency	LDA ± prophylactic LMWH
3. History of thrombotic APS	LDA + therapeutic LMWH
Recurrent obstetric APS despite treatment	LDA + Therapeutic LMWH ± HCQ ± low dose prednisone

Abbreviations: aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; LDA, low dose aspirin; LMWH, low molecular weight heparin; HCQ, hydroxychloroquine.
LDA must be started before conception. LMWH must be continued up to 6 weeks after delivery.

therapy fails despite a target INR is lacking. Viable options include higher intensity warfarin therapy (target INR, 3–4), switch to LMWH, the addition of LDA, antimalarials,[68] statins, or a combination of these approaches.[55]

Since the introduction on the market of direct oral anticoagulants (DOACs) in 2010, they received increasing attention due to the obvious advantages in terms of quality of life for patients who have to follow a long-term, often lifetime, VKA treatment and have to come every 2–3 weeks to the clinic to get an INR determination. The recent TRAPS trial analyzed the efficacy of rivaroxaban, a direct factor X inhibitor, in comparison with warfarin for prevention of thrombosis recurrence in triple aPL positive patients with previous arterial thrombosis, showing an excess of arterial thrombosis in patients on rivaroxaban. Therefore, DOACs are not recommended in patients with arterial thrombosis.[69]

Prevention of pregnancy complications in asymptomatic patients with aPL, especially those with high risk profile, is based on LDA (75–100 mg per day), even though good evidence is lacking.[55] Pregnant women with previous obstetric APS should be treated with a combination of LDA and a prophylactic dose of unfractionated or LMWH,[70] with a live birth rate of about 75%,[71] even though the quality of evidence is low.[72] LDA should be preferably started prior to conception, and heparin should be added as soon as pregnancy is confirmed. LMWH is preferred for practical reasons. Oral anticoagulants should be discontinued at conception because of teratogenicity between 6 and 14 weeks of gestation. Heparin should be continued

up to 6 weeks after delivery to prevent maternal thrombosis, given the increased thromboembolic risk in puerperium. In case of recurrent pregnancy morbidity despite combination therapy, increasing heparin dose to therapeutic dose, addition of hydroxychloroquine[73] or low-dose prednisolone[74] in the first trimester may be considered. Intravenous immunoglobulins are an option in refractory cases,[75] albeit results are contradictory.[76] Even though statins are not typically used in pregnancy, a case-control study which analyzed the use of pravastatin with standard of care in APS patients with pre-eclampsia and/or intrauterine growth restriction showed no progression compared to LDA and LMWH.[77] The putative mechanism of action has been investigated in a very recent study and it seems to be increased nitric oxide synthesis.[78] In women with a history of thrombotic APS, a combination treatment of LDA and heparin at therapeutic dosage during pregnancy is recommended, regardless of obstetric history.

Since long term risk of thrombosis for women with obstetrical APS is lower than the risk for women whose syndrome-defining event was thrombotic,[79] long-term antithrombotic therapy for women who have a history of obstetrical APS but no other risk factors for thrombosis is not recommended.

A prompt and aggressive treatment is critical in case of catastrophic APS, and the current standard of care is the so-called triple therapy, a combination of anticoagulants, glucocorticoids, and plasma exchange.[80] Intravenous immunoglobulins (1–2 g/kg, given over a period of 2–5 days) are often associated to the triple therapy and, as well as rituximab, are an option for refractory cases.[81] Complement inhibition (e.g. eculizumab) may also be an option for refractory cases.[81] Given the rarity of the syndrome, non-controlled studies have been done, and the proposed therapies are based on low-quality evidence.

Finally, the so-called non-criteria manifestations represent a gray area of treatment guidelines. Thrombocytopenia is pretty exclusively mild-to-moderate and does not require medical treatment. In the rare case of severe thrombocytopenia (platelets below 20,000 per cubic millimeter), treatment is based on glucocorticoids with or without intravenous immunoglobulins if indicated.[82] Splenectomy is not a first-line treatment because of the increased risk of thrombosis for patients with the APS who undergo surgery.[57] Second-line therapies include mycophenolate mofetil, cyclophosphamide, and azathioprine with evidence coming from case series and observational studies.[82] Rituximab[83] and thrombopoietin receptor agonists[84] are indicated in refractory cases. First-line treatment for autoimmune hemolytic anemia in APS consists of high-dose corticosteroids, while traditional immunosuppressants, rituximab, or splenectomy have been used with varying success as second-line treatments in refractory cases.[85]

Evidence-based recommendations for the management of heart valve disease in APS are lacking. An earlier consensus report concluded that oral anticoagulation does not halt the development or progression of valve lesions, while prophylactic LDA may be considered in asymptomatic aPL-positive individuals with valve disease.[85] Anticoagulation is recommended in patients with thromboembolic episodes attributed to valve disease and can be considered in case of vegetations due to the increased risk of thromboembolic stroke.[86]

There is no consensus about the treatment of neurologic manifestations associated to APS. Various case reports showed efficacy of antiplatelet and anticoagulant treatment, while the role of conventional immunosuppression is not clear.[87] Case reports showed successful treatment of aPL-associated chorea with hydroxychloroquine, mycophenolate mofetil or intravenous immunoglobulins, but prospective studies are needed to examine their efficacy.

APS-related nephropathy is usually slowly progressive, histologically characterized by fibrous intimal hyperplasia, fibrocellular arterial occlusion, focal cortical atrophy and tubular thyroidization, and has no standard treatment. Anticoagulation is indicated in case of history of thrombotic APS, but its role in the evolution of renal function is unknown, owing to the limited number of patients and limited follow-up period in the majority of case series.[85] Acute renal failure is typically associated with thrombotic microangiopathy and can be treated with rituximab,[83] eculizumab[88] and plasma exchange.[89] In any case of aPL-associated nephropathy, strict control of arterial hypertension and proteinuria with angiotensin-converting enzyme inhibitors and angiotensin-receptor blockers is highly recommended.

Not specific treatments are usually needed for *livedo reticularis* or *livedo racemosa*. Livedoid vasculopathy is usually refractory to glucocorticoids; LDA, dipyridamole, clopidogrel, pentoxifylline, sildenafil, intravenous immunoglobulins, tissue plasminogen activator, hyperbaric oxygen therapy, or a combination of these interventions, with or without anticoagulant therapy, have been used.[57,90]

Sometimes, patients who initially tested positive for aPL can become persistently negative. In such cases the question arises of whether it is possible to withdraw anticoagulant treatment. Coloma-Bazzan et al.[91] described a series of 11 patients who presented no new thrombotic episode during a 20 month-follow-up after withdrawal of anticoagulation, suggesting that anticoagulation can be safely withdrawn in selected patients. However, discontinuation of VKA treatment in patients who became persistently negative to aPL needs further evidence.

Unmet needs

All assays routinely used to detect aPL show methodological shortcomings and lack of standardization. Harmonization of working conditions using automated systems may contribute to a reduction in interlaboratory variation[92] and validation of several non-criteria antibodies assays, such as prothrombin, phosphatidylserine–prothrombin complex, domain 1, phosphatidic acid, annexin A5, aCL and aβ2GPI IgA.[49]

Since the current classification criteria do not incorporate the full spectrum of clinical findings for the APS, an international effort is under way to develop a more comprehensive classification, with the use of the same methods that were used to develop the most recent classification criteria for SLE.[93]

There are several areas of uncertainty in the management of APS in which evidence is scarce or nonexistent, such as treatment of non-criteria manifestations, seronegative APS and refractory cases of thrombotic and obstetric APS.

Treatment of obstetric APS with current standard of care results in live-birth rates above 70%, which means that about 30% of women continue to have pregnancy complications. A multicenter randomized controlled trial of hydroxychloroquine (associated to standard of care) versus placebo to improve pregnancy outcome in women with aPL (HYPATIA)[94] is ongoing and results are awaited.

Last but not least, the possibility of withdrawal of anticoagulation in selected cases of thrombotic APS in which assays for aPL become persistently negative is another gray area where evidence is scarce, and further studies are warranted.

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Appendix A. Supplementary material

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The presence of non-criteria manifestations negatively affects the prognosis of seronegative antiphospholipid syndrome patients: a multicenter study

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Abstract

Background: Seronegative antiphospholipid syndrome (SN-APS) is often defined as the presence of APS criteria manifestations, negative antiphospholipid antibodies (aPL), and coexistence of APS non-criteria manifestations. Nevertheless, the impact of these non-criteria features is still unclear. On a different note, the relevance of one single aPL positive determination in patients with APS manifestations is another domain with limited evidence. We aim to compare the course of SN-APS and single-positive aPL (SP-aPL) patients with that of individuals with APS manifestations without non-criteria features/aPL positivity (controls).

Methods: Retrospective analysis of patients with thrombosis/obstetric morbidity assessed in two European hospitals between 2005 and 2020. Patients were divided into SN-APS, SP-aPL, and control groups. Clinical characteristics, comorbidities, and therapies were compared.

Results: A total of 82 patients were included in the SN-APS group, 88 in the SP-aPL group, and 185 in the control group. In Cox regression model, SN-APS displayed more thrombosis recurrence than controls (HR 3.8, 95% CI 2.2–6.5, $p < 0.001$) even when adjusting for the presence of hereditary thrombophilia, systemic lupus erythematosus, or contraceptive hormonal treatment. In SP-aPL, the difference in thrombosis recurrence did not reach statistical significance ($p = 0.078$). Indefinite anticoagulation ($p < 0.001$ and $p = 0.008$, respectively) and vitamin K antagonist (VKA) use ($p < 0.001$ in both cases) were more common in SN-APS/SP-aPL.

Conclusion: SN-APS displayed more thrombosis recurrence, indefinite anticoagulation, and VKA use than controls without non-criteria manifestations. The presence of such features in patients with thrombosis and negative aPL may negatively impact their clinical course.

Keywords: Antiphospholipid syndrome, Antiphospholipid antibodies, Seronegative, Single positive, Non-criteria manifestations

Introduction

The classification criteria for definite antiphospholipid syndrome (APS) [1] are currently facing a revision, with new criteria under development [2]. The call for an update derives from different aspects surrounding the disease, including the existence of numerous patients

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with a suspicion of APS but not classified as such according to the current criteria. These patients are often referred to as "non-criteria" APS, a label that encompasses a wide range of clinical and laboratory presentations [3].

A subgroup of these patients correspond to the so-called seronegative APS (SN-APS), a term initially defined as "patients with migraine, stroke, several previous miscarriages, thrombocytopenia, and *livedo reticularis*, whose antiphospholipid antibodies (aPL) tests are doggedly negative [4]." Other definitions have been presented, and in a previous publication, we address the different descriptions present in the literature [3], with most publications [5–7] including not only the presence of APS manifestations and aPL negativity, but also the presence of non-criteria manifestations (i.e., clinical manifestations fairly prevalent in APS patients but not included in the classification criteria). The latter comprise both non-obstetric (e.g., thrombocytopenia, valvular heart disease, *livedo reticularis*) and obstetric manifestations (e.g., two spontaneous abortions, late pregnancy morbidity) [8]. The relevance of such manifestations in seronegative patients resides in the fact that they represent additional evidence to reinforce a possible APS diagnosis, instead of alternative diagnoses such as a different thrombophilia or an idiopathic event. Nevertheless, either due to the rarity of the disease or the difficulty to identify and categorize these patients, there are no studies addressing the impact of these non-criteria manifestations as a whole in the clinical course and prognosis of patients exhibiting clinical manifestations of APS.

Another cluster of patients which raises doubts in clinical practice are those in whom aPL testing is positive in only one occasion. The classification criteria for definite APS [1] require aPL positivity on two or more occasions at least 12 weeks apart, and state that classification as APS should be avoided if the positive aPL determination and clinical manifestations are separated by less than 12 weeks. The rationale behind these caveats includes the difficulty to exclude a false-positive aPL elevation due to other causes in the setting of one positive determination (e.g., infection, malignancy, or drugs) [9–11] and the possibility of transient aPL elevation during the acute phase of an event [12, 13]. However, despite a systematic review reporting a similar recurrence rate to the general population in individuals with venous thromboembolism or stroke and only one single aPL determination [14], the included primary studies are scarce and outdated and have important methodological limitations, such as still not including anti-beta-2-glycoprotein I (anti-β2GPI) antibodies or inadequate aPL positivity cut-offs [15–18].

For the abovementioned reasons, much is still unknown and uncertain regarding SN-APS and single-positive (SP-aPL) individuals. Our aim was to describe a cohort of both subsets of patients, and to compare them with patients with thrombosis or pregnancy morbidity but lacking other criteria to be included in these groups (i.e., without non-criteria features or aPL positivity). Herewith, we intend to evaluate the impact of the presence of non-criteria manifestations of APS and SP-aPL in patients with manifestations of APS (either thromboembolic events or pregnancy morbidity). A deeper understanding of the influence of these traits could change the current management and therapy of these individuals and contribute to an eventual inclusion of some additional features in the classification criteria for APS.

Methods

Study design

Patient selection

We retrospectively reviewed the clinical records of patients assessed in the Autoimmune Diseases, Internal Medicine, Thrombophilia and Obstetrics Departments of two tertiary European hospitals—University Hospital Center of São João (Oporto, Portugal) and Hospital Clínic of Barcelona (Barcelona, Catalonia, Spain)—between January 2005 and December 2020, selecting all those with thrombosis and/or obstetric morbidity fulfilling the APS clinical classification criteria [1], but not fulfilling laboratory criteria. Patients with a major risk factor for a thrombotic event (e.g., recent major surgery, bone fracture, cancer) were excluded. Patients taking oral contraceptive pill (OCP) were excluded if the medication had been started less than 1 year previously to the event. Patients were then divided into the three following groups:

- *Seronegative APS* (using the definition of the nomenclature recently proposed by our research team for non-criteria APS) [3]: patients with thrombosis or obstetric morbidity fulfilling APS classification criteria [1], plus the presence of "non-criteria" manifestations [at least (i) one obstetric, (ii) one *major* non-obstetric, or (iii) two *minor* non-obstetric manifestations—see Table 1], with persistently negative aPL, and exclusion of other thrombophilias. However, the presence of hereditary thrombophilias (i.e., factor V Leiden mutation, prothrombin G20210A mutation, protein C, protein S, or antithrombin deficiency) was accepted if judged as not justifying the whole clinical presentation of the patient.
- *Single-positive aPL (SP-aPL) group*: patients with thrombosis or obstetric morbidity fulfilling APS classification criteria [1] with only one single positive aPL result (regardless of occurring during or outside

Table 1 Included "non-criteria" manifestations of APS [adapted from [3]]

Non-obstetric manifestations		**Obstetric**
Major[a]		Infertility
Acute ischemic encephalopathy	Adrenal hemorrhage	Late IUGR (after 34 weeks)
APS nephropathy	Cardiac microvascular disease	Late pre-eclampsia (after 34 weeks)
Chorea	Evans syndrome	Placental abruption
Livedo reticularis/racemosa	Livedoid vasculopathy	Placental hematoma
Longitudinal myelitis	Pulmonary hemorrhage	Preterm birth (>34 to <37 weeks)
Superficial vein thrombosis	Thrombocytopenia	Puerperal pre-eclampsia
Valvular heart disease		Two or more unexplained in vitro fertilization failures
Minor[a]		Two unexplained spontaneous abortions <10 weeks
Amaurosis fugax	Brain MRI white matter lesions	
Cognitive dysfunction	Coombs' test positivity	
Hemolytic anemia	Ischemic necrosis of bone	
Migraine	Pseudo-multiple sclerosis	
Pulmonary hypertension	Raynaud's phenomenon	
Seizures	Sensorineural hearing loss	
Splinter hemorrhages		

APS Antiphospholipid syndrome, *IUGR* Intrauterine growth restriction

[a] We considered as *major* manifestations those *suggested, recommended, or strongly recommended* to be included as part of the APS criteria revision in the report of the *14th International Congress on Antiphospholipid Antibodies Technical Task Force on APS Clinical Features* [7] and those occurring in higher frequency in the cases categorized as "highly likely APS" in Phase III of the *Development of New International Classification Criteria for Antiphospholipid Syndrome* [19]

the acute phase of the event). Patients were excluded if an evident cause for the positivity was identified, such as infection, malignancy, or drugs.

– *Control group*: patients with thrombosis or obstetric morbidity fulfilling APS classification criteria [1], with persistently negative aPL, without non-criteria manifestations fulfilling the criteria for seronegative APS. The presence of hereditary thrombophilias (i.e., factor V Leiden mutation, prothrombin G20210A mutation, protein C, protein S, or antithrombin deficiency) was accepted in order to establish an adequate parallelism with the SN-APS group.

Our aim was to establish a comparison between the SP-aPL and SN-APS groups and the control group regarding demographic and clinical characteristics, namely recurrence of events. The study received approval from the Hospital Clínic Ethics Committee (HCB/2020/1259).

Data collection and definition of variables

We collected information from each patient on demographic data, type of clinical manifestations (thrombotic, obstetric, or both), specific clinical manifestations [thrombosis: arterial, venous, or both; stroke, transient ischemic accident (TIA), acute myocardial infarction, limb ischemia, deep vein thrombosis (DVT), pulmonary embolism (PE), cerebral vein thrombosis (CVT), and retinal vessels thrombosis; obstetric: one or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, one or more premature births of a morphologically normal neonate before the 34th week of gestation, three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, placental ischemia]; recurrence of events; number of thrombotic events; number of spontaneous abortions; presence and type of "non-criteria" manifestations (in the case of SN-APS); aPL positivity and profile (in the case of the SP-aPL group); associated autoimmune diseases (AID); presence of autoantibodies [antinuclear antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA)] and complement consumption; cardiovascular risk factors (arterial hypertension, dyslipidemia, hyperuricemia, obesity, smoking); and presence of risk factors for thrombosis (OCP and hereditary thrombophilia: factor V Leiden mutation, prothrombin G20210A mutation, protein C, protein S, or antithrombin deficiency). A possible future progression towards systemic lupus erythematosus (SLE), based on clinical manifestations and autoantibodies' profile, was also noted.

In addition, we collected information on the treatments each patient was under, including indefinite therapy with oral anticoagulation [vitamin K antagonists (VKA) or direct oral anticoagulants (DOAC)], antiplatelet agents or hydroxychloroquine, anticoagulation duration, and recurrence under treatment. In the case of obstetric manifestations, treatment of at least one pregnancy with low-dose aspirin (LDA) monotherapy,

low molecular weight heparin (LMWH) monotherapy, LDA/LMWH combination, or hydroxychloroquine was noted.

Statistical analysis

Continuous variables were described by means and standard deviations or by medians and interquartile ranges (IQR), while categorical variables were described using absolute and relative frequencies. We built univariable logistic regression models to compare SN-APS patients versus controls and SP-aPL patients versus controls. Tested independent variables included clinical manifestations, recurrence, comorbidities, and treatment options. Multivariable analyses were performed when adjustment for confounders was considered required. Seronegative APS patients and SP-aPL patients were also compared against controls regarding the development of thrombosis recurrence—we built univariable and multivariable Cox regression models (adjusting for the presence of hereditary thrombophilia, SLE and OCP—we could not adjust for additional factors due to sample size limitations), considering the time between the first thrombotic event until the development of a second thrombotic event (or, if such event did not occur, the date of the last registered outpatient visit). In addition, Kaplan-Meier curves were obtained.

Exponentials of logistic regression coefficients were interpreted as odds ratio (OR) and exponentials of Cox regression coefficients were interpreted as hazard ratios (HR). Exponentials of regression coefficients were calculated along with their respective 95% confidence intervals (CI). Values of $p<0.05$ were considered statistically significant. Data were analyzed using SPSS Statistics 26.0 (IBM Corp, Armonk, NY).

Results

Patient characteristics

Three hundred and fifty-five patients were included in the analysis: 82 in the SN-APS group, 88 in the SP-aPL group, and 185 in the control group (161 without and 24 with hereditary thrombophilia). Patient characteristics and demographic data are summarized in Table 2, and the non-criteria manifestations of the SN-APS group in Table 3. Groups displayed no significant difference in age ($p=0.519$) and age of first event ($p=0.241$). In univariable regression analyses, a significantly lower frequency of males was observed in the SN-APS group in comparison to the control group (OR=0.5, 95% CI=0.3–0.9, $p=0.022$), with no difference ($p=0.120$) when adjusting for the presence of obstetric patients in each group; on the other hand, no significant differences on gender distribution were found between the SP-aPL and control groups ($p=0.107$). Complete results are available in Table 2.

Comorbidities

Concomitant AID was more common in the SN-APS group (OR=2.4, 95% CI 1.1–5.1, $p=0.026$) than in the control group, with no significant difference between SP-aPL and control patients ($p=0.322$) (Table 2). Both SN-APS and SP-aPL patients displayed less use of OCP compared with the control group (OR 0.3, 95% CI 0.15–0.5, $p<0.001$, and OR 0.4, 95% CI 0.2–0.7, $p=0.002$, respectively).

Positive ANA were more common in the SN-APS/SP-aPL groups (OR=3.3, 95% CI 1.8–5.9, $p<0.001$, and OR=2.0, 95% CI 1.1–3.6, p 0.026, respectively) than in the control group, even when adjusting for the presence of associated AID (OR=3.1, 95% CI 1.7–5.6, $p<0.001$, and OR=1.9, 95% CI 1.1–3.5, $p=0.033$, respectively). Complement consumption was more common in the SN-APS group (OR=5.4, 95% CI 1.4–21.2, $p=0.016$), but this difference was not maintained when adjusting for the presence of associated AID ($p=0.067$). No difference was found in the proportion of patients in which a progression towards a diagnosis of SLE was suspected between SN-APS ($p=0.828$)/SP-aPL ($p=0.278$) and the control group. Complete results are available in Table 2.

Differences in clinical manifestations among groups

SN-APS and SP-aPL groups were associated with a higher frequency of obstetric manifestations in comparison with the control group (OR 3.0, 95% CI 1.5–6.0, $p=0.002$, and OR 4.3, 95% CI 2.2–8.2, $p<0.001$, respectively). Only one patient in the control group (0.5%) displayed both thrombotic and obstetric manifestations, a feature present in 6.1% and 5.7% of patients in the SN-APS ($p=0.025$) and SP-aPL ($p=0.029$) groups, respectively.

Concerning obstetric manifestations, no significant differences were found between SN-APS, SP-aPL and control patients. Additionally, no significant difference was observed in the recurrence of obstetric events and number of abortions between SN-APS/SP-aPL patients and the control group. Complete results are available in Table 4.

No significant differences were found between SN-APS/SP-aPL and the control groups in the frequency of venous thrombosis ($p=0.698$ and $p=0.208$, respectively). On the other hand, the SP-aPL group displayed significantly less arterial thrombosis than the control group (OR=0.4, 95% CI 0.2–0.8, $p=0.007$), with no significant difference between SN-APS and control patients ($p=0.711$). Concerning specific thrombotic manifestations, SP-aPL patients displayed significantly less frequency of stroke (OR=0.3, 95% CI 0.1–0.7, $p=0.008$)

Table 2 Patient characteristics, demographic data, and comorbidities

Patient group	Control group ($n=185$)	Seronegative APS ($n=82$)	*P*-value (SN-APS vs. controls)	Single-positive aPL ($n=88$)	*P*-value (SP-APS vs. controls)
Sex (female) (*n*, %)	123 (66.5)	66 (80.5)	$p=0.022$	67 (76.1)	$p=0.107$
Age (median, IQR)	45.0 (38.0–50.0)	45.5 (37.0–53.3)	$p=0.105$	44 (39.0–51.0)	$p=0.519$
Age at first event (median, IQR)	37.5 (29.0–43.0)	35 (30.0–40.4)	$p=0.609$	35 (27.0–43.0)	$p=0.241$
Type of manifestations					
Thrombosis only (*n*, %)	166 (89.7)	61 (74.4)	$p=0.002$	59 (67.0)	$p<0.001$
Obstetric morbidity only (*n*, %)	18 (9.7)	16 (19.5)	$p=0.022$	24 (27.3)	$p<0.001$
Both (*n*, %)	1 (0.5)	5 (6.1)	$p=0.025$	5 (5.7)	$p=0.029$
aPL profile (one determination)					
Anti-β2GPI	-	-	-	45 (51.1)	-
LA	-	-	-	35 (39.7)	-
aCL	-	-	-	24 (27.3)	-
Double positive	-	-	-	17 (19.3)	-
Triple positive	-	-	-	1 (1.1)	-
Associated AID (*n*, %)	16 (8.6)	15 (18.3)	$p=0.026$	11 (12.5)	$p=0.322$
SLE	2 (1.1)	6 (7.3)	$p=0.017$	2 (2.3)	$p=0.454$
Plausible evolution to SLE	4 (2.2)	2/76 (2.6)	$p=0.828$	4/86 (4.7)	$p=0.278$
Autoantibodies (*n*, %)					
Antinuclear antibodies	34/142 (23.9)	37/73 (50.7)	$p<0.001$	29/76 (38.2)	$p=0.026$
Cardiovascular risk factors (*n*, %)					
Diabetes	9 (4.9(	3 (3.7)	$p=0.662$	1 (1.1)	$p=0.160$
Smoker	66 (35.7)	27 (32.9)	$p=0.664$	19 (21.6)	$p=0.02$
Arterial hypertension	23 (12.4)	17 (20.7)	$p=0.083$	13 (14.8)	$p=0.594$
Obesity	39 (21.1)	15 (18.3)	$p=0.601$	16 (18.2)	$p=0.577$
Dyslipidaemia	56 (30.3)	26 (31.7)	$p=0.814$	26 (29.5)	$p=0.461$
Hyperuricemia	5 (2.7)	6 (7.3)	$p=0.091$	3 (3.4)	$p=0.707$
Other prothrombotic risk factors (*n*, %)					
Hereditary thrombophilia	24 (13.0)	6 (7.3)	$p=0.214$	12 (13.6)	$p=0.768$
Oral contraceptive pill	76 (41.1)	19 (23.2)	$p<0.001$	24 (27.3)	$p=0.002$

aCL Anticardiolipin antibodies, *AID* Autoimmune disease, *aPL* Antiphospholipid antibodies, *APS* Antiphospholipid syndrome, *IQR* Interquartile range, *LA* Lupus anticoagulant, *SLE* Systemic lupus erythematosus

but more frequent cerebral vein thrombosis (OR = 2.2, 95% CI 1.01–4.7, $p=0.045$) than patients from the control group. No significant differences were found in the remaining clinical manifestations between SN-APS/SP-aPL and the control group. Complete results are available in Table 4.

Thrombosis recurrence

Regarding thrombosis recurrence, in a Cox regression model, SN-APS associated with significantly higher chances of recurrence than the control group (HR = 3.8, 95% CI = 2.2–6.5, $p<0.001$) (Fig. 1). Similar results were observed after adjusting for the presence of hereditary thrombophilia (HR 3.8, 95% CI 2.1–6.6, $p<0.001$), associated SLE (HR 3.8, 95% CI 2.2–6.6, $p<0.001$) or OCP (HR 5.7, 95% CI 2.6–12.6, $p<0.001$). In the case of SP-aPL group, we observed a non-significant trend for a higher chance of recurrence both in unadjusted analysis (HR 1.8, 95% CI 0.9–3.4, $p=0.078$) (Fig. 2) and after adjusting for the presence of hereditary thrombophilia (HR 1.8, 95% CI 0.9–3.5, $p=0.078$), associated SLE (OR 1.9, 95% CI 0.98–3.5, $p=0.06$) or OCP (HR 2.3, 95% CI 0.97–5.3, $p=0.057$). The mean number of thrombotic events was higher in both SN-APS and SP-aPL groups in comparison with the control group (regression coefficient = 0.6, 95% CI 0.4–0.7, $p<0.001$, and regression coefficient = 0.2, 95% CI 0.1–0.3, $p=0.004$, respectively). No patient in the control group displayed recurrence under anticoagulation, a feature present in 10.6% and 4.7% of patients in the SN-APS and SP-aPL groups, respectively.

When assessing SN-APS patients among themselves, no particular non-criteria manifestation was specifically associated with thrombosis recurrence. When

Table 3 "Non-criteria" clinical manifestations present in the seronegative antiphospholipid syndrome group

Clinical manifestation	
Non-obstetric (*n*, %)	***n*=82**
Migraine	23 (28.0)
Brain MRI white matter lesions	21 (25.6)
Superficial vein thrombosis	19 (23.2)
Thrombocytopenia	17 (20.7)
Livedo reticularis	10 (12.2)
Valvular heart disease	8 (9.8)
Raynaud's phenomenon	6 (7.3)
Seizures	6 (7.3)
Coombs' positivity	5 (6.1)
Memory lapses	5 (6.1)
Transverse myelitis	3 (3.7)
Hemolytic anemia	3 (3.7)
Cognitive dysfunction	3 (3.7)
Pseudo-multiple sclerosis	3 (3.7)
Cardiac microvascular disease	3 (3.7)
Skin ulcers	2 (2.4)
APS nephropathy	2 (2.4)
Livedoid vasculopathy	1 (1.2)
Obstetric	***n*=66**
Two spontaneous abortions <10 weeks	11 (16.7)
Late IUGR (>34 weeks)	5 (7.6)
Infertility	4 (6.1)
Premature birth between 34 and 37 weeks	3 (4.5)
Placental abruption	3 (4.5)
≥ 2 or more IVF failures	2 (3.0)
Puerperal preeclampsia	1 (1.5)
Late preeclampsia (>34 weeks)	1 (1.5)
Placental hematoma	1 (1.5)
Puerperal Thrombosis	1 (1.5)

APS Antiphospholipid syndrome, *IUGR* Intrauterine growth restriction, *IVF* In vitro fertilization, *MRI* Magnetic resonance imaging

selecting SN-APS patients with specific non-criteria features and comparing them with the control group, various manifestations were statistically associated with recurrence, albeit the OR's displayed wide confidence intervals, hinting a low estimate precision due to small sample size: thrombocytopenia (OR 9.9, 95% CI 3.2–30.5, $p<0.001$), brain white matter lesions (OR 11.3, 95% CI 4.0–31.8, $p<0.001$), migraine (OR 9.9, 95% CI 3.6–26.9, $p<0.001$), superficial vein thrombosis (OR 3.3, 95% CI 1.1–9.7, $p=0.03$), and seizures (OR 9.9, 95% CI 1.6–62.5, $p=0.015$).

Differences in treatment among groups

In patients with thrombosis, indefinite anticoagulation was more common both in SN-APS and SP-aPL groups comparing with the control group (OR 3.2, 95% CI 1.8–5.8, $p<0.001$, and OR 2.2, 95% CI 1.2–4.0, $p=0.008$, respectively). Additionally, a longer global duration of anticoagulation was observed in SN-APS (regression coefficient = 46.4; 95% CI 33.0–55.1, $p<0.001$) and SP-aPL patients (regression coefficient = 20.8; 95% CI 4.7–36.9, $p=0.012$) in comparison with the control group. In anticoagulated patients, anticoagulation with a VKA (instead of a DOAC) was more common in the SN-APS/SP-aPL groups than in the control group (OR = 3.5, 95% CI 1.9–6.6, $p<0.001$, and OR = 3.7, 95% CI 2.0–7.0, $p<0.001$, respectively). In patients with obstetric manifestations, no significant difference was observed in pregnancy treatment between SN-APS/SP-aPL patients and the control group. Complete results are available in Table 4.

Discussion

Even though various non-criteria manifestations are being considered for inclusion in the new Classification Criteria for APS currently under development [2], there are few studies specifically tackling the relevance of these features [7, 20]. To our knowledge, this is the first study to address the impact of non-criteria APS manifestations as a whole in seronegative patients with APS criteria manifestations. The additional inclusion of a group of patients with single aPL positivity provides further data on a controversial domain for which there is limited evidence available.

In our series, patients with non-criteria manifestations (SN-APS) displayed a higher frequency of obstetric morbidity and concurrent obstetric and thrombotic manifestations in comparison with controls. Additionally, ANA positivity was more prevalent even when taking into account the presence of other AID, hinting a possible role as a marker of autoimmunity in these patients. When focusing on patients with thrombosis, although the type of events did not differ from controls, a significantly higher number of events and thrombosis recurrence was observed in patients with non-criteria manifestations, a deed still sustained even after adjusting for various relevant confounders (i.e., contraceptive pill use, associated SLE or hereditary thrombophilia, follow-up duration). A previous work described similar prevalence of thrombosis recurrence between patients with SN-APS (defined as clinical manifestations of APS but testing negative for criteria aPL plus the presence of at least two non-criteria manifestations) and definite APS patients [6]. Our data sheds new light on the potential impact of non-criteria manifestations on the prognosis of these patients; it is curious to notice that this information might already influence daily clinical practice, as these patients in our

Table 4 Patient clinical manifestations and treatment

Clinical manifestations	Control group (*n* = 185)	Seronegative APS (*n* = 82)	*P*-value (SN-APS vs. controls)	Single-positive aPL (*n* = 88)	*P*-value (SP-APS vs. controls)
Thrombosis	***N* = 167**	***n* = 66**		***n* = 64**	
Number of thrombotic events (median, IQR)	1 [1–1]	1.5 [1–2]	$p<0.001$	1 [1–2]	$p=0.004$
Arterial thrombosis only (*n*, %)	77 (46.1)	24 (36.4)	$p=0.268$	14 (21.9)	$p=0.007$
Venous thrombosis only (*n*, %)	79 (47.3)	33 (50.0)	$p=0.698$	43 (67.2)	$p=0.208$
Both arterial and venous thrombosis (*n*, %)	11 (6.6)	9 (13.6)	$p=0.156$	7 (10.9)	$p=0.533$
Stroke (*n*, %)	48 (28.7)	22 (33.3)	$p=0.894$	9 (14.1)	$p=0.008$
TIA (*n*, %)	9 (5.4)	8 (12.1)	$p=0.408$	3 (4.7)	$p=0.581$
Limb ischemia (*n*, %)	6 (3.6)	6 (9.1)	$p=0.783$	3 (4.7)	$p=0.699$
Acute myocardial infarction (*n*, %)	21 (12.6)	6 (9.1)	$p=0.189$	9 (14.1)	$p=0.669$
Pulmonary embolism (*n*, %)	27 (16.2)	18 (27.3)	$p=0.262$	18 (28.1)	$p=0.136$
DVT (*n*, %)	56 (33.5)	28 (42.4)	$p=0.157$	21 (32.8)	$p=0.656$
Cerebral vein thrombosis (*n*, %)	19 (11.4)	6 (10.6)	$p=0.228$	14 (21.9)	$p=0.045$
Retinal vessels thrombosis (*n*, %)	3 (1.8)	2 (3.0)	$p=0.349$	2 (3.1)	$p=0.827$
Obstetric	***N* = 19**	***n* = 21**		***n* = 29**	
Number of abortions (median, IQR)	3.0 [1–5]	3 [1.5–4]	$p=0.473$	3 [1–4]	$p=0.384$
More than three abortions <10 weeks (*n*, %)	10 (52.6)	5 (23.8)	$p=0.065$	15 (48.3)	$p=0.951$
Miscarriage >10 weeks (*n*, %)	8 (42.1)	14 (66.7)	$p=0.123$	11 (37.9)	$p=0.773$
Prematurity <34 weeks (*n*, %)	3 (15.8)	5 (23.8)	$p=0.529$	3 (10.3)	$p=0.579$
Placental ischemia (*n*, %)	5 (26.3)	12 (57.1)	$p=0.054$	8 (27.6)	$p=0.923$
Treatment					
Thrombosis patients	***n* = 167**	***n* = 66**		***n* = 64**	
Indefinite anticoagulation (*n*, %)	54 (32.3)	40 (60.6)	$p<0.001$	33 (51.6)	$p=0.008$
Vitamin K antagonist (*n*, %)	29 (17.4)	28 (42.4)	$p<0.001$	28 (43.8)	$p<0.001$
Direct oral anticoagulant (*n*, %)	25 (15.0)	12 (18.2)	$p=0.546$	5 (7.8)	$p=0.155$
Antiplatelet therapy (*n*, %)	68 (40.7)	23 (34.8)	$p=0.408$	13 (20.3)	$p=0.004$
Hydroxychloroquine (*n*, %)	1 (0.6)	5 (7.6)	$p=0.018$	5 (7.8)	$p=0.017$
Anticoagulation duration [median (months), IQR]	20.5 [11–73.8]	74.5 [23.3–114.8]	$p<0.001$	61 [14–113]	$p=0.013$
Obstetric patients (during pregnancy) (*n*, %)	***n* = 18**	***n* = 21**		***n* = 29**	
Any treatment	13 (72.2)	11 (52.4)	$p=0.209$	16 (55.2)	$p=0.246$
Aspirin monotherapy	2 (11.1)	0 (0)	$p=0.998$	3 (10.3)	$p=0.934$
LMWH/aspirin combination	11 (61.1)	10 (47.6)	$p=0.401$	13 (44.8)	$p=0.280$
Hydroxychloroquine	6 (33.3)	3 (14.3)	$p=0.169$	3 (10.3)	$p=0.063$

TIA Transitory ischemic attack, *aPL* Antiphospholipid antibodies, *APS* Antiphospholipid syndrome, *IQR* Interquartile range, *LMWH* Low-molecular-weight heparin

cohort were also more frequently under indefinite anticoagulation, displayed longer anticoagulation duration, and a higher use of VKA instead of DOAC.

Regarding the impact of specific non-criteria manifestations on thrombosis recurrence, the significance of the observed associations is undermined by the small sample size possibly leading to estimates of low precision and misleading high magnitude. Nevertheless, there is already some evidence in the literature portraying a possible role of these manifestations. In the case of thrombocytopenia, although previous data provided conflicting results, most publications increasingly support a potential impact of this feature in APS prognosis. While two studies found no significant difference in thrombosis recurrence among APS patients with and without thrombocytopenia [21, 22], a study of 138 patients with aPL positivity and thrombocytopenia (i.e., fulfilling laboratory but not clinical criteria of APS) described a five times higher risk

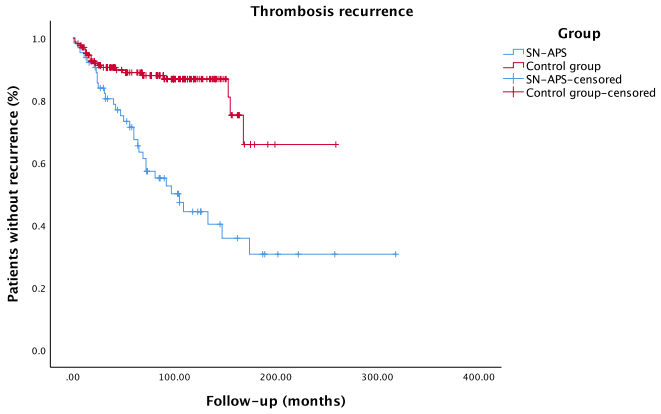

Fig. 1 Kaplan-Meier curve of overall survival (absence of thrombosis recurrence) of seronegative antiphospholipid syndrome and controls

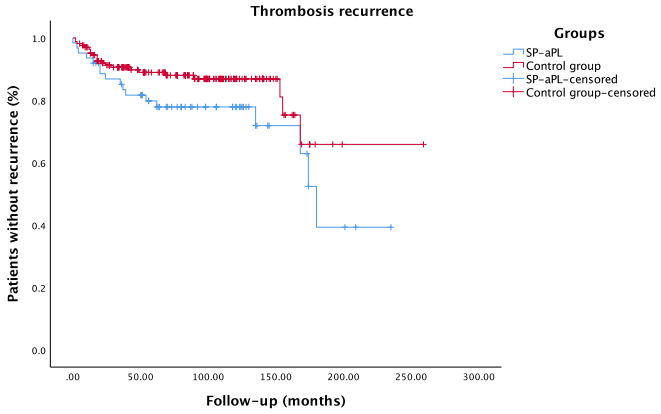

Fig. 2 Kaplan-Meier curve of overall survival (absence of thrombosis recurrence) of single-positive aPL patients and controls

of future thrombosis in these patients compared with those with normal platelet counts [23], and another publication described that, in aPL-positive patients, those with a low platelet count developed thrombosis more frequently than those without [24]. Moreover, a study found significantly higher adjusted Global APS Score (aGAPSS) values in APS patients with thrombocytopenia when compared to patients without non-criteria manifestations [25]. Concerning *livedo reticularis*, a previous work reported an increased frequency of this feature in patients with arterial events and decreased frequency in those with venous events [8, 26]. In respect to superficial vein thrombosis, in a prospective study of patients with SLE and/or aPL, its presence carried a hazard ratio of 7.45 for the occurrence of thromboembolic events, suggesting a possible prognostic significance [27]. Relative to brain white matter lesions, APS patients frequently display abnormalities on neuroimaging studies, most

commonly focal subcortical white matter areas of signal hyperintensity [28, 29]. It is not always clear whether these lesions represent ischemia or inflammation [28, 29], with hints pointing towards an APS diagnosis including smaller lesions on MRI, frequently located in the subcortical area, with stability over time and possible improvement with anticoagulation therapy [30]. The evidence of a potential impact of these lesions in patients' prognosis gains relevance as their significance is still debated in APS. There is a controversial possibility linking white matter lesions with the presence cognitive impairment, but while some MRI studies in APS patients with neurological symptoms display high frequency of infarcts, others focusing specifically on cognition did not demonstrate an increased number of infarcts in APS patients with cognitive deficits comparing with controls [28].

Regarding SP-aPL patients, in a similar fashion to SN-APS, they also displayed a higher frequency of obstetric criteria manifestations and concurrent obstetric and thrombotic manifestations in comparison with controls. Focusing on thrombosis, even though the mean number of events was higher in SP-aPL, recurrence was not significantly different from controls. This is in line with the notion that one single aPL positive determination is not associated with increased recurrent thrombosis, as stated in the previous systematic review reporting, in these patients, a similar recurrence rate to the general population [14].

Lastly, the comparison of the characteristics and treatment of obstetric patients between SN-APS/SP-aPL patients and the control revealed no significant differences, but the small sample undermines the extraction of significant and generalizable conclusions.

This work encompasses significant strengths but also limitations. First, as these patients did not have definite diagnosis, they were managed according to their physician's judgment and not respecting a predefined protocol, leading to potential disparities in their treatment. The treatment options might also reflect specific management approaches of the studied centers and not be easily comparable with other institutions. Additionally, the retrospective design and the fact that both SN-APS and SP-aPL groups include quite heterogeneous individuals (i.e., patients with varied non-criteria manifestations and single positivity of different antibodies) weaken the extrapolation of obtained results. Nevertheless, considering the rarity of the entity and the difficulties in identifying these patients, a retrospective design is a feasible first approach to gather initial data in a domain with practically no available evidence. This is also valid regarding the different non-criteria manifestations. The report of the *14th International Congress on Antiphospholipid Antibodies Technical Task Force on APS Clinical Features* reviewed the literature devoted to some of these non-criteria manifestations, and the sparsity of data regarding their impact was clear. Even in the new criteria under development [2], part of the decision to include or not these non-criteria manifestations in the preliminary criteria included a share of eminence-based assessment, as experts classified clinical scenarios with these features as "highly likely" or "equivocal or unlikely" APS. Therefore, the fact that our work included patients from two different centers, with clearly defined manifestations and inclusion criteria, and an adjustment for relevant confounders when comparing with the control group, constitutes a pertinent, though initial, effort to provide data in a domain with limited and heterogenous guiding evidence.

These results carry clinical implications, suggesting that the presence of non-criteria manifestations negatively affects the prognosis of SN-APS. This could imply a potential need for a more thorough follow-up and aggressive management of these patients, with earlier and prolonged anticoagulation. Conversely, the presence of only one single aPL positive determination does not seem to dictate increased risk of recurrent thrombosis, serving as a reinforcement to the current practice of managing these patients in a similar fashion to the general population. However, confirmation of these results should be obtained in future prospective, ideally multicenter studies (considering the scarcity of these patients), ideally focusing on specific non-criteria manifestations.

Conclusion

SN-APS patients displayed more thrombosis recurrence, indefinite anticoagulation, use of VKA (instead of DOAC), and longer anticoagulation duration than controls without non-criteria manifestations. SP-aPL patients did not display significantly higher thrombosis recurrence in comparison with controls. The presence of non-criteria manifestations in patients with thrombosis and negative aPL may negatively impact the clinical course of these patients and confer a poorer prognosis.

Authors' contributions

GPR participated in the study design, data acquisition/analysis, and manuscript draft. BSP participated in the study design, data analysis and manuscript draft. EF participated in the study design, data acquisition/analysis, and manuscript draft. OA, GB, PB, RC, and GE participated in the study design and manuscript draft/revision. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study received approval from the Hospital Clínic Ethics Committee (HCB/2020/1259).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Immunosuppression use in primary antiphospholipid antibody-positive patients: Descriptive analysis of the AntiPhospholipid Syndrome Alliance for Clinical Trials and InternatiOnal Networking (APS ACTION) Clinical Database and Repository ("Registry")

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Abstract

Background/Purpose: APS ACTION Registry was created to study the outcomes of patients with persistently positive antiphospholipid antibodies (aPL) with or without other systemic autoimmune disease (SAIDx). Given that immunosuppression (IS) is used for certain aPL manifestations, for example, thrombocytopenia (TP), our primary objective was to describe the indications for IS in aPL-positive patients without other SAIDx. Secondly, we report the type of IS used in patients with selected microvascular or non-thrombotic aPL manifestations.

Methods: An online database is used to collect clinical data. The inclusion criteria are positive aPL based on the laboratory section of the APS Classification Criteria, tested at least twice within one year prior to enrollment. Patients are followed every 12 ± 3 months. For this descriptive retrospective and prospective analysis, we included aPL-positive patients without other SAIDx and excluded those with new SAIDx classification during follow-up. For each patient, we retrieved clinical data at baseline and follow-up including selected aPL manifestations (diffuse alveolar hemorrhage [DAH], antiphospholipid-nephropathy [aPL-N], livedoid vasculopathy [LV]-related skin ulcers, TP, autoimmune hemolytic anemia [AIHA], cardiac valve disease [VD]), and IS medications.

Results: Of 899 patients enrolled, 537 were included in this analysis (mean age 45 ± 13 years, female 377 [70%], APS Classification in 438 [82%], and at least one selected microvascular or non-thrombotic aPL manifestation in 141 (26%)). Of 537 patients, 76 (14%) were reported to use IS (ever), and 41/76 (54%) received IS primarily for selected aPL manifestation. In six of 8 (75%) DAH patients, 6/19 (32%) aPL-N, 4/28 (14%) LV, 25/88 (28%) TP, 6/11 (55%) AIHA, and 1/43 (2%) VD, the IS (excluding corticosteroids/hydroxychloroquine) indication was specific for selected aPL manifestation.

Conclusion: In our international cohort, 14% of aPL-positive patients without other SAIDx were reported to receive IS; the indication was at least one of the selected microvascular and/or non-thrombotic aPL-related manifestations in half. Thrombocytopenia was the most frequent among those selected aPL-related manifestations; however, approximately one-third received IS specifically for that indication. Diffuse alveolar hemorrhage was frequently treated with IS followed by AIHA and aPL-N. Systematic controlled studies are urgently needed to better define the role of IS in APS.

Keywords

antiphospholipid syndrome, antiphospholipid antibodies, immunosuppression, non-criteria manifestations

Background

Antiphospholipid syndrome (APS) is characterized by thrombosis and/or pregnancy morbidity in association with antiphospholipid antibodies (aPL), lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and anti-β_2 glycoprotein-I antibodies (aβ_2GPI).[1] APS may exist in its primary form when it occurs in patients without systemic autoimmune disease (SAIDx), or in association with other autoinflammatory disorders, particularly systemic lupus erythematosus (SLE).[2]

The Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION) was created in 2010 specifically to conduct large-scale multicenter clinical studies and trials in persistently aPL-positive patients. The goal of the APS ACTION Clinical Database and Repository ("Registry") is to study the natural course of persistently aPL-positive patients with or without other SAIDx over at least 10 years.[3]

Immunosuppression (IS) has been increasingly used in primary APS, specifically for microvascular disease, for example, diffuse alveolar hemorrhage (DAH), aPL-nephropathy (aPL-N), and hematologic non-thrombotic manifestations such as thrombocytopenia (TP).[4] However, there are no randomized control studies, and very limited number of systematic studies, to support the use of IS in aPL-positive patients without other SAIDx. Thus, our primary objective was to describe the general indications for IS medications in aPL-positive patients without other SAIDx. Secondly, we report the type of IS used in patients with selected microvascular or non-thrombotic aPL-related manifestations.

Methods

The inclusion criteria for the APS ACTION registry are positive aPL based on the updated Sapporo classification criteria at least twice within 1 year prior to enrollment. Patients are followed every 12 ± 3 months with clinical data and blood collection. Antiphospholipid antibody-specific medical history (including microvascular or non-thrombotic aPL-related manifestations), aPL/APS-related medications (anticoagulant/antiplatelet medications, hydroxychloroquine (HCQ), intravenous immunoglobulin (IVIG), plasma exchange, rituximab (RTX), azathioprine (AZT), corticosteroids (CS), cyclophosphamide, cyclosporine, methotrexate (MTX), mycophenolate mofetil (MMF), and "other" IS medications), and blood samples (for aPL-positivity confirmation) are collected at registry entry. At each annual follow-up visit, clinical data for the new aPL-related events and new SAIDx, blood

samples, and medication changes are collected. The registry data are managed using REDCap electronic data capture tool, a secure, web-based system designed to support research studies.[5]

In this descriptive retrospective and prospective analysis of the registry, we only included aPL-positive patients without other SAIDx and excluded those with catastrophic APS (CAPS) (given IS is part of the acute CAPS management) or with new SAIDx classification during follow-up (given the possibility of IS use part of the SAIDx management). We identified all patients who have ever received IS at baseline and/or during prospective follow-up, as well as investigator-reported indications for IS use and attribution of IS to selected microvascular or non-thrombotic aPL-related manifestations. For the purposes of this study, CS and HCQ use were not counted as IS medications, and only selected microvascular or non-thrombotic aPL-related manifestations were analyzed: DAH based on bronchoscopy/bronchoalveolar lavage and/or biopsy, aPL-N (biopsy-proven) and cardiac valve disease (VD) based on the definitions included in the 2006 revised Sapporo APS classification criteria report,[6] livedoid vasculopathy (LV)-related skin ulcers, TP defined as a platelet count of <100,000 per microliter tested twice at least 12 weeks apart, and autoimmune hemolytic anemia (AIHA) defined as anemia with the presence of hemolysis and with a positive direct antiglobulin test (DAT).

Data were summarized in a descriptive fashion; mean ± SD (SD) was used for continuous variables.

Results

As of July 2021, 899 patients were included in the registry; five were excluded due to CAPS and 357 (40%) were excluded due to another SAIDx at baseline (344) or during follow-up (13 [11 SLE and two rheumatoid arthritis). Of the remaining 537 patients (mean age at entry: 45 ± 13 years; 70% female; 70% white; 438 [82%] met the APS Classification Criteria; and 141 (26%) had at least one of the selected microvascular and/or non-thrombotic aPL-related manifestation), 76 (14%) used IS (ever) (excluding CS and HCQ) (Table 1).

Based on investigator-reported indications, of 76 IS users, 41 (54%) were treated primarily because of their selected aPL-related manifestation (16 patients had more than one selected aPL-related manifestations simultaneously or at different time points), whereas 35/76 (46%) received IS for other potential indications (Table 1 Footnote). Of note, 16 [46%] of the latter group also had one or more of the selected aPL-related manifestations (1 aPL-N, 7 LV, 7 TP, 1 AIHA, and 2 VD), although IS use was not reported for that indication. The number of patients fulfilling three of 11 American College of Rheumatology SLE Classification Criteria, that is, "lupus-like disease" was 16/41 in the former group and 6/35 in the latter group.

In a subgroup analysis of 141 (26%) patients with at least one selected microvascular and/or non-thrombotic manifestations reported at baseline and during the follow-up, (a) eight patients had DAH and 6 (75%) of these received IS for this indication (most commonly used medications were IVIG and/or RTX); (b) 19 (13%) had aPL-N and 6 (32%) received IS for this indication (MMF and/or RTX); (c) 28 (20%) had LV and 4 (14%) received IS for this indication (RTX); (d) 88 (62%) had TP and 25 (28%) received IS for this indication (IVIG and/or RTX); (e) 11 (8%) had AIHA and 6 (55%) received IS for this indication (IVIG and/or AZT); and (f) 43 (30%) had VD and 1 (2%) received IS for this indication (IVIG) (Table 1).

Discussion

In this descriptive retrospective and prospective analysis of our international cohort of aPL-positive patients without other SAIDx and CAPS, 76 (14%) of the cohort received IS medications other than CS and HCQ. The indication was at least one of the selected microvascular and/or non-thrombotic aPL-related manifestations (DAH, aPL-N, LV, TP, AIHA, and/or VD) in half of these patients. Although TP was the most common, DAH, aPL-N, and AIHA were frequently treated with IS.

There is no uniform approach to the management of microvascular or non-thrombotic APS, most probably due to heterogeneous organ involvement with different severity, lack of controlled studies, or compelling evidence supporting any treatment strategy. The only published systematic assessment of IS in APS has been a pilot prospective uncontrolled small (n: 19) study of RTX (the RITAPS study) for aPL-positive patients with microvascular disease or hematologic involvement.[7] This study suggested that despite causing no substantial change in aPL profiles, RTX is effective in some aPL-positive patients with TP, aPL-related skin ulcers, kidney disease, and cognitive dysfunction. The use of other traditional (e.g., MMF, AZT, or cyclophosphamide) and non-traditional (e.g., sirolimus and eculizumab) IS agents in APS is mostly based on case reports[8–11] and expert/consensus opinion.[4,12,13] In our analysis, the most commonly used IS medications were IVIG followed by RTX and MMF; the relatively higher proportion of patients treated with IS for specific aPL-related manifestations were those with DAH (75%) and AIHA (55%).

DAH is a rare manifestation of APS, which generally responds to CS. However, flares during CS tapering is common, and many patients require a steroid-sparing IS agent to achieve full remission. Based on a literature review of 66 patients with primary APS (excluding CAPS), cyclophosphamide- or RTX-based regimens achieve the

Table 1. Patients with Selected Microvascular and/or Non-thrombotic Manifestations (MV-NTM) (immunosuppressive [IS] medications were recorded in 76 patients; indication was for MV-NTM in 41 and "other"[a] in 35).

# of patients	DAH	aPL-N	LV	TP	AIHA	VD
Baseline	5	15	25	84	11	37
Follow-up	3	4	3	4	0	6
Total						
Alone or together with another MV-NTM	8[b]	19[b]	28[b]	88[b]	11[b]	43[b]
Alone as the only MV-NTM	4	4	16	70	6	25
Immunosuppression use (ever)	6	9	13	34	7	9
Immunosuppression use for MV-NTM[c]	6 (75%)	6 (32%)	4 (14%)	25 (28%)	6 (55%)	1 (2%)
IVIG	4	1	1	18	4	1
Rituximab (RTX)	5	4	3	14	2	0
Mycophenolate mofetil (MMF)	3	5	1	5	0	0
Azathioprine (AZT)	0	2	1	5	3	0
Plasma exchange (PE)	1	0	0	2	0	0
Cyclophosphamide (CYC)	1	1	0	3	0	0
Belimumab (BEL)	0	1	1	1	0	0
Eculizumab (ECU)	0	0	0	2	0	0
Sirolimus (SIR)	0	0	0	2	0	0
Other[d]	1	0	2	1	0	0
Hydroxychloroquine use (total)	5	10	19	41	6	16

Abbreviation: DAH: diffuse alveolar hemorrhage; aPL-N: aPL-nephropathy; LV: livedoid vasculopathy; TP: persistent thrombocytopenia < 100 × 10^9/l; HA: hemolytic anemia; and VD: cardiac valve disease.

[a]Immunosuppressive indications reported other than selected microvascular and non-thrombotic manifestations were (35/76): Lupus-like clinical features with musculoskeletal and/or hematologic involvement without SLE classification (n: 6, methotrexate [MTX], AZT); heparin-induced TP (n:1, IVIG); peripheral artery ischemia (n:1, IVIG, RTX); cognitive dysfunction (n:1, RTX, MMF); HELLP (hemolysis, elevated liver enzyme, and low platelet) syndrome (n:2, PE, IVIG); vasculitis (n:3, AZT, CYC, MMF, MTX); hidradenitis suppurativa (n:1, adalimumab); post-CVA acute renal failure(n:1, PE); interstitial lung disease (n:2, IVIG, RTX, MMF, CYC); pregnancy morbidity resistant to traditional management (n:2, IVIG); peripheral artery bypass surgery (n:1, eculizumab); primary biliary cirrhosis/autoimmune hepatitis/Crohn's disease (n:3, AZT); myasthenia gravis (n:1, AZT); renal transplant thrombotic microangiopathy/hepatopulmonary syndrome (n:2, CYC, MMF, tacrolimus); idiopathic pachymeningitis encephalopathy (n:2, AZT, RTX, CYC); dystonia/neuropathy (n:3, IVIG, PE, RTX, AZT, MMF, MTX); in vitro fertilization co-adjuvant treatment (n;1, IVIG); anticoagulation refractory TIA (n:1, RTX); and atopic dermatitis/alopecia (n:1, MMF).

[b]Corticosteroid use was reported in 3 DAH patients, 4 aPL-N, 4 LV, 19 TP, 3 AIHA, and 1 VD.

[c]16 patients had more than one MV-NTM simultaneously or at different time points.

[d]Abatacept, MTX, danazol, and tacrolimus.

highest remission rates (50%); other strategies include IVIG, plasmapheresis, MMF, and/or AZT.[14] Based on our small numbers, the most commonly used IS for DAH was RTX followed by IVIG and MMF.

Antiphospholipid antibody-associated nephropathy, which develops in less than 5% of aPL-positive patients, can present as acute or chronic disease.[15,16] Chronic aPL-N is usually slowly progressive, with no proven treatment. The use of anticoagulation in this scenario is controversial;[4] and there have been anecdotal reports of successful CS, cyclophosphamide, MMF, or RTX use.[7,9] Strong conclusions regarding the effectiveness of any of these regimens are difficult given the lack of systematic studies. One-third of our registry patients with aPL-N received IS, most commonly MMF and RTX, which supports the fact that international centers experienced in APS have different strategies while managing these patients.

Skin manifestations of aPL vary from livedo reticularis/racemosa to LV-related skin ulcerations.[17] For LV, CS are less preferable due to the risk of infection. For patients failing conservative management, RTX is an option;[4,7] in addition to the complete response of five RTX-treated patients with aPL-related skin ulcers in the RITAPS trial,[7] another primary APS patient with recurrent skin ulcers was reported to receive belimumab with partial improvement.[18] In our cohort, the most commonly used IS for LV was RTX; however, the majority of our cohort did not receive IS, which may be due to different management strategies of the centers or the severity of the LV presentation.

Twenty percent of aPL-positive patients develop mild-to-moderate TP;[19] however, TP usually does not require any treatment because the degree of TP is generally above 30–50 × 10^9 L.[20] For severe TP, CS and/or IVIG are first line treatments.[19] Azathioprine, MMF, or RTX are considered in CS-resistant cases.[20–23] In our registry, TP was the most frequent among those selected microvascular or non-thrombotic aPL manifestations; for patients

requiring treatment, the most common strategy was IVIG followed by RTX.

APL may be associated with the formation of autoantibodies directed against erythrocyte antigens, leading to premature destruction of red blood cells.[21] Almost 5% of aPL-positive patients develop DAT-positive AIHA,[19] which is usually treated with CS, AZT, MMF, RTX, or splenectomy.[20,24] In our study, almost half of the AIHA patients required treatment and the most commonly used IS was IVIG followed by AZT.

Valvular heart disease (vegetations and/or valve thickening) is the most common aPL-related cardiac manifestation. Depending on the definitions and the echocardiography method, that is, transthoracic versus transesophageal, 10%–50% of aPL-positive patients may develop VD.[25] Both aortic and mitral insufficiencies are common and require valve replacement in severe cases.[20] Cardiac valve thickening increases the risk for arterial/embolic events. Corticosteroids and anticoagulation generally do not lead to regression of cardiac valve lesions, but antithrombotic treatment is usually administered to decrease risk of embolic events, despite low evidence associated with outcome.[20] We found only one patient who received IS specifically for VD.

Based on a recent descriptive analysis of the APS ACTION Registry, TP, AIHA but not aPL-N, LV, or VD is observed more commonly in aPL-positive SLE patients, compared to those without SLE.[15] Similarly, CS, HCQ, AZT, cyclophosphamide, MTX, and MMF, but not IVIG, RTX, or plasma exchange use was more common in aPL-positive SLE patients. Thus, our previous and current registry analyses demonstrate that IS is part of the APS management strategy, independent of SLE Classification or SLE clinical features. We believe that IS has a role in the management of aPL-positive patients with selected clinical phenotypes, mainly microvascular APS and non-thrombotic APS; however, we are also aware that despite theoretical and preclinical evidence, clinical studies supporting the role of IS in APS is limited.[4]

Our study has several limitations including the retrospective baseline data collection, which may not provide the most accurate information about each IS medication. The number of patients with some of the selected aPL-related manifestations is relatively small. Furthermore, we cannot comment on the use of CS and HCQ for selected microvascular or non-thrombotic aPL-related manifestations included in our study given that these medications are commonly used for other indications (similarly we cannot comment how CS and/or HCQ use affected the decision-making regarding the IS use). Another limitation is an inability to indicate IS effect on aPL titers recognizing contradictory reports appear in the literature.[7] Lastly, our retrospective/prospective study design did not allow investigation of the effectiveness of IS medications; however, further studies are planned based on the prospective registry data. Despite these limitations, APS ACTION Registry has a heterogeneous group of aPL-positive patients from tertiary referral centers, representing a real-world experience; and this study will serve as a model for future analysis of the data and hopefully help build a future research agenda.

In conclusion, in our multi-center international cohort, 14% of aPL-positive patients without other systemic autoimmune diseases were reported to receive IS for selected aPL-related manifestations or other indications. Systematic studies and randomized controlled trials are urgently needed to better define the role of IS in APS.

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Statement for ethics approval

This study obtained approval from HSS APS ACTION IRB # 2014-252. All participants gave informed consent.

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Article

Early Prediction of Adverse Pregnancy Outcome in Women with Systemic Lupus Erythematosus, Antiphospholipid Syndrome, or Non-Criteria Obstetric Antiphospholipid Syndrome

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Abstract: A prospectively study of pregnant women with systemic lupus erythematosus (SLE), antiphospholipid syndrome, or non-criteria obstetric antiphospholipid syndrome was conducted to describe the characteristics of women followed in a referral unit and to derive a predictive tool for adverse pregnancy outcome (APO). Demographic characteristics, treatments, SLE activity, and flares were recorded. Laboratory data included a complete blood cell count, protein-to-creatinine urinary ratio (Pr/Cr ratio), complement, anti dsDNA, anti-SSA/Ro, anti-SSB/La, and antiphospholipid antibodies status. A stepwise regression was used to identify baseline characteristics available before pregnancy and during the 1st trimester that were most predictive of APO and to create the predictive model. A total of 217 pregnancies were included. One or more APO occurred in 45 (20.7%) women. A baseline model including non-Caucasian ethnicity (OR 2.78; 95% CI [1.16–6.62]), smoking (OR 4.43; 95% CI [1.74–11.29]), pregestational hypertension (OR 16.13; 95% CI [4.06–64.02]), and pregestational corticosteroids treatment OR 2.98; 95% CI [1.30–6.87]) yielded an AUC of 0.78 (95% CI [0.70–0.86]). Among first-trimester parameters, only Pr/Cr ratio improved the model fit, but the predictive performance was not significantly improved (AUC of 0.78 vs. 0.81; $p = 0.16$). Better biomarkers need to be developed to efficiently stratify pregnant women with the most common autoimmune diseases.

Keywords: systemic lupus erythematosus; antiphospholipid syndrome; non criteria obstetric antiphospholipid syndrome; pregnancy outcome; prediction; autoimmune disease

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1. Introduction

Most autoimmune diseases are no longer an absolute contraindication for pregnancy. Advances in management of systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) have provided a better quality of life of women at childbearing age and a consequent increase in the pregnancy rate among these women [1]. A group of special interest are those women who do not meet all clinic or laboratory diagnostic criteria for APS, which has been defined as non-criteria obstetric APS (NC-OAPS) [2]. Scarce data exist regarding their characteristics and prognostic factors despite representing a considerable number of women followed in high-risk obstetric units [3]. Despite management improvements, pregnancies in women with SLE, APS, or NC-OAPS still present a higher perinatal morbidity compared to the general population, especially related to placental insufficiency, such as preeclampsia (PE), intrauterine growth retardation (IUGR), or perinatal death [4–6]. The pathophysiology underlying obstetric morbidity remains elusive [7]. In fact, pregnancy is an exceptional situation in which multiple systems must successfully adapt to obtain

optimal outcomes. Clinical or subclinical inflammation, activation of the complement, the presence of autoantibodies [8], hormonal dysfunction, and immune disturbances might contribute to alter these complex balance [9–12].

The reported risk factors for adverse pregnancy outcomes (PO) include presence of lupus anticoagulant (LAC), antihypertensive use, physician global assessment score greater than 1, and low platelet count [13]. Considering women with obstetric APS, LAC positivity seems to be the strongest predictor of APO [14,15], and triple aPL positivity has been associated to an especially high risk of pregnancy complications and thrombosis [16]. More recently, aberrant activation of the alternate complement pathway has also been associated to APO [9]. More specific mechanisms, such as incomplete downregulation of SLE-associated transcriptional networks, including type-l interferon (INF-I), have also been related to SLE complications during pregnancy [17]. Women with NC-OAPS have not been included in randomized clinical trials or in observational registries, and therefore, information about their risk of obstetric recurrence is scarce.

Some studies have attempted to identify predictive variables, obtaining models with limited predictive capacity and diverse results [18–20]. We conducted a study based on a prospectively collected database of pregnant women with SLE, APS, or NC-OAPS in order to accurately describe the characteristics of women with the aforementioned autoimmune diseases and who were followed in a referral unit and to derive from these characteristics a predictive tool for adverse pregnancy outcome that can help clinicians in risk counselling and tailoring the pregnancy follow-up.

2. Materials and Methods

2.1. Study Design and Population

This is an observational cohort study based on a prospectively collected database of pregnant women with autoimmune diseases followed in a high-risk obstetric unit of a tertiary hospital between 2010 and 2019. Pregnant women diagnosed with SLE, APS, or NC-OAPS were included. Each pregnancy of the included patients was registered and analyzed as an independent episode. The diagnosis of SLE was established according to the criteria of the latest update of the American College of Rheumatology/EUropean League Against Rheumatology (ACR/EULAR) [21]. The diagnosis of APS was established according to Sydney updated criteria [22]. NC-OAPS was defined as aPL-related obstetric complications not fulfilling clinical and/or the laboratory classification criteria for APS [2].

The study protocol was approved by the local Ethics Committee (HCB/2020/0184), and all participants signed informed consent. All women were treated according to a specific protocol that included the schedule of visits, ultrasounds, and laboratory tests. Patients with aPL received treatment according to the latest recommendations [23–25].

2.2. Measures

Demographic characteristics, previous obstetric outcomes and episodes of thrombosis, pre-pregnancy arterial hypertension, mode of conception, presence of other autoimmune diseases, and treatments were recorded. Regarding SLE activity, SLE flare during pregnancy or puerperium was defined as onset or worsening of signs/symptoms of SLE in any organ or system attributed to the activity of the disease and that conditioned a change in the treatment [25]. In addition, Systemic Lupus Erythematosus Pregnancy Disease Activity Index (SLEPDAI) [26] and Lupus Low Disease Activity State (LLDAS) [27] were calculated as well as adjusted GAPSS (Global Anti-Phospholipid Syndrome Score) [28], which included presence of dyslipidemia, arterial hypertension, and status of LAC, aCL, and aB2GPI antibodies. Accumulated organ involvement of SLE (skin, articular, serosa, hematologic system, kidney, and nervous system) was also registered.

Laboratory data included in each trimester of gestation a complete blood cell count, protein-to-creatinine urinary ratio (Pr/Cr ratio), C3, C4, and anti-dsDNA antibodies. Status of anti-SSA/Ro (anti-Ro) and anti-SSB/La (anti-La) antibodies were registered. Regarding aPL, anticardiolipin antibodies (aCL), anti-B2-glycoprotein I antibodies (anti-β2GPI), and

LAC that tested positive at least twice on two or more consecutive occasions at least 12 weeks apart with titers >40 GPL/MPL (99th centile) and/or >40 AU, respectively, were recorded as criteria aPL positivity. Persistent aPL at low titers and those with medium-high aPL titers or LAC but not persistently positive (only one positive determination) were classified as non-criteria aPL positivity.

Pregnancy and adverse pregnancy outcomes were recorded. APO were defined as follows: (a) fetal death > 10 weeks of gestation not explainable by chromosomal abnormalities, anatomical malformations, or congenital infections; (b) neonatal death before hospital discharge due to complications of prematurity and/or placental insufficiency; (c) preterm birth < 37 weeks due to placental insufficiency, gestational hypertension, or preeclampsia; (d) small for gestational age newborn (<percentile 5) in the absence of anatomical malformations or genetic alterations; and (e) preeclampsia during gestation or puerperium.

2.3. Statistical Analysis

The data were collected and entered into a database for analysis using the STATA software (version 13.1; Texas, College Station). The distribution of data was evaluated using the Shapiro–Wilk normality test. Missing values in the first trimester protein-to-creatinine urinary ratio (n = 21) were imputed from maternal age and second-trimester values by expectation-maximization (EM) method assuming a normal distribution and checking for the randomness of missing values by Little's statistic. The MVA-package of IBM SPSS 23.0 was used. The statistical significance of differences in continuous data was calculated using the Student's t-test or the Mann–Whitney U-test for normally and not-normally distributed data, respectively. The categorical data was analyzed using Fisher's exact test. Multivariable analysis was performed by selecting variables with $p < 0.1$ in the univariate analysis as potential predictors of APO. Logistic regression was applied to assess the OR and 95% CIs of APO for all potential predictors. A stepwise regression was then used ($p < 0.05$ for the forward and $p < 0.10$ for the backward steps) to identify baseline characteristics available before pregnancy that were most predictive of APO and to create the predictive model. All the models were runed by penalized maximum likelihood method, which is more robust in samples with rare events. From individual risks, receiver-operating characteristics (ROC) curves were plotted. The area under the receiver-operating characteristics curve (AUC) was used to assess the discrimination of the logistic model obtained. The same procedure was applied to identify and generate a model with variables available during the 1st trimester of pregnancy to assess if the addition of some variable to the pregestational model might improve the predictive ability.

3. Results

A total of 188 women and 217 pregnancies were included in the study. The distribution of the diagnoses and incidence of APO according to the diagnosis is shown in Table 1. One or more APO occurred in 45 (20.7%) women. Baseline characteristics, medical and obstetric history, treatments, laboratory parameters, and SLE type are shown in Table 2. Regarding the presence of other autoimmune diseases other than SLE, APS, or NC-OAPS, there were 14 women who had another autoimmune disease. Specifically, there were 10 women with Sjogren syndrome: 9 of them in the SLE group and 1 in the NC-OAPS group. Three women also presented with systemic sclerosis: two in the SLE and one in the SLE+ aPL group. One woman in the SLE+ aPL group also suffered idiopathic juvenile arthritis. Therefore, most of the comorbidities were found in SLE group rather than in the APS or NC-OAPS patients. There were no differences in APO rate among women with more than one autoimmune disease. The distribution of APO are shown in Table 3. Obstetric follow-up and pregnancy outcomes are also shown in Table 3.

Table 1. Distribution of pregnancies included and incidence of APO according to the diagnosis.

		Total *n* = 217	With APO *n* = 45	*p*
Diagnosis	APS	41	9 (22)	0.70
	NC-OAPS	42	9 (21.4)	
	SLE	100	18 (18)	
	SLE + aPL	26	6 (23.1)	
	SLE + APS	8	3 (37.5)	

Abbreviations: APO, adverse pregnancy outcomes; APS, Antiphospholipid syndrome; NC-OAPS, non-criteria obstetric antiphospholipid syndrome; SLE, Systemic Lupus Erithematosus; aPL, antiphospholipid antibodies. Data given as *n* (%).

Table 2. Characteristics of study population and their association with adverse pregnancy outcome.

	Total *n* = 217	With APO *n* = 45	Without APO *n* = 172	*p*
Maternal age	36.5 (33.5–39.5)	36.5 (33.5–39.5)	36.5 (33.5–39.5)	0.83
Body mass index	22.5 (20.5–24.7)	22.8 (20.7–25)	21.6 (19.2–23.7)	0.42
Non-Caucasian	39 (18)	15 (33.3)	24 (14)	0.01
Smoking	29 (13.4)	12 (26.7)	17 (9.9)	0.01
Assisted reproductive technique	39 (18)	12 (26.7)	27 (15.7)	0.08
Previous adverse pregnancy outcome	26 (12)	6 (13.3)	20 (11.6)	0.8
Previous thrombosis	18 (8.3)	4 (8.9)	14 (8.1)	0.8
Pre-gestational hypertension	15 (6.9)	12 (26.7)	3 (1.7)	<0.001
Other autoimmune diseases	14 (6.4)	3 (6.6)	11 (6.4)	0.94
LDA	150 (69.1)	38 (80)	114 (66.3)	0.1
LMWH	88 (40.6)	20 (44.4)	68 (39.5)	0.61
Corticosteroids beginning pregnancy	46 (21.2)	18 (40)	28 (16.3)	0.002
Corticosteroids dose (mg)	5 (5–10)	5 (3.3–10)	5 (5–10)	0.19
Hydroxychloroquine treatment	126 (58.1)	27 (60)	99 (57.6)	0.87
GAPSS	0 (0–4)	0 (0–4)	0 (0–4)	0.25
LLDAS	119 (54.8)	22 (48.9)	97 (56.4)	0.4
SLEPDAI	2 (0–4)	2 (0–4)	2 (0–2)	0.03
Anti-Ro/anti-La antibodies	57 (26.3)	11 (24.4)	46 (26.7)	0.85
Anti-dsDNA antibodies	78 (35.9)	19 (42.2)	59 (34.3)	0.38
Low levels of complement (C3 or C4)	55 (25.4)	17 (37.8)	39 (22.1)	0.04
Lupus anticoagulant Negative No criteria Criteria	 176 (81.1) 10 (4.3) 31 (14.6)	 32 (71.1) 4 (8.9) 9 (20)	 144 (83.7) 6 (3.5) 22 (12.8)	0.11
aCL IgM Negative No criteria Criteria	 180 (83) 8 (3.7) 29 (13.4)	 36 (80) 2 (4.4) 7 (15.6)	 144 (83.7) 6 (3.5) 22 (12.8)	0.71
aCL IgG Negative No criteria Criteria	 166 (76.5) 22 (10.1) 29 (13.4)	 36 (80) 6 (13) 3 (6.7)	 130 (75.6) 16 (9.3) 26 (15.1)	0.25

Table 2. *Cont.*

	Total n = 217	With APO n = 45	Without APO n = 172	p
Anti-β2GPI IgM Negative No criteria Criteria	186 (85.7) 15 (6.9) 16 (7.4)	37 (82.2) 4 (8.9) 4 (8.9)	149 (86.6) 11 (6.4) 12 (7)	0.67
Anti-β2GPI IgG Negative No criteria Criteria	196 (90.3) 9 (4.2) 12 (5.5)	43 (95.6) 1 (2.2) 1 (2.2)	153 (89) 8 (4.7) 11 (6.4)	0.57
Triple aPL positivity	13 (6)	4 (8.9)	9 (5.2)	0.36
SLE type Musculoskeletal Dermatologic Hematologic Renal Serosa Neurological	 101 (46.5) 96 (44.2) 53 (24.4) 34 (15.7) 26 (12) 3 (1.4)	 21 (46.7) 22 (48.9) 11 (24.4) 12 (26.7) 9 (20) 0 (0)	 80 (46.5) 74 (43.0) 42 (24.4) 22 (12.8) 17 (9.9) 3 (1.7)	 0.5 1 1 0.04 0.07 1
Protein/creatinine urinary ratio at 1T	77 (55–109)	93 (57–162)	74 (54–97)	0.02

Abbreviations: APO, Adverse pregnancy outcome; LDA, low-dose aspirin; LMWH, low-molecular-weight heparin; LLDAS, low-level disease activity state; GAPSS, Global Anti-Phospholipid Syndrome Score; LLDAS, Lupus Low Disease Activity State; SLEPDAI, Systemic Lupus Erythematosus Disease Activity Index; aCL, anticardiolipin antibodies; β2GPI, anti-B2-glycoprotein; aPL, antiphospholipid antibodies; SLE, Systemic Lupus Erithematosus; 1T, first trimester. Data given as *n* (%) or median (IQR).

Table 3. Obstetric follow-up and pregnancy outcomes.

	Total n = 217	With APO n = 45	Without APO n = 172	p
Pregnancy loss 10–24 weeks	4 (1.8)			-
Fetal loss > 24 weeks	4 (1.8)			-
Neonatal death	3 (1.4)			-
Preeclampsia	18 (8.3)			-
Placental abruption	1 (0.5)			-
Preterm birth	19 (8.8)			-
Small for gestational age	28 (13.3)			-
Onset of labor Spontaneous Induction Elective caesarean section	65 (31) 104 (49.5) 41 (19.5)	7 (16.3) 25 (58.1) 11 (25.6)	58 (34.7) 79 (47.3) 30 (18)	0.06
Mode of delivery Vaginal Non-elective caesarean section Elective caesarean section	122 (58.4) 46 (22) 41 (19.6)	17 (38.6) 16 (36.4) 11 (25)	105 (63.6) 30 (18,2) 30 (18.2)	0.01
Gestational age at delivery	39.3 (37.8–40)	37.1 (34–38)	39.6 (38.7–40.1)	<0.001
Birthweight	3090 (2700–3400)	2250 (1608–2653)	3155 (2980–3460)	<0.001
Thrombosis gestation or puerperium	3 (1.4)	1 (2.2)	2 (1.2)	0.5
SLE flare gestation or puerperium	13 (6)	6 (13.3)	7 (4.1)	0.03
Protein/creatinine urinary ratio at 2T	93 (63–130)	93 (72–168)	91 (62–122)	0.17
Protein/creatinine urinary ratio at 3T	114 (74–182)	216 (115–597)	100 (72–144)	<0.001

Abbreviations: APO, Adverse pregnancy outcome; SLE, Systemic Lupus Erythematosus; 1T, first trimester; 2T, second trimester; 3T, third trimester. Data given as *n* (%) or median (IQR).

No differences were found regarding maternal age, body mass index, mode of conception and previous APO, or thrombosis episodes between study groups. Among the baseline epidemiological characteristics, non-Caucasian ethnicity (33.3% vs. 14%; $p = 0.01$), smoking (26.7% vs. 9.9%; $p = 0.01$), and pre-gestational hypertension (26.7% vs. 1.7%; $p < 0.001$) were significantly more prevalent in the APO group. Regarding laboratory parameters, hypocomplementemia (low C3 or C4) (37.8% vs. 22.1%; $p = 0.04$) and Pr/Cr ratio in the 1st trimester (93–74; $p = 0.02$) and 3rd trimester (216–100; $p < 0.001$) were significantly associated to APO. Regarding treatments, only the use of corticosteroids treatment at the beginning of pregnancy was significantly more prevalent in APO group (40% vs. 16.3%; $p = 0.002$). However, mean corticosteroids dose was similar between APO and non-APO group (10 vs. 7.4; $p = 0.19$). No association was found with the other treatments, namely LDA, LMWH, and hydroxychloroquine. In this cohort, almost 70% of women were treated with low dose of aspirin, 40% with low-molecular-weight heparin (any dose), and almost 60% with hydroxychloroquine. Regarding SLE activity, only SLEPDAI score was significantly higher in the APO group. Kidney involvement was the only SLE organ involvement significantly associated to APO. Surprisingly, in our cohort, aPL positivity (meeting or not APS criteria, persistent LAC, double or triple aPL positivity) of any type was not associated to APO. As expected, APO group had more caesarean sections and SLE flares.

A baseline model including non-Caucasian ethnicity (OR 2.78; 95% CI [1.16–6.62]), smoking (OR 4.43; 95% CI [1.74–11.29]), pregestational hypertension (OR 16.13; 95% CI [4.06–64.02]), and pregestational corticosteroids treatment (OR 2.98; 95% CI [1.30–6.87]) yielded an AUC of 0.78 (95% CI, 0.70–0.86). Among the potential first-trimester laboratory predictors, only Pr/Cr ratio (log_{10}-trasnformed) (OR 1.92; 95% CI [1.13–3.26]); $p = 0.021$) significantly improved the model fit (R^2-Naelgerkerke 0.35 vs. 0.32; chi-square $p < 0.001$). However, the predictive performance was not significantly improved (AUC of 0.78 vs. 0.81; $p = 0.16$). Figure 1 shows the ROC curves. Calibration plots are shown as a Supplementary Material (Figure S1). The final multi-variable weighted models of predictors of adverse pregnancy outcomes are shown in Supplementary Material (Table S2). Details regarding the predictive performance of APO for fixed false-positive rate cut-offs are shown in Supplementary Material (Table S3). Interval validation step using bootstrapping to adjust for overfitting/optimism are also shown in Supplementary Material (Table S4).

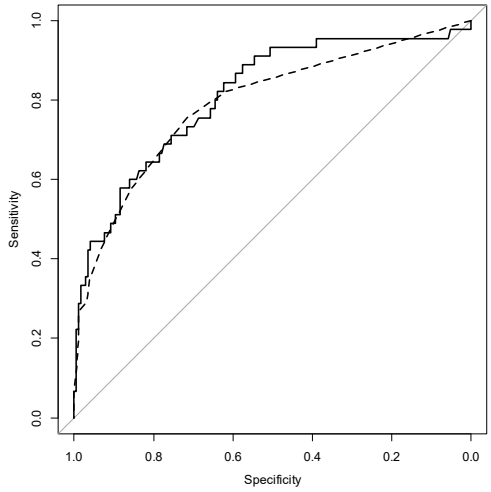

Figure 1. Comparison of ROC curves between the baseline model (dashed line) and the model including urinary protein (solid line).

4. Discussion

In this study, we profiled the demographic, clinical, and laboratory characteristics of women representing the clinical spectrum of women with autoimmune diseases attended in a referral unit. The status of the AID before or at the beginning of the pregnancy has proven to be a key aspect in order to obtain optimal pregnancy outcomes [13]. In this study, a predictive model of adverse pregnancy outcomes was developed in order to counsel women with SLE, APS, and NC-OAPS. For a screen positive rate of 50%, a baseline model that can be used even before pregnancy presents a detection rate of 83% for adverse outcome. This could be translated into the clinical practice by the tool being able to label 50% of the women with the more common autoimmune diseases as low-risk for adverse outcome (8%), while the other 50% of women labeled as high-risk would concentrate 83% of the adverse outcomes.

Regarding the demographic characteristics, only non-Caucasic ethnicity and smoking were significantly associated to adverse pregnancy outcomes. Similar were pregestational hypertension and corticosteroid treatment at the beginning of pregnancy, probably reflecting a non-stable disease before gestation. However, disease activity and its relation to poor outcomes was not reflected by LLDAS index or anti-dsDNA positivity. Only hypocomplementemia (C3 or C4) was associated to APO even though it was not finally included in the model. Similarly, Pr/Cr ratio at the 1st and 3rd trimesters was associated to APO, which might reflect some degree of kidney involvement, a marker of worse prognosis in women with SLE [29,30]. However, this parameter did not independently add predictive capacity to the baseline model.

Another finding to remark is the absence of association between any APO and the positivity of any of antiphospholipid antibodies regardless of whether they met the diagnostic criteria for APS or not. These results differ from other studies, in which lupus anticoagulant positivity was the main predictor of adverse pregnancy outcome in women with aPL [14,19]. GAPPS score was also explored as a marker of APO in order to examine possible shared pathophysiological mechanisms between thrombosis and APO, but no association was found. In our cohort, only 9.7% of women with SLE (+/− APS or aPL) developed a SLE flare during pregnancy or puerperium, which is inferior to the rates reported in previous studies [31]. This could be explained by the fact that most of the women presented a stable disease at time of conception, with a median SLEPDAI score of 2. Most of them had a pre-conception consultation with counseling on the risks of pregnancy and treatment optimization. Furthermore, the follow-up was carried out in a specialized consultation, where a specialist in maternal fetal medicine and a specialist in autoimmune diseases jointly saw the patient and made agreed therapeutic decisions. Altogether, our predictive models have a goodness-of-fit far from optimal (explaining only about one-third of the uncertainty to present an adverse pregnancy outcome), which highlights the need to explore new biomarkers that would help individualize the management of these pregnant women, thus improving their perinatal results.

It is worth mentioning the inclusion of women with NC-OAPS, who have been routinely excluded from studies despite representing approximately one-quarter of the pregnant women with aPL positivity referred to high-risk units. The inclusion of this group in our model enhances the external validity. In our cohort, 21% of women with NC-OAPS presented an APO, which agrees with data from previous studies and confirms that women with such autoimmune diseases still present worse perinatal results. Regarding study limitations, sample size is small due to the relatively low prevalence of AID in pregnant women. When the number of events is small, model overfitting can be a problem. An overfitted model tends to demonstrate poor predictive accuracy when applied to new data, but this small-events problem may also undermine external validation. Although we applied frequentist shrinkage methods to alleviate overfitting (penalized methods), we acknowledge that the small number of events in our cohorts may still result in an underpowered validation. It also worth mentioning that each one of the pregnancies from the recruited women were included and analyzed as independent events. However, only

twenty-nine women presented two episodes or pregnancies. No women with more than two pregnancies were included.

However, advances in management of AID have provided a better quality of life with a consequent increase in the number of pregnancies among these patients, a tendency that will continue to grow in the upcoming years.

5. Conclusions

To conclude, until new and better APO biomarkers are developed, a model including ethnicity, smoking status, pre-gestational hypertension, and corticosteroids treatment at the beginning of pregnancy could efficiently stratify pregnant women with the most common autoimmune diseases according to their risk for subsequently presenting an adverse pregnancy outcome.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11226822/s1, Figure S1. Calibration plot; Table S2. Final multivariable weighted models of predictors of adverse pregnancy outcomes; Table S3. Predictive performance (%) of adverse pregnancy outcome for fixed false-positive rate cut-offs; Table S4. Interval validation step using bootstrapping to adjust for overfitting/optimism.

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