The antiphospholipid syndrome – often overlooked cause of vascular occlusions?

E. Svenungsson & A. Antovic

From the Department of Medicine Solna, Division of Rheumatology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

Abstract. Svenungsson E, Antovic A (Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden). The antiphospholipid syndrome – often overlooked cause of vascular occlusions? (Review). J Intern Med 2020; 287: 349–372.

The antiphospholipid syndrome (APS) was fully recognized as a clinical entity in the early 1980s. Still, more than 30 years later, the epidemiology of APS is not well described, and furthermore, APS remains a challenge in terms of both diagnostic issues and clinical praxis involving a wide range of specialties. To date, there are no diagnostic criteria for APS. The present classification criteria rely on a combination of clinical manifestations and persistently positive tests for antiphospholipid antibodies (aPL). Clinical symptoms comprise vascular thrombosis, which can affect any vascular bed, including venous, microvascular and arterial vessels, and a set of pregnancy morbidities including early and late miscarriages, foetal death and preeclampsia. APS is more frequent among patients with other autoimmune diseases, and it is especially common in systemic lupus erythematosus (SLE). Importantly, APS symptoms can present in almost any medical specialty, but general knowledge and most previous clinical studies have essentially been confined to haematology, rheumatology and obstetrics/gynaecology. However, recent data demonstrate a relatively high prevalence of aPL also in patients from the general population who suffer from vascular occlusions or pregnancy complications. It is important that these patients are recognized by the general health care since APS is a treatable condition. This review aims to summarize the present knowledge on the history, pathogenesis, clinical manifestations and treatment of APS in order to urge a wide range of clinicians to consider comprehensive assessment of all patients where the diagnosis APS may be conceivable.

Keywords: antiphospholipid syndrome, antiphospholipid antibodies, thrombosis, pregnancy morbidity, anticardiolipin, anti-β2-glycoprotein I, venous thromboembolism, stroke, myocardial infarction.

Abbreviations: aCL, anticardiolipin antibodies; AnxA V, Annexin A V; aPL, antiphospholipid antibodies; APL, IgA antiphospholipid units/mL (APL, according to Harris standard); APLN, antiphospholipid antibody associated nephropathy; APS, antiphospholipid syndrome; aPS/PT, antiphosphatidylserine/antiprothrombin antibodies; aPTT, activated partial thromboplastin time, a test for lupus anticoagulant; C, complement factor; CAPS, catastrophic antiphospholipid syndrome, sometimes referred to as Asherson’s syndrome; CCP, complement control protein; DIC, disseminated intravascular coagulation; DOAC, direct oral anticoagulant drug; dRVVT, diluted Russell viper venom test, a test for lupus anticoagulant; DVT, deep venous thrombosis; ELISA, enzyme-linked immunosorbent assays; EULAR, European League Against Rheumatism; GPL, IgG antiphospholipid units/mL, according to Harris standard; HELLP, hemolysis, elevated liver enzymes and low platelet count; HIT, heparin induced thrombocytopenia; HLA, human leukocyte antigen; Ig, immunoglobulin; INR, international normalized ratio; LA, lupus anticoagulant; LDA, Low dose aspirin; LMWH, Low Molecular Weight Heparin; LPS, lipopolysaccharide; MI, myocardial infarction; MP, microparticles; MPL, IgM antiphospholipid units/mL, according to Harris standard; pAPS, primary antiphospholipid syndrome, APS without any other autoimmune disease; PE, pulmonary embolism; SLE, systemic lupus erythematosus; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura; VKA, vitamin K antagonists; VTE, venous thromboembolism including deep venous thrombosis and pulmonary embolism; β2GPI, β2glycoprotein I.
History

The diagnostic history of aPL/APS starts in 1906 with Wasserman’s test for syphilis, where cardiolipin, extracted from bovine muscle or heart, is used as antigen (Fig. 1). Wasserman’s test is however not specific for syphilis, and it was soon noted that also patients with SLE, malaria or tuberculosis could have positive test results [1]. The in vitro anticoagulant phenomenon, later referred to as the lupus anticoagulant (LA), was first recognized as a clotting inhibitor (circulating anticoagulant) in two patients with SLE by Conley and Hartmann in 1952 [2]. These initial patients presented with bleeding, and both were diagnosed with false-positive Wasserman’s test. A circulating anticoagulant antibody was described by Laurell and Nilsson in 1957 [3], and the name lupus anticoagulant was introduced by Feinstein in 1972 [4]. It is in several ways a misnomer since most LA positive patients do not have lupus and follow-up studies have surprisingly observed that patients positive in the LA test, primarily suffer from enhanced risk of thrombosis and not bleeding [5]. In 1975, Nilsson et al first reported the association between LA and recurrent abortions [6].

The combination of a positive LA test together with recurrent thromboses and foetal loss was established by several groups in the early 1980s [7, 8]. Harris et al demonstrated in 1983 that the commonly observed overlap between a positive test for syphilis and a positive LA test could be explained by antibodies targeting the negatively charged phospholipid and cardiolipin, which is used as antigen in the syphilis test, thus explaining the previous observations with false-positive tests for syphilis among patients with autoimmunity. They developed a solid-phase assay to detect antcardiolipin antibodies (aCL) and could through use of this assay identify a subset of SLE patients with LA positivity and previous venous and/or arterial thrombosis [9]. Based on this test, the syndrome was originally named the Anticardiolipin syndrome [10] and later changed to the Antiphospholipid syndrome (APS) [11].

In 1990, a major step forward was concurrently taken by three independent groups when they described the necessity of a protein cofactor for the binding of aCL to cardiolipin on the enzyme-linked immunosorbent assays (ELISA). The cofactor was identified as β2glycoprotein I (β2GPI), an abundant 50-KDa plasma glycoprotein [12–14]. β2GPI readily binds to phospholipids and functions as a scavenger molecule. It is today believed to be the major antigen in APS [15].

In 1992, Asherson described the most severe form of APS, the catastrophic APS (CAPS), an acute life-threatening, rapidly progressing disorder of multiple thromboses often affecting micro vessels in vital organs [16].

The first classification criteria for definite APS were published in 1999 by an expert workshop in Sapporo, the Sapporo criteria [17]. These criteria were later revised to their current status in Sydney 2006 [18].

During the last 10 years, several high-risk aPL profiles have been identified, especially triple aPL positivity [19, 20], LA positivity [21] and

Fig. 1  Timeline, illustrating important discoveries contributing to the diagnosis of APS.
antibodies targeting the tail, Domain1, of the β2GP1 molecule [22, 23]. These antibody profiles have all been associated with higher risks of both thrombosis and pregnancy complications.

Present definition

The Sydney classification criteria are constructed for research categorization, and though often used as such they should not be regarded as strict diagnostic criteria. According to them, APS is present when persistently positive aPL occur together with a set of defined clinical manifestations (Box 1 for facts) [18]. Persistent aPL positivity is defined as repeatedly positive tests ≥ 12 weeks apart. Five tests, the LA, aCL IgG or IgM and αβ2GP1 IgG or IgM are included in the Sydney criteria. Clinical manifestations include venous, arterial or microvascular thrombosis and/or obstetric morbidities. Finally, not more than 5 years should elapse between the testing and the clinical event. See Box 1 for more detailed definition.

Epidemiology

Reliable epidemiological studies regarding aPL and APS are still essentially lacking. Testing for aPL is unfortunately not common practice for any of the clinical manifestations included in the criteria, possibly with the exclusion of pregnancy morbidities. Moreover, assays for testing have for many years not been well standardized and large cohort studies have, with few exceptions, not been performed in a structured way.

In a very recent population-based study, the incidence of APS was estimated to be 2.1/100 000 among persons >18 years old. Estimated prevalence was 50/100 000. Interestingly, frequencies were similar for both sexes in this study [24], contrasting to most previous studies where females are usually more frequent. Based on a recent meta-analyses, the prevalence of aPL has been estimated to be approximately 10% in venous thromboembolism, 14% in ischaemic stroke, 11% in myocardial infarction and 6% in women with pregnancy morbidity [25]. However, these figures include studies with severe shortcomings, such as older, nonstandardized laboratory methods, and in a majority of these studies, aPL testing was only performed once.

Frequencies of aPL in the general population are also uncertain; by definition, the cut-off for positivity is set at the 99% of the local general population or at medium/high levels, defined as ≥40 GPL/MPL for aCL [18]. The GPL/MPL units depend on the Harris standard [26], but when possible, it is recommended to use the 99% cut-off [27].

Occurrences between 1% and 5% have been reported, with higher frequencies among patients with older age, infections, malignancies, chronic inflammatory and autoimmune diseases, especially SLE [28].

However, in many of these settings, such as infections, aPL are thought to be transient and assumed not to be associated with clinical events. In other autoimmune diseases, especially in SLE, aPL are more common and persistent; thus, 30-50% of SLE patients are reported to be positive for at least one of the aPL tests [28, 29].
To summarize, there is a clear need for new and representative epidemiologic studies on the occurrence of both aPL and APS.

Clinical manifestations

The major clinical manifestations of APS, venous thromboembolism (VTE), stroke, myocardial infarction (MI) and pregnancy complications are common in the general population, but despite estimated aPL frequencies that generally exceed 10% for the most common atherothrombotic manifestations [25], aPL are not part of general screening guidelines for cardiovascular risk factors. To date, the largest cross-sectional study on APS patients is the Euro-Phospholipid Project Group which reported in 2002 on clinical and serologic manifestations in 1000 APS patients fulfilling the Sapporo criteria [17] from 50 tertiary hospitals in 13 countries. In this study, 82% were females and the average age at first APS symptom was 34 years. Of the ‘criteria manifestations’, the most common initial symptoms were VTE 41%, comprising deep venous thromboses (DVT) and pulmonary embolism (PE), followed by stroke 13%, foetal loss 8%, skin ulcers 4% and myocardial infarction 3%. Approximately half, 53%, were primary (p)APS (i.e. APS without any other autoimmune disease), and 41% were associated with SLE or SLE-like disease [30]. During 10-year follow-up, 9% died at mean age of 59 years, and it was apparent that arterial events prevailed over venous during the follow-up period [31], possibly indicating that standard APS treatment with warfarin gives better protection from venous than arterial events.

Venous thromboembolism

Venous thromboembolism, including DVT and PE, is the most common manifestation of APS, and it is often also the first symptom. A recent meta-analysis estimated that on average 10% of DVTs are positive for aPL [25], although both higher [32] and lower figures have been reported [33]. The risk for VTE rises when several aPL tests are positive and especially if one of them is the LA [33]. Furthermore, in comparison with aPL negative patients, aPL-positive patients with VTE and positive aCL were at high risk for mortality during four years of follow-up and the risk for recurrent thrombosis was also elevated, especially shortly after warfarin withdrawal [32].

To what extent aPL precede and predict VTE in the general population is still controversial. An early study within the physicians’ health study demonstrated a positive association [34], which was not confirmed in a large population-based health study from Norway [35]. It is likely that differences with regard to nonstandardized tests and patient selections can explain these discrepant results.

Arterial thrombosis

There are numerous reports on positive associations between ischaemic arterial events and aPL [36–43], but also some negative [44–46]. It is furthermore clear that there is an interplay with the occurrence of traditional CVD risk factors such as age, gender, smoking, hyperlipidemia, diabetes and hypertension. Oral oestrogens and smoking have in particular been demonstrated to add additional risk for vascular events in patients with aPL [39, 47].

Of arterial occlusions, stroke is most commonly reported, an association already observed in the first publications on the syndrome [8]. Over the years, studies on the contribution of aPL to the risk of ischaemic strokes in the general population are not fully consistent, some could not confirm an independent association [46], while others did so [36, 39, 43]. In general, associations have been more convincing among younger patients [43] and patients with SLE. Urbanus et al demonstrated a strong positive association between LA positivity and previous strokes among women who were <50 years old. Ethnic differences may also play a role, and it has been noted that strokes are especially frequent in APS patients from Asia. In a single-centre Japanese cohort, 61% of APS cases presented with cerebral infarction [48].

With regard to strokes, it is important to remember that valvular heart disease is one of the more common ‘non-criteria’ manifestations associated with aPL [49] and simultaneously an important risk factor for thromboembolic stroke.

Myocardial infarctions (MIs) have been less frequent in cross-sectional APS studies, and MI has therefore not been regarded as a common manifestation of APS. Previous studies have usually been focused on younger patients with MI, and they report conflicting results. In an early study, Hamsten et al investigated men with MI who were <45 years old at 3, 12 and 36 months post-MI [50]. They reported elevated levels of IgG aCL on two or more occasions in 21%, and they also observed...
that aCL positivity was a risk factor for new cardiovascular events. Meroni et al detected aβ2GPI positivity (either IgG and/or IgM) 3–12 months after MI in 14.5% of 172 women <45 years old as compared to 1% in controls [38]. Urbanus et al investigated 203 women (<50 years) several years after a first MI. LA positivity was present in 3% of patients, versus 0.6% in controls, whereas anti-CL or anti-β2GPI did not differ between cases with MI and controls [39]. We recently reported that 11% of 805 consecutive patients with a with first-time MI, <75 years old, were positive for aPL (aCL and/or anti-β2GPI) of the IgG, but not IgM or IgA, isotype. In contrast only 1% of 805 matched controls were aPL IgG positive [37]. A recent meta-analysis also found that patients with coronary artery disease and IgG aPL are at higher risk of recurrent events during 2 years of follow-up, supporting a role for IgG aPL [51].

Peripheral arterial disease is less studied with respect to aPL, but there are several reports of positive associations both in the general population [52, 53] and among patients with SLE, especially when peripheral arterial disease occurs at a younger age [54]. aPL positivity was also predictive of a more severe prognosis considering outcomes such as critical limb ischaemia and mortality [52, 53, 55].

**Microvascular thrombosis**

Small vessel occlusions have gained less attention as manifestations of APS. They are often part of the severe rapidly progressive catastrophic APS (CAPS), but may also occur isolated and in more chronic presentations. To diagnose these conditions, biopsies are usually required.

Kidneys and skin are often affected, two locations where it is also possible to perform biopsies.

In the kidneys, aPL-associated nephropathy (APLN) comprises acute conditions such as thrombotic microangiopathy (TMA) and more chronic lesions with vascular damage that are often difficult to delineate from kidney damage caused by hypertension. APLN was recently reviewed [56], and notably, this condition is similar in SLE and pAPS, but cannot from the clinical presentation be distinguished from lupus nephritis, unless a biopsy is performed. Tektonidou et al demonstrated that these lesions are common in SLE patients with aPL and that they are associated with hypertension and impaired renal function [57]. Similar observations were observed in 150 SLE patients from Thailand and in 162 Mexican SLE patients who had been subject to renal biopsies [58, 59]. In our SLE cohort in Stockholm, APLN was present in 14% of all renal biopsies from SLE patients presenting with signs of renal involvement. In half of them, 7%, APLN occurred together with lupus nephritis, but in the other 7%, APLN occurred isolated [60].

In the skin, microvascular thrombosis may cause ulcerations, gangrenes and subungual splinter haemorrhages and thrombophlebitis [61].

Other small vessel occlusions are difficult to diagnose by biopsy, including osteonecrosis, hearing loss and a variety of brain syndromes. These manifestations may be underdiagnosed, and, though difficult to prove, may also constitute microvascular manifestations of APS.

**The catastrophic antiphospholipid syndrome (CAPS)**

The CAPS constitutes < 1% of all APS cases. It was initially described by Ronald Asherson in 1992 [16] and is sometimes referred to as Asherson’s syndrome. CAPS is a dramatic severe ‘thrombotic storm’, with thrombotic lesions in multiple locations occurring within a short period of time. International consensus criteria from 2003 [62] state that for classification of a definite CAPS diagnosis, four criteria have to be fulfilled; (i) involvement of three or more organ systems, (ii) presentation within a week, (iii) biopsy confirming small vessel occlusion in at least one organ and (iv) aPL positivity. Patients may however present with acute APS-related symptoms, but do not fulfil all four criteria. In these cases, they should, depending on symptoms, be considered as probable CAPS and be treated accordingly (Box 2, for more details).

Small vessel occlusions dominate the clinical CAPS picture, but thromboses in large vessels may also occur. A major source of knowledge about CAPS comes from the CAPS registry, an online registry which has collected more than 500 international case reports. Information on initiating and descriptive factors, treatments and outcomes is collected in this registry (https://ontocrf.grupocostaisa.com/web/caps), and all who care for these patients are asked to report their cases. The last updated cumulative data state that the average age at diagnosis is 38 years and 69% are female. The most common
organs to be affected are kidneys (73%), lungs (60%), brain (56%), heart (50%) and skin (47%). Mortality rates have declined over the years but are still very high, 37%. A key observation is that precipitating factors, ‘a second hit’, especially infections (49%), but also surgery (17%), malignancies (16%), contraceptives (10%) and pregnancy-related complications (8%), precede CAPS in a majority of cases. Patients with autoimmune diseases, especially SLE, were overrepresented (40%) and these patients had a more severe prognosis [63].

Importantly, all CAPS patients should be subject to intensive multidisciplinary care. Presentation is often within emergency medicine, and it is important to exclude other conditions with similar symptoms such as infections and sepsis with associated disseminated intravascular coagulation (DIC), heparin-induced thrombocytopenia (HIT), thrombotic thrombocytopenic purpura (TTP) and haemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome during pregnancy [64].

Obstetric manifestations

The obstetric subset of APS is defined by persistent positivity for aPL together with either early recurrent pregnancy loss, early foetal death, still birth or premature birth prior to the 34th gestational weeks due to preeclampsia, eclampsia and placental insufficiency. Thus, obstetric APS is recognized as the most frequent acquired risk factor for a treatable cause of recurrent pregnancy loss and represents an important health burden for women of childbearing age [65].

Risk for adverse pregnancy outcomes described in the classification criteria is highest in patients with isolated positive LA [21, 66] and in patients having simultaneous presence of aCL, αβ2GPI antibodies and LA, that is ‘triple positivity’ [21, 66–70]. Additionally, ethnicity and clinical features such as a concomitant SLE diagnosis [66, 71] and history of vascular thrombosis [68, 72] are associated with a higher risk for pregnancy morbidity in women with APS. In the prospective PROMISSE study comprising 385 SLE patients, adverse pregnancy outcome affected 19%, with an enhanced risk for women who were LA positive at baseline, OR 8.3 (95% CI 3.6–19.3) [21]. In the cohort of 30 patients with primary APS, we observed that women with obstetric APS had higher risk for adverse pregnancy outcomes during the next pregnancy compared to women with thrombotic APS, with highest risk among triple positive patients, despite anticoagulant treatment [73].

APS in SLE

aPL and APS are maybe best studied among patients with SLE. Approximately 30–50 % are positive for at least one aPL test, while around half of these 15 % develop APS according to classification criteria.

Prospective studies demonstrate that aPL predict a more severe prognosis among SLE patients, for example higher rates of mortality [74, 75], clinical APS manifestations such as arterial [76–79] and venous thrombosis [80] and pregnancy morbidity [21, 81]. Heart valve disease is common in SLE and strongly associated with aPL, especially with LA positivity [82]. There are also numerous reports that the aPL-positive subgroup in SLE is affected

| Clinical symptoms | Laboratory/histopathological findings |
|-------------------|--------------------------------------|
| 1 Involvement of 3 organ systems/tissues | 3 Laboratory confirmation |
| 2 Manifestations within 7 days | Positive test for LA, aCL or anti-β2GPI (IgG or IgM) and confirmed within 6 weeks |
| 3 Laboratory confirmation | 4 Histopathological confirmation |
| 4 Histopathological confirmation | Small vessel occlusion (thrombosis) in at least one tissue |

Definite CAPS: All 4 criteria

Probable CAPS:
(1) All 4 criteria but only two organs involved
(2) Confirmation of aPL not possible due to death
(3) Criteria 1, 2 and 4
(4) Criteria 1, 3 and 4, and 3rd event within 1 month, despite anticoagulation

Box 2 Diagnosis of the catastrophic antiphospholipid syndrome (CAPS)
with more severe organ damage, with significant contributions from the vascular and neurological domains [83–85].

Noncriteria manifestations

Heart valves

Heart valve disease, including Libman-Sacks endocarditis [86], is common in SLE and there is convincing evidence for an association with aPL. In a large meta-analysis comprising 23 studies, Zuily et al demonstrated that positive aPL, in particular LA positivity, was 3–5.5 times more common in 508 SLE patients with than in 988 without heart valve involvement [82]. Similar findings were reported by Farzaneh-Far who also demonstrated that mitral valve nodules and mitral regurgitation was the most common valve findings in aPL positive SLE patients [49]. Turiel et al performed a prospective transesophageal study in 56 patients with pAPS. Mitral valve thickening occurred in more than half and embolic sources were detected in 25%. Both were associated with high aCL IgG titres, and the majority remained and were predictive of new thrombotic events in the five-year follow-up examination [87, 88]. Taken together, aPL+/APS patients should be routinely investigated for heart murmurs. Further investigation with echocardiography is indicated in all who have abnormal auscultatory findings or arterial occlusions.

Neurologic manifestations

In addition to stroke, many other neurological symptoms have been linked to aPL/APS including cognitive disorders, seizures, migraines, chorea and transverse myelitis, as recently reviewed by Graf [89].

Cognitive dysfunction and dementia have been reported in aPL-positive patients both with and without SLE, positive associations with aCL IgG, livedo reticularis and white matter lesions on magnetic resonance imaging (MRI) were observed [90, 91]. In a recent study, using functional MRI, enhanced cortical activation in the frontal lobes was observed in aPL positive patients during undertakings involving working memory and executive tasks [92].

Headaches and migraine, though often inconsistently defined, are reported in 20–40% of patients with aPL/APS. Conversely, Cavestro et al found that 12% of 284 patients with migraine were positive for aPL, as compared to 3% of controls. Interestingly, there are many case reports where headaches have promptly disappeared following anticoagulant therapy with both heparin and warfarin [93, 94].

Autoimmune mechanisms have recently been more tightly associated with epilepsy/seizures and both APS and SLE were associated with epilepsy in a large epidemiological registry study [95]. Seizures occur in approximately 5–12% of patients with SLE, and they are consistently included as part of the immunologic criteria in SLE [96–98]. aPL and APS are overrepresented among SLE patients with seizures according to several studies are [98–100]. Positive aCL IgG titres were also recently reported in patients from the general population with severe refractory focal epilepsy [101].

Fig. 2 Livedo reticularis is often, but not exclusively, associated with antiphospholipid antibodies. Pictures are published with the permission from the patients.
Skin

Livedo reticularis/racemosa (Fig. 2) are a characteristic, but nonspecific, cutaneous manifestations present in around 25% of APS patients. They manifest as stippled red or bluish discoloration of the skin with a net-like pattern. Livedo often affects the upper and lower extremities (cutis marmorata), while in pathologic cases, livedo can involve the skin of the trunk and buttocks. A coarser more irregular and often more widespread similar pattern is referred to as livedo racemosa [61]. In 1965, before the recognition of APS as a clinical entity, Sneddon described the association between livedo reticularis and cerebrovascular lesions, referred to as Sneddon’s syndrome [102]. Approximately half of these patients are aPL positive [61], and in general, livedo reticularis/racemosa are strongly associated with the arterial and microangiopathic subtypes of APS [61, 103].

Skin ulcerations were observed in 5.5% of the 1000 European patients with APS, as a presenting manifestation in 3.9% [30]. While postphlebitic ulcers usually occur in patients with recurrent phlebitis of the leg after many years of follow-up, ulcerations resulting from circumscribed skin necrosis are frequently a presenting feature of APS. These ulcers are painful, 0.5–3 cm in diameter and develop commonly around the ankles or on the feet, often preceded by necrotizing purpura and/or livedo reticularis of the legs.

Digital gangrene is also an important and dangerous manifestation of APS, presented in around 3.3–7.5% of APS cases [30, 95].

Antigens in Clinical Practice

\[ \text{\( \beta_2 \) Glycoprotein-1 (\( \beta_2 \)GPI) } \]

- Also called apolipoprotein H
- Synthesized mainly by hepatocytes
- Abundant plasma protein
- Scavenger molecule binding to phospholipids
- Reduced (circular) and oxidized (J-shaped) conformational state

\[ \text{Cardiolipin (CL) } \]

- Negatively charged phospholipid
- Synthesized in inner mitochondria membrane
- A major constituent of inner mitochondrial membrane
- Also common in bacterial membranes
- Role in electron transport chain and ATP production
- Role in cytochrome C release and induction of apoptosis
- Conserves mitochondrial pH by trapping protons

Fig. 3 \( \beta_2 \)Glycoprotein-1 and Cardiolipin are the two specific antigens which are used in clinical practice today, both are also included in the ‘Sidney APS criteria’.
Antigens, antibodies and laboratory tests

The antigens

As described above, cardiolipin was the first antigen used in specific aPL assays (Fig. 3). Cardiolipin is a universal component of mitochondria in all eukaryotes, but it can also be found in some bacterial membranes. It is a dimeric phospholipid which is a major constituent (20%) of the inner mitochondria membrane. Cardiolipin is important for the respiratory chain in mitochondria, and it is especially plentiful in metabolically active cells, such as myocytes in the heart and muscles [104].

β2GPI, also known as apolipoprotein H, is now believed to be the major antigen in APS (Fig. 3). It is an abundant, evolutionally well-preserved protein that was first described in 1961. β2GPI is primarily synthesized by hepatocytes. Structurally, it belongs to the complement control protein (CCP) superfamily. In similarity to the proteins in this family, β2GPI is composed of repeating protein stretches (domains). β2GPI is made up of five domains of about 60 amino acids, (domains I–V, Fig. 3). Normally, β2GPI circulates in the blood in a closed circular conformation, but it is known that during certain conditions such as systemic inflammation, the molecule ‘opens up’ to a J shape, thus exposing otherwise cryptic epitopes, primarily on Domain I, which are known targets for anti-β2GPI antibodies [15].

β2GPI is primarily synthesized by hepatocytes. Structurally, it belongs to the complement control protein (CCP) superfamily. In similarity to the proteins in this family, β2GPI is composed of repeating protein stretches (domains). β2GPI is made up of five domains of about 60 amino acids, (domains I–V, Fig. 3). Normally, β2GPI circulates in the blood in a closed circular conformation, but it is known that during certain conditions such as systemic inflammation, the molecule ‘opens up’ to a J shape, thus exposing otherwise cryptic epitopes, primarily on Domain I, which are known targets for anti-β2GPI antibodies [15].

The function of β2GPI has for many years been an enigma. Genetic deficiency of β2GPI is not associated with any particular symptoms. Mounting evidence now indicate that β2GPI plays an important role as a scavenger molecule, removing bacteria, microparticles, dead and dying cells from the circulation [105, 106].

The LA test is functional and not dependent on a specific autoantibody. It is however widely accepted that it is primarily β2GPI antibodies together with antibodies targeting another phospholipid–protein complex, phosphatidylserine/prothrombin (aPS/PT), that are responsible for LA activity. Prothrombin is in similarity to β2GPI a phospholipid binding protein. Flecker et al described already in 1988 that antiprothrombin antibodies had lupus anticoagulant activity [107], and several studies thereafter have confirmed this observation [108, 109] and also linked aPS/PT antibodies to clinical symptoms of APS [110, 111]. Presently, there is an ongoing discussion on the clinical value of aPS/PT antibodies and if they should be included in APS criteria.

More than 20 other possible target proteins/phospholipids for ‘aPL’ have been described, including annexin V, annexin II, phosphatidylserine, thrombomodulin, antithrombin, protein C, protein S, oxidized low-density lipoproteins, tissue plasminogen...
activator, but the clinical significance of these antibodies have not been well studied.

Antibodies and laboratory tests

APS classification requires persistent positivity in at least one of three antiphospholipid tests: lupus anticoagulant (LA) and/or medium to high titres of IgG or IgM isotypes of anti-β2-glycoprotein-I antibodies (anti-β2GPI) and/or anticardiolipin (aCL) antibodies [18]. IgA antibodies can also be detected for each test, but are not available at all laboratories, and they are not part of the classification criteria for APS [18].

The term ‘LA’ stipulates an inhibitor of in vitro coagulation with unique features, as this inhibitor does not affect activities of individual coagulation factors and is seldom associated with bleeding. The phenomenon is caused by antibodies that interfere with the assembly of components of the coagulation cascade on a phospholipid surface, and adding excess phospholipids to the test system should neutralize the antibodies effect on clotting times. (Fig. 4) The laboratory assays currently used for assessment of LA do not meet the standards of good laboratory test practice, thus international guidelines for LA-testing have proposed that a combination of two tests is used [112]. Accordingly, LA is determined using two different principles. A sensitive activated partial thromboplastin time (aPTT) and the diluted Russell viper venom test (dRVVT). Both tests should be used simultaneously for detection of LA. If a test is prolonged, the sample is first mixed with the anticoagulant (LA) and/or medium to high titres of antibodies targeting Domain 1 of the β2GPI molecule, that is the nonphospholipid binding tail of the molecule, are more pathogenic [23, 116, 117], and these antibodies are also associated with the more severe ‘triple positive’ aPL pattern [22].

Positive LA tests are usually caused by anti-β2GPI or anti-PS/PT antibodies [107, 108, 110], but other antibodies targeting other proteins with affinity for anionic phospholipids and/or associated proteins have been demonstrated to contribute to positive LA tests [109]. LA caused by anti-β2GPI and LA associated with anti-PS/PT seem to identify two different subgroups of patients where anti-β2GPI-dependent LA appears to be characteristic of a subgroup with more pronounced thrombotic risk [118, 119].

Heparin, low-molecular-weight heparin (LMWH), warfarin and direct oral anticoagulants can interfere with LA testing [112, 120]. Therefore, testing for LA is not recommended in patients receiving these drugs, or should be performed with caution when necessary. In contrast, testing for aCL and anti-β2GPI antibodies is unaffected by anticoagulation therapy (Box 3).

What is the origin/triggering factors for the occurrence of aPL

Genetics

Reports of familial aggregation of aPL/APS appeared early [121], indicating that the genetic background plays role in the pathogenesis. Though large and representative genetic studies are still lacking, the human leucocyte antigens (HLA)-DR4 and DRw53 were recently considered to have prominent HLA associations in genetic studies of APS patients [122]. Other important HLA gene associations are HLA-DRB1*04, *07, *13, *09 and HLADQB1*0302, all have been associated with aPL production in APS patients of varying ethnicities, with or without SLE [122–124]. The number of complement factor (C)4A or C4B null alleles was also linked to aCL production in black American populations [125]. Islam et al performed a recent systematic review of genetic risk factors specifically for primary APS and reported 22 polymorphisms, 16 of which were associated with thrombotic APS and the majority of them were located along the coagulation and
polymorphism in domain V of \( \beta_2 \)GPI, causing a compositional protein change, exchanging valine for leucine at position 247, has also been associated with anti-\( \beta_2 \)GPI antibodies in patients of Asian origin [127, 128]. The \( \text{STAT4} \) risk allele for SLE and other autoimmune diseases was associated with aPL and ischaemic cerebrovascular lesions both in APS secondary to SLE and also in pAPS, [129, 130].

**Infections**

Infections and associated systemic inflammation are known 'second hits', which can trigger clinical APS events in aPL positive individuals. But, it is also known that infections can cause de novo production of aPL in previously aPL negative patients. Generally, these antibodies are assumed to be transient and not associated with clinical events. However, CAPS is commonly preceded by infections and this observation among others indicate that ‘infection-associated aPL’ may not always be as harmless as previously thought. These issues need to be addressed in large and representative longitudinal studies.

Molecular mimicry has been suggested as one possible mechanism behind the occurrence of aPL, Blank et al identified a hexa peptide (TLRVYK) in common bacteria (\( \text{Haemophilus influenzae} \) and \( \text{Neisseria gonorrhoeae} \)) which is recognized by anti-\( \beta_2 \)GPI antibodies. When mice were immunized with this peptide they developed antibodies, that after purification and concentration could cause APS like symptoms when transferred to previously healthy mice [131, 132].

**Smoking and environment**

A more or less convincing association between smoking and the occurrence of aPL has been demonstrated by several studies, in the general population [133]. We also observed a positive relationship in our studies of SLE patients, this was especially striking among patients who were former smokers and LA positivity/aPL of the IgG isotype [47]. In our study of SLE patients and in two studies investigating young women from the general population with vascular events, there was a higher risk of arterial events event among aPL positive women who were also smokers [38, 39].

**Malignancies**

There are a number of case reports on aPL/APS occurring in association with malignancies.
covering a broad range of different tumours including lymphomas, renal cell carcinoma and lung cancer [134]. It is thus important to remember that APS can be the first manifestation of a malignancy.

What do we know about mechanisms causing aPL-related symptoms

Underlying mechanisms that can explain the association between aPL and the clinical symptoms of APS are to date not well understood and there is also growing evidence that the pathogenesis of thromboses and pregnancy morbidities differ. Several possible mechanisms have been proposed, as summarized below.

Are aPL pathogenic??

Convincing evidence that aPL are pathogenic was presented already in 1990 by Branch et al who demonstrated that injection of purified IgG from women with obstetric APS caused foetal loss, necrosis with depositions of IgG and fibrin in the decidua of pregnant mice, while injection of IgG from women with uncomplicated pregnancies had no such effects [135].

Passive transfer of aPL antibodies can thus cause foetal loss in animals. On the other hand, it has been observed that the mere presence/transfer of aPL is not sufficient to cause thrombosis in experimental animals. However, if the animals are primed with either a vascular injury [136, 137] or with systemic inflammation, e.g. by prior injection of pro-inflammatory lipopolysaccharide (LPS) [138], clotting will occur after aPL transfer, and the thrombi will grow larger and dissolve slower than in the absence of aPL. Also in the clinic, several observations support that aPL in humans need a ‘second hit’ to become thrombogenic, most clearly demonstrated in the CAPS where infections, trauma, warfarin withdrawal or surgery are examples of triggering factors that precede CAPS in a majority of cases [63].

Obstetric APS versus thrombotic APS

There is a growing body of evidence regarding the mechanisms by which aPL induce a procoagulant state and abnormal cellular proliferation and differentiation in placental tissues causing the typical clinical features of APS. Early miscarriages are, based on animal studies, believed to be caused by implantation failure [139]. Late pregnancy complications, including pre-eclampsia, intrauterine growth restriction due to placenta insufficiency and still birth, are considered more specific for aPL/APS and are dependent on other mechanisms. These manifestations are generally believed to be caused by abnormal placentation and failure of the spiral arteries to adjust to the growing foetus leading to ischaemic injuries. \( \beta_2 \)GPI is synthesized by and abundantly expressed on placenta trophoblasts and maternal endothelial cells [140]. In vitro studies demonstrate that anti-\( \beta_2 \)GPI can interfere with angiogenesis and trophoblast migration [141, 142]. aPL can also activate complement, where the alternative complement pathway was recently demonstrated to be important for obstetric APS [143], leading to activation of the final complement pathway which results in a harmful local inflammation mediated through the recruitment of proinflammatory neutrophils and monocytes [143, 144].

Thus, the pathophysiological mechanisms behind the vascular and obstetric presentations of APS seem to differ. In thrombotic APS a triggering ‘second hit’ is crucial for thrombi to occur, while in obstetric APS the mere presence of aPL seem to suffice and impaired development of the placenta, rather than thrombosis, is believed to be the major pathophysiological problem. The inflammatory aspects of aPL-induced thrombotic complications are less studied and have mainly focused on complement activation.

The role of complement activation

Multiple studies point towards the importance of the complement system for development of both vascular and obstetric APS. The complement system is a part of the innate immune system composed of two activation pathways, the classical (along with the lectin) and the alternative, both resulting in generation of C5 convertase which activates component C5 into C5a, involved in chemotaxis, and C5b, that initiates the membrane attack on the surface of foreign and also endogenous cells such as endothelial cells [145].

Holers et al transferred human aPL into pregnant mice and they could demonstrate that complement activation is necessary for aPL to cause pregnancy loss. Importantly, the aPL injections did not lead to foetal loss or growth retardation when complement activation was blocked by either antibodies or genetically in mice knocked-out for C3 or C5 [146].
Studies in humans have reported abnormal plasma levels of the terminal C5b-9 complex, in APS patients with ischaemic stroke [147]. Further studies have reported signs of complement activation both in pAPS [148] and in patients with APS secondary to SLE [149]. There are also several reports that complement degradation products, C4d, are abundant on platelets and erythrocytes and associated with aPL/APS and thrombosis [150–153]. A recent proteomic study detected several complement products, among them the terminal C5b-9 complex, in clots formed in vitro from patients with APS [154]. Another important observation is that treatment with complement inhibitors have been successful in a number of severe APS/CAPS cases, as summarized by Chaturvedi et al. [155]. Despite promising case reports, no clinical trials have to date been performed using complement inhibition in APS.

The role of systemic inflammation and cellular activation

Several pathophysiological mechanisms may contribute to the procoagulant phenotype induced by aPL such as activation of platelets, monocytes, and endothelial cells as well as diminished function of natural anticoagulant and fibrinolytic systems. This has been described as the 'first hit', which leads to thrombosis only in the presence of a second provoking factor often also called the 'second hit'. The most important inciting factor ('second hit') is considered to be a systemic inflammatory process, serving as a necessary link between the aPL positive procoagulant phenotype and definite thrombus formation.

When aPL bind to platelets they induce platelet activation, leading to reduced platelet size and the formation of microparticles. Platelet activation is frequent in SLE and activated platelets provide a cellular surface where complement-fixing antibodies can bind [150, 156, 157]. Once activated the complement cascade will produce pro-inflammatory split products, C3a and C5a, which initiate chemotaxis and local inflammation, further promoting a pro-coagulant state through activation of platelets, monocytes and endothelial cells [158–160].

The adaptive immune system

The production of aPL is a key feature of APS, and β2GPI is believed to be the main antigen for these antibodies. β2GPI is known to bind to dead and dying cells as a scavenger molecule and experimental studies indicate that interactions between β2GPI and phospholipids on cellular debris can cause conformational changes in the β2GPI molecule, which seem to be important for the recognition by immune system and the production of autoantibodies. Many questions remain regarding the role of the adaptive immune system in APS. It is however clear that specific β2GPI reactive T cells are present in both APS and SLE [161, 162], and that they can, in an HLA dependent manner, provide help to autoantigen specific B-cells to produce αβ2GPI antibodies, as recently reviewed by Rauch et al. [163].

Disrupting the anti-coagulant Annexin V shield

Annexin AV (Anx-AV) is an abundant phospholipid-binding protein. Its high affinity for anionic phospholipids is used experimentally to detect apoptotic cells and microparticles that expose negatively charged phosphatidyl serine (PS) on the outer membrane. Under normal physiological circumstances Anx-AV forms a crystalline lattice that covers bare phospholipid surfaces. This is an important anti-coagulant mechanism which can be compared to a ‘band aid’ that protects anionic pro-coagulant phospholipids, e.g. PS, from contact with the circulating coagulation enzymes. Rand et al demonstrated that the Anx-AV content was markedly reduced on villi from women with obstetric APS and it has also been demonstrated that aPL can reduce Anx-AV binding on cultured trophoblasts and endothelial cells in vitro [164, 165]. This ability of aPL to disrupt the anticoagulant Anx-AV shield, is believed to produce gaps where thrombogenic anionic phospholipids are exposed to the circulating coagulation enzymes, thus facilitating activation of the coagulation cascade and thrombus formation [166, 167].

Microparticles

Microparticles (MPs) are small membrane surrounded structures that are released during cell activation or apoptosis through a blebbing process [168]. Though the technologies for measurement of particles are still not well standardized, MPs are more common in APS than in matched controls [169]. MPs can be released from any cell population and on their surface they commonly carry negatively charged PS, an eat me signal for phagocytes that normally quickly clear MPs from the circulation [170]. β2GPI, an abundant scavenger molecule in the circulation, and simultaneously the most
important antigen for aPL [15], is a ‘sticky protein’ that specifically binds to PS on MPs. In vitro studies demonstrate that that endothelial MPs can be released after stimulation by anti-β2GPI antibodies [171]. We recently noted that the subset of SLE patients that are positive for aPL/anti-β2GPI antibodies had, despite high numbers of total circulating MPs, lower concentrations of β2GPI-expressing MPs as compared both to aPL negative SLE patients and healthy controls [172]. These results imply that PS-β2GPI complexes, known to be upregulated in SLE MPs [173], could be hidden from the immune system on MP surfaces by the binding anti-β2GPI. We suggested that this ‘shielding’ could disturb important scavenging mechanisms and eventually lead to accumulation of circulating cellular debris, which may activate alternative clearance pathways, and also serve as antigen triggers for autoantibody production in predisposed individuals [172].

Clot Composition and fibrinolysis

Unfavourably altered fibrin morphology is proposed as a novel pro-thrombotic mechanism in the pathophysiology of APS. Fibrin clots made from plasma samples from APS patients are dense, composed of thin fibres and with small intrinsic pores and these are more difficult to lyse as demonstrated by Celinska-Lowenhoff [174] and confirmed in our group [175] (Fig. 5). Recently, the composition of fibrin clots from APS patients revealed the predominance of bone marrow proteoglycan, complement components C5-C9, immunoglobulins, apolipoprotein B-100, platelet-derived proteins, and thrombospondin-1 [154]. Increased levels of thrombin activatable fibrinolysis inhibitor in patients with APS and its relation to C5a inhibition could be additional mechanism contributing to prothrombotic state in APS, as postulated by our group [176].

Primary prevention in asymptomatic carriers of antiphospholipid antibodies

In a prospective study of triple-positive aPL carriers a 5.3% annual incidence of thromboembolism was observed, not significantly diminished using aspirin administered in a non-controlled manner [20]. In two meta-analyses by Arnaud et al. Low dose aspirin (LDA) protected from arterial but not venous events among asymptomatic aPL carriers [180]. Furthermore, treatment with low dose aspirin (LDA, 75–100 mg) is recommended to carriers with high risk aPL profiles according to EULAR recommendations [178]. In high-risk situations, such as surgery and hospitalization, prophylaxis with Low Molecular Weight Heparin (LMWH) or LDA may be considered. The use of oestrogen-containing oral contraceptives is a major risk factor for arterial thrombotic events in young women with aPL and should not be used and smoking should be strongly discouraged since it seems to potentiate the thrombotic risk in aPL positive patients.

Secondary prevention of VTE

Anticoagulant therapy reduces the risk of recurrent VTE in APS patients by 80–90% [181]. Patients with definite APS and a first VTE should be treated with vitamin K antagonists (VKA), targeting international normalized ratio (INR) ranging from 2.0 to 3.0 [178, 181]. Two randomized controlled trials compared higher intensity anticoagulation (INR target range of 3.1–4.0) to standard-intensity VKA but the higher intensity treatment did not lower the rate of recurrence [182, 183].

Due to the substantial and persistent risk of recurrent thrombosis, current recommendations recommend indefinite anticoagulation after the first aPL-associated VTE [178]. Still, if VTE occurs in a setting with a strong transient precipitating risk factor and if there is only a single positive aPL test, a limited duration of treatment can be considered [178, 181].

Secondary prevention of arterial events

In patients with definite APS and first arterial thrombosis long-term treatment with VKA is recommended targeting INR 2-3 or 3-4, depending on individual’s risk profile for recurrent thrombosis and bleeding risk [147]. Patients with arterial thrombosis due to APS have higher risk for
### Box 4 Summary of treatment guidelines

| Primary prevention | Secondary prevention venous thromboembolism |
|--------------------|---------------------------------------------|
| High risk aPL profile | ldASA |
| Unprovoked VTE | Long term treatment with vitamin K antagonists (VKA), INR target 2-3 |
| Provoked VTE and/or low risk profile | Shorter VKA treatment may be considered in selected cases |

#### Secondary prevention arterial events

| Ischemic stroke, MI or other arterial thrombosis | Long term VKA treatment with INR target 2-3, or INR 3-4, considering individual risks for e.g. bleeding |
| Thrombotic recurrence, arterial or venous, despite VKA treatment to target | Long term VKA treatment, increase INR target to 3-4, or add ldASA (75–100 mg) to VKA with INR 2-3, or change to LMWH |

#### Prevention during pregnancy

| Individualized treatment, depending on previous history and aPL profile. | Combination of ldASA (75–100 mg) and low molecular weight heparin (LMWH), dosages vary with previous risk profile, should be cared for at specialized maternity care units. |
| Should be monitored together with specialist in maternity care | Low dose steroids and hydroxychloroquine can be considered in difficult cases |

#### Post partum

| | Continue ldASA and LMWH for at least 6 weeks as thrombotic prevention |

VTE = venous thromboembolism, including venous thrombosis and pulmonary embolism, VKA = vitamin K antagonists, INR = international normalized ratio, MI = myocardial infarction, ld ASA = low-dose acetylsalicylic acid (75–100 mg), LMWH = low-molecular-weight heparin.

---

**Fig. 5** *Electron microscopy photography illustrating the fibrin clot density in a normal control subject and in a patient with APS.*
recurrence than patients with venous thrombosis [184]. However, data from previous studies showed that there was no statistically significant difference in thrombosis recurrences between treatment with VKA with a target INR of 3-4 and treatment with INR of 2-3 [178, 180, 185, 186]. Combined treatment with VKA with an INR target range of 2–3 and antiplatelet therapy with LDA can be considered as an alternative [147]. Interestingly a small recent retrospective study from Japan reported that dual antiplatelet therapy gave good protection from arterial events and no increase in adverse events as compared to warfarin [187]. However further studies are needed before such treatment can be applied in a larger scale.

**Recurrences, despite secondary prevention**

The recurrence of thrombotic manifestations was studied by Pengo et al. in triple positive patients demonstrating cumulative incidence of thromboembolic events of 12% after one year, 26% after five years and 44% after 10 years, despite ongoing anticoagulation [19]. There is limited evidence on the management of recurrent thrombosis in these patients. The common practice is to increase the INR target to 3-4, after evaluating the adherence to VKA treatment, or add LDA to the ongoing VKA treatment with target INR 2-3, or switching to LMWH [188]. In refractory cases, combining anticoagulation with immunosuppressive and alternative therapies can be considered (e. g. rituximab, hydroxychloroquine and statins) [189–192].

**CAPS Treatment**

For patients with CAPS, treatment should be carried out by an interdisciplinary team often with access to intensive care unit. The compiled data from the CAPS registry demonstrate the best outcome for patients who received a combined therapeutic approach, including anticoagulation, plasma exchange and/or intravenous immunoglobulin (IVIG) and glucocorticoids [63, 193–196]. It is also important to promptly treat/correct all triggering factors such as infections.

**Caution regarding treatment with direct oral anticoagulant drugs (DOACs)**

There is limited evidence about the effectiveness and safety of DOACs in patients with APS. In a first randomized clinical trial comparing rivaroxaban versus warfarin during 6 months in patients with venous thrombotic APS, there was no difference in outcomes for venous thrombosis [197], but this study was underpowered for clinical events. The TRAPS trial [198] of rivaroxaban versus warfarin in patients with APS and triple aPL positivity was prematurely terminated due to an excess of thromboembolic events (mostly arterial) in the rivaroxaban arm. Based on the trial rivaroxaban should not be used in triple aPL-positive patients with APS. In addition, an ongoing trial of apixaban in APS (Apixaban for the Secondary Prevention of Thromboembolism among patients with the Antiphospholipid Syndrome ((ASTRO-APS)) (ClinicalTrials.gov identifier: NCT02295475) was recently modified after evaluation of their initial data to exclude patients with arterial thrombosis. Based on these data and those from case series reporting arterial thrombosis recurrences in patients with APS treated with DOACs [199], use of DOACs are currently not recommended in patients with definite APS and arterial events.

DOACs may be considered in exceptional cases in patients with difficulty achieving a target INR of 2–3 despite compliance with VKA or in those who have contraindications to VKA. Importantly, switching from treatment with VKA to DOACs due to low adherence to VKA or INR monitoring should specifically be avoided, since adherence is even more important for DOAC therapy as compared to VKA.

**Complement inhibition**

Treatments targeting the complement pathways might be an attractive alternative for refractory APS patients. Until now, there are only case reports on the usage of eculizumab, a recombinant monoclonal antibody inhibiting activation of C5 in the terminal complement pathway in patients with thrombotic microangiopathy (TMA) due to APS or in patients with the CAPS [200–204]. The recent, so far largest, report of 9 cases with secondary TMA due to SLE/APS presents promising data on significant improvement in hematological outcomes and renal function following treatment with eculizumab [205]. Future clinical trials are necessary to explore the role of complement blocking therapies in APS.

**Management of obstetric APS**

Using combined treatment including LDA (75–100 mg day$^{-1}$) and LMWH or unfractionated heparin in prophylactic, intermediate or therapeutic
dose, depending on risk profile, has led to live births in 70% of women with previous aPL-related pregnancy complications [206]. Therefore, this treatment regimen is recommended as a current standard of care treatment in patients with obstetric APS [177, 178].

In severe cases with recurrent late pregnancy morbidity, despite treatment with LDA and LMWH, low dose steroids (10 mg Prednisolone) and hydroxychloroquine may be added [147]. In severe treatment-resistant cases intravenous gamma globulin (IvIg) or repeated plasma exchange/apheresis could, based on small case series with favourable outcomes, be considered [207].

After delivery, it is important to continue treatment with LMWH for 6–12 weeks to protect the mother from thrombotic events during this high-risk period.

Future perspectives
Recent studies demonstrate that aPL may be more common in the general population than previously appreciated. More reliable techniques are now available to measure aPL antibodies on a large scale and future studies should address their role as cardiovascular risk factors in unselected populations. Not all antibodies are associated with events and we need to learn which subset, levels or combinations of antibodies are risk factors for clinical symptoms.

It has also been assumed, though never properly studied, that transient antibodies commonly associated with infections, are not associated with risk of clinical APS manifestations. aPL positivity also seems to fluctuate in some individuals, especially among SLE patients, and we do not today know how to treat these patients.

In general, the role of aPL positivity in SLE deserves more attention. According to our results aPL positive SLE patients may constitute a distinct subset characterized not only by autoantibodies but also by distinct activated molecular pathways and biomarkers [208]. Genetic and other observations demonstrate that the aPL positive SLE subset shares many features with primary APS, including the same need for anticoagulant treatment.

Microangiopathic conditions affecting the kidneys occur both in SLE and in pAPS and these disorders are today a clinical challenge where treatment guidelines are lacking. Collaborative studies are needed to address what is the best treatment for these patients.

Furthermore, the underlying mechanisms in thrombotic and obstetric APS seem to differ and these differences need to be further described and understood.

There is also a number of aPL associated manifestations such as heart valve disease, aPL associated nephropathy, migraine, cognitive function where the connection to aPL/APS needs to be better described.

Anticoagulant therapy with VKA remains the conventional therapy in patients with thrombotic APS, whereas in obstetric APS the first line treatment is low-molecular-weight heparin in combination with low-dose aspirin. Initial studies report that DOACs are not effective treatment in high risk APS patients, but we need to understand why and to continue the search for better and safer treatments.

Conflict of interest
The authors declare that they have no conflict of interest.

References
1 Bialynicki-Birula R. The 100th anniversary of Wassermann-Neisser-Bruck reaction. Clin Dermatol. 2008; 26: 79–88.
2 Conley CL, Hartmann RC. A hemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. J Lab Clin Invest 1952; 31: 621–2.
3 Laurell AB, Nilsson IM. Hypergammaglobulinemia, circulating anticoagulant, and biologic false positive Wassermann reaction; a study in two cases. J Lab Clin Med 1957; 49: 694–707.
4 Feinstein DI, Rapaport SI. Acquired inhibitors of blood coagulation. Prog Hemost Thromb 1972; 1: 75–95.
5 Bowie EJ, Thompson JH Jr, Pascuzzi CA, Owen CA Jr. Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. J Lab Clin Med 1963; 62: 416–30.
6 Nilsson IM, Astedt B, Hedner U, Berezin D. Intrauterine death and circulating anticoagulant (antithromboplastin). Acta Med Scand 1975; 197: 153–9.
7 Carreras LO, Vermynen JG. “Lupus” anticoagulant and thrombosis—possible role of inhibition of prostacyclin formation. Thromb Haemost 1982; 48: 38–40.
8 Hughes G. Thrombosis, abortion, cerebral disease and the lupus antikoagulant. Br Med J 1983; 287: 1088–9.
The antiphospholipid syndrome / E. Svenungsson and A. Antovic

9 Harris EN, Gharavi AE, Boey ML et al. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983; 2: 1211–4.

10 Hughes GR, Harris NN, Gharavi AE. The anticardiolipin syndrome. J Rheumatol 1986; 13: 486–9.

11 Alarcón-Segovia D, Delezé M. Orias CV, al e. Antiphospholip antibodies and the antiphospholip syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. Medicine 1989; 68: 353–65.

12 Galli M, Comfurius P, Maassen C et al. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 1990; 335: 1544–7.

13 McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Antiphospholip antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 1990; 87: 4120–4.

14 Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Koike T. Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. Lancet 1990; 336: 177–8.

15 de Groot PG, Meijers JC. beta(2)-Glycoprotein I: evolution, structure and function. J Thromb Haemost 2011; 9: 1275–84.

16 Asherson RA. The catastrophic antiphospholipid syndrome. J Rheumatol 1992; 19: 508–12.

17 Wilson WA, Gharavi AE, Koike T et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arthritis Rheum 1999; 42: 1309–11.

18 Miyakik 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.

19 Pengo V, Ruffatti A, Legnani C et al. Clinical course of high-risk patients diagnosed with antiphospholipid syndrome. J Thromb Haemost 2010; 8: 237–42.

20 Pengo V, Ruffatti A, Legnani C et al. Incidence of a first thromboembolic event in asymptomatic carriers of high-risk antiphospholipid antibody profile: a multicenter prospective study. Blood 2011; 118: 4714–8.

21 Buyon JP, Kim MY, Guerra MM et al. Predictors of pregnancy outcomes in patients with lupus: a cohort study. Ann Intern Med 2015; 163: 153–63.

22 Pengo V, Ruffatti A, Tonello M et al. Antiphospholipid syndrome: antibodies to Domain I of beta2-glycoprotein 1 correctly classify patients at risk. J Thromb Haemost 2015; 13: 782–7.

23 de Laat B, Pengo V, Pabinger I et al. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. J Thromb Haemost 2009; 7: 1767–73.

24 Duarte-Garcia A, Pham MM, Crowson CS et al. The epidemiology of antiphospholipid syndrome. A population-based study. Arthritis Rheumatol 2019; 71: 1545–52.

25 Andreoli L, Chighizola CB, Banzato A, Pons-Estel GJ, Ramire de Jesus G, Erkan D. Estimated frequency of antiphospholip antibodies in patients with pregnancy morbidity, stroke, myocardial infarction, and deep vein thrombosis: a critical review of the literature. Arthritis Care Res (Hoboken) 2013; 65: 1869–73.

26 Pierangeli SS, Favaloro EJ, Lakos G et al. Standards and reference materials for the antcardiolipin and anti-beta2-glycoprotein I assays: a report of recommendations from the APL Task Force at the 13th International Congress on Antiphospholipid Antibodies. Clin Chim Acta 2012; 413: 1358–60.

27 Devreese KM, Pierangeli SS, de Laat B et al. Testing for antiphospholip antibodies with solid phase assays: guidance from the SSC of the ISTH. J Thromb Haemost 2014; 12: 792–5.

28 Petri M. Epidemiology of the antiphospholip antibody syndrome. J Autoimmun 2000; 15: 145–51.

29 Vikerfors A, Johansson AB, Gustafsson JT et al. Clinical manifestations and anti-phospholipid antibodies in 712 patients with systemic lupus erythematosus: evaluation of two diagnostic assays. Rheumatology 2013; 52: 501–9.

30 Cervera R, Piette JC, Font J et al. Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. Arthritis Rheum 2002; 46: 1019–27.

31 Cervera R, Serrano R, Pons-Estel GJ et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. Ann Rheum Dis 2015; 74: 1011–8.

32 Schulman S, Svenungsson E, Granqvist S. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. Am J Med 1998; 104: 332–8.

33 de Groot PG, Lutters B, Derksen RH, Lisman T, Meijers JC, Rosendaal FR. Lupus anticoagulants and the risk of a first episode of deep venous thrombosis. J Thromb Haemost 2005; 3: 1993–7.

34 Ginsburg KS, Liang MH, Newcomer L et al. Antiphospholipin antibodies and the Risk for Ischemic Stroke and Venous Thrombosis. Ann Intern Med 1992; 117: 997–1002.

35 Naess IA, Christiansen SC, Cannegieter SC, Rosendaal FR, Hammerstroem J. A prospective study of anticardiolipin antibodies as a risk factor for venous thrombosis in a general population (the HUNT study). J Thromb Haemost 2006; 4: 44–9.

36 Brey RL, Abbott RD, Curb JD et al. beta(2)-Glycoprotein 1-dependent anticardiolipin antibodies and risk of ischemic stroke and myocardial infarction: the honolulu heart program. Stroke 2001; 32: 1701–6.

37 Grosso G, Sippl N, Kjellstrom B et al. Antiphospholipid antibodies in patients with myocardial infarction. Ann Intern Med 2018; 170: 277.

38 Meroni PL, Peyvandi F, Foco L et al. Anti-beta 2 glycoprotein I antibodies and the risk of myocardial infarction in young premenopausal women. J Thromb Haemost 2007; 5: 2421–8.

39 Urbanus RT, Siegerink B, Roest M, Rosendaal FR, de Groot PG, Algra A. Antiphospholipid antibodies and risk of myocardial infarction and ischaemic stroke in young women in the RATIO study: a case-control study. Lancet Neurol 2009; 8: 998–1005.

40 Zuckerman E, Touibi E, Shiran A et al. Anticardiolipin antibodies and acute myocardial infarction in non-systemic lupus erythematosus patients: a controlled prospective study. Am J Med 1996; 103: 381–6.

41 Bili A, Moss AJ, Francis CW, Zareba W, Watelet LF, Sanz I. Anticardiolipin antibodies and recurrent coronary events: a prospective study of 1150 patients. Thrombogenic Factors,
and Recurrent Coronary Events Investigators. Circulation 2000; 102: 1258–63.
42 Tuhrim S, Rand JH, Wu XX et al. Elevated anticirodilin antibody titer is a stroke risk factor in a multietnic population independent of isotype or degree of positivity. Stroke 1999; 30: 1561–5.
43 Brey RL, Stallworth CL, McGlasson DL et al. Antiphospholipid antibodies and stroke in young women. Stroke 2002; 33: 2396–400.
44 Ahmed E, Stegmayr B, Trifunovic J, Weinehall L, Hallmans G. Antiphospholipid antibody in an unselected stroke population. Lancet 1994; 344: 452–6.
45 Ahmed E, Stegmayr B, Trifunovic J, Weinehall L, Hallmans G, Lefvert AK. Anticardiolipin antibodies are not an independent risk factor for stroke: an incident case-referent study nested within the MONICA and Vasterbotten cohort project. Stroke 2000; 31: 1289–93.
46 Gustafsson JT, Gunnarsson I, Kallberg H et al. Cigarette smoking, antiphospholipid antibodies and vascular events in Systemic Lupus Erythematosus. Ann Rheum Dis 2015; 74: 1537–43.
47 Sletnes KE, Smith P, Abdelnoor M, Arnesen H, Wisloff F. Antibodies to cardiolipin in young survivors of myocardial infarction: an association with recurrent cardiovascular events. A systematic review and Bayesian meta-regression analysis. Circulation 2014; 130: 1710–20.
48 Erdozain JG, Villar I, Nieto J, Ruiz-Arruzca I, Ruiz-Irastorza G. Predictors of peripheral arterial disease in SLE change with patient’s age. Lupus Sci Med 2017; 4: e000190.
49 Gavier B, Vazquez F, Gandara E. Antiphospholipid antibodies and lower extremity peripheral arterial disease – a systematic review and meta-analysis. Vasa 2016; 45: 325–30.
50 Tektonidou MG. Antiphospholipid syndrome nephropathy: from pathogenesis to treatment. Front Immunol 2018; 9: 1181.
51 Tektonidou MG, Sotsiou F, Nakopoulou L, Vlahoyianopoulos PG, Moutsopoulos HM. Antiphospholipid syndrome nephropathy in patients with systemic lupus erythematosus and antiphospholipid antibodies: prevalence, clinical associations, and long-term outcome. Arthritis Rheum 2004; 50: 2569–79.
52 Cheunsuchon B, Rungkaew P, Chawanasuntorapej R, Pattaragarn A, Parichatikanond P. Prevalence and clinicopathologic findings of antiphospholipid syndrome nephropathy in Thai systemic lupus erythematosus patients who underwent renal biopsies. Nephrol (Carlton) 2007; 12: 474–80.
53 Miranda JM, Jara LJ, Calleja C, Saavedra MA, Bustamante RM, Angeles U. Clinical significance of antiphospholipid syndrome nephropathy (APSN) in patients with systemic lupus erythematosus (SLE). Reumtol Clin 2009; 5: 209–13.
54 Gerhardsson S, Sundelin B, Zickert A, Padyukov L, Svenungsson E, Gunnarsson I. Histological antiphospholipid-associated nephropathy versus lupus nephritis in patients with systemic lupus erythematosus: an observational cross-sectional study with longitudinal follow-up. Arthritis Res Ther 2015; 17: 109.
55 Asherson RA, Cervera R, de Groot PG et al. Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. Lupus 2003; 12: 530–4.
56 Rodriguez-Pinto I, Moitinho M, Santacreu I et al. Catastrophic antiphospholipid syndrome (CAPS): Descriptive analysis of 500 patients from the International CAPS Registry. Autoimmun Rev 2016; 15: 1120–24.
57 Cervera R, Rodriguez-Pinto I, Colafrancesco S et al. 14th International congress on antiphospholipid antibodies task force report on catastrophic antiphospholipid syndrome. Autoimmun Rev 2014; 13: 699–707.
58 Schreiber K, Sciascia S, de Groot PG et al. Antiphospholipid syndrome. Nat Rev Dis Primers 2018; 4: 17103.
59 Abou-Nassar K, Carrier M, Ramsay T, Rodger MA. The association between antiphospholipid antibodies and placenta mediated complications: a systematic review and meta-analysis. Thromb Res 2011; 128: 77–85.
60 Chauveau C, Galanapau JD, Alonso S et al. Observational study of pregnant women with a previous spontaneous abortion before the 10th gestation week with and without antiphospholipid antibodies. J Thromb Haemost 2010; 8: 699–706.
61 Ruffatti A, Calligaro A, Hoxha A et al. Laboratory and clinical features of pregnant women with antiphospholipid syndrome and neonatal outcome. Arthritis Care Res (Hoboken) 2010; 62: 302–7.
62 Ruffatti A, Tonello M, Visentin MS et al. Risk factors for pregnancy failure in patients with anti-phospholipid syndrome treated with conventional therapies: a multicentre, case-control study. Rheumatology (Oxford) 2011; 50: 1684–9.
63 Stone S, Hunt BJ, Khamashta MA, Bewley SJ, Nelson-Piercy C. Primary antiphospholipid syndrome in pregnancy: an analysis of outcome in a cohort of 33 women treated with a rigorous protocol. J Thromb Haemost 2005; 3: 243–5.
64 Danowski A, de Azevedo MN, de Souza Papi JA, Petri M. Determinants of risk for venous and arterial thrombosis in primary antiphospholipid syndrome and in antiphospholipid syndrome with systemic lupus erythematosus. J Rheumatol 2009; 36: 1195–9.
65 Fredi M, Andreoli L, Aggogeri E et al. Risk factors for adverse maternal and fetal outcomes in women with confirmed aPL
The antiphospholipid syndrome / E. Svenungsson and A. Antovic

positivity: results from a multicenter study of 283 pregnancies. Front Immunol 2018; 9: 864.
73 Hogden A, Antovic A, Berg E, Bremke M, Chaireti R. Obstetric outcomes in patients with primary thrombotic and obstetric antiphospholipid syndrome and its relation to the antiphospholipid antibody profile. Lupus 2019; 28: 868–77.
74 Drenkard C, Villa AR, Alarcón-Segovia D, Pérez-Vázquez ME. Influence of the antiphospholipid syndrome in the survival of patients with systemic lupus erythematosus. J Rheumatol 1994; 21: 1067–72.
75 Gustafsson J, Simard JF, Gunnarsson I et al. Risk factors for cardiovascular mortality in patients with systemic lupus erythematosus, a prospective cohort study. Arthritis Res Ther 2012; 14: R46.
76 Bengtsson C, Ohman ML, Nived O, Rantapaa Dahlqvist S. Cardiovascular event in systemic lupus erythematosus in northern Sweden: incidence and predictors in a 7-year follow-up study. Lupus 2012; 21: 452–9.
77 Gustafsson J, Gunnarsson I, Borjesson O et al. Predictors of the first cardiovascular event in patients with systemic lupus erythematosus - a prospective cohort study. Arthritis Res Ther 2009; 11: R186.
78 Magder LS, Petri M. Incidence of and risk factors for adverse cardiovascular events among patients with systemic lupus erythematosus. Am J Epidemiol 2012; 176: 708–19.
79 Tolozza SM, Urbe AG, McGwin G Jr et al. Systemic lupus erythematosus in a multiethnic US cohort (LUMINA). XXIII. Baseline predictors of vascular events. Arthritis Rheum 2004; 50: 3947–57.
80 Calvo-Alen J, Tolozza SM, Fernandez M et al. Systemic lupus erythematosus in a multiethnic US cohort (LUMINA). XXV. Smoking, older age, disease activity, lupus anticoagulant, and glucocorticoid dose as risk factors for the occurrence of venous thrombosis in lupus patients. Arthritis Rheumatol 2005; 52: 2060–8.
81 Mankee A, Petri M, Magder LS. Lupus anticoagulant, disease activity and low complement in the first trimester are predictive of pregnancy loss. Lupus Sci Med 2015; 2: e000095.
82 Zuily S, Regnaut V, Selton-Suty C et al. Increased risk for heart valve disease associated with antiphospholipid antibodies in patients with systemic lupus erythematosus: meta-analysis of echocardiographic studies. Circulation 2011; 124: 215–24.
83 Artim-Esen B, Cene E, Sahinkaya Y et al. Cluster analysis of autoantibodies in 852 patients with systemic lupus erythematosus from a single center. J Rheumatol 2014; 41: 1304–10.
84 Petri M, Purvey S, Fang H, Magder LS. Predictors of organ damage in systemic lupus erythematosus: the Hopkins Lupus Cohort. Arthritis Rheum 2012; 64: 4021–8.
85 Taraborelli M, Leuenberger L, Lazzaroni MG et al. The contribution of antiphospholipid antibodies to organ damage in systemic lupus erythematosus. Lupus 2016; 25: 1365–8.
86 Libman E. A hitherto undescribed form of valvular and mural endocarditis. Arch Intern Med 1924; 33: 701–37.
87 Turiel M, Sarzi-Puttini P, Peretti R et al. Five-year follow-up by transesophageal echocardiographic studies in primary antiphospholipid syndrome. Am J Cardiol 2005; 96: 574–9.
88 Turiel M, Sarzi-Puttini P, Peretti R et al. Thrombotic risk factors in primary antiphospholipid syndrome: a 5-year prospective study. Stroke 2005; 36: 1490–4.
89 Graf J. Central nervous system manifestations of antiphospholipid syndrome. Rheum Dis Clin North Am 2017; 43: 547–60.
90 Hanly JG, Hong C, Smith S, Fisk JD. A prospective analysis of cognitive function and anticardiolipin antibodies in systemic lupus erythematosus. Arthritis Rheum 1999; 42: 728–34.
91 Tektonidou MG, Varsou N, Kotoulas G, Antoniou A, Moutsopoulos HM. Cognitive deficits in patients with antiphospholipid syndrome: association with clinical, laboratory, and brain magnetic resonance imaging findings. Arch Intern Med 2006; 166: 2278–84.
92 Kozora E, Ulug AM, Erkan D et al. Functional magnetic resonance imaging of working memory and executive dysfunction in systemic lupus erythematosus and antiphospholipid antibody-positive patients. Arthritis Care Res (Hoboken) 2016; 68: 1655–63.
93 Hughes GR, Cuadrado MJ, Khamashta MA, Sanna G. Headache and memory loss: rapid response to heparin in the antiphospholipid syndrome. Lupus 2001; 10: 778.
94 Noureddine MHA, Haydar AA, Berjawi A et al. Antiphospholipid syndrome (APS) revisited: Would migrain headaches be included in future classification criteria? Immunol Res 2017; 65: 230–41.
95 Ong MS, Kohane IS, Cai T, Gorman MP, Mandl KD. Population-level evidence for an autoimmune etiology of epilepsy. JAMA Neurol 2014; 71: 569–74.
96 Tan EM, Cohen AS, Fries JF et al. The 1982 revised criteria for classification of systemic lupus erythematosus. Arthritis Rheum 1982; 25: 1271–7.
97 Aringer M, Costenbader K, Dalich D et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. Ann Rheum Dis 2019; 78: 1400–12.
98 Hopia L, Andersson M, Svennungsson E, Khademi M, Piel F, Tomson T. Epilepsy in Systemic lupus erythematosus: Prevalence and risk factors. Eur J Neurol 2019; 26: 297–307.
99 Appenzeller S, Cendes F, Costallat LT. Epileptic seizures in systemic lupus erythematosus. Neurology 2004; 63: 1808–12.
100 Hanly JG, Urowitz MB, Su L et al. Seizure disorders in systemic lupus erythematosus results from an international, prospective, inception cohort study. Ann Rheum Dis 2012; 71: 1502–9.
101 Limatainen S, Peltola M, Fallah M, Kharazmi E, Haapala AM, Peltola J. The high prevalence of antiphospholipid antibodies in refractory focal epilepsy is related to recurrent seizures. Eur J Neurol 2009; 16: 134–41.
102 Sneddon IB. Cerebro-vascular lesions and livedo reticularis. Br J Dermatol 1965; 77: 180–5.
103 Frances C. Dermatological manifestations of Hughes’ antiphospholipid antibody syndrome. Lupus 2010; 19: 1071–7.
104 Ren M, Phoon CK, Schlame M. Metabolism and function of mitochondrial cardiolipin. Prog Lipid Res 2014; 55: 1–16.
105 Balasubramanian K, Schroit AJ. Characterization of phosphatidylinerine-dependent beta2-glycoprotein I macrophage interactions. Implications for apoptotic cell clearance by phagocytes. J Biol Chem 1998; 273: 29272–7.
106 Andreoli L, Fredi M, Nalli C, Franceschini F, Meroni PL, Tincani A. Antiphospholipid antibodies mediate autoimmunity against dying cells. *Autoimmunity* 2013; **46**:302–6.

107 Fleck RA, Rapaport SI, Rao LV. Anti-prothrombin antibodies and the lupus anticoagulant. *Blood* 1988; **72**:512–9.

108 Bevers EM, Galli M, Barbui T, Comfurius P, Zwaal RF. Lupus anticoagulant IgG’s (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb Haemost* 1991; **66**:629–32.

109 Oosting JD, Derksen RH, Bobbink IW, Hackeng TM, Bouma BN, de Groot PG. Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: an explanation for their pathogenic mechanism? *Blood* 1993; **81**:2618–25.

110 Atsumi T, Ieko M, Bertolaccini ML et al. Association of autoantibodies against the phosphatidyserine-prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. *Arthritis Rheum* 2000; **43**:1982–93.

111 Amengual O, Forastiero R, Sugiuira-Ogasawara M et al. Evaluation of phosphatidyserine-dependent antiprothrombin antibody testing for the diagnosis of antiphospholipid syndrome: results of an international multicentre study. *Lupus* 2017; **26**:266–76.

112 Pengo V, Tripodi A, Reber G et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost* 2009; **7**:1737–48.

113 Favalaro EJ, Wong RC, Silvestrini R, McEvoy R, Jovanovich S, Roberts-Thomson F. A multilaboratory peer assessment quality assurance program-based evaluation of anticardiolipin antibody, and beta2-glycoprotein I antibody testing. *Semin Thromb Hemost* 2005; **31**:73–84.

114 Matsuura E, Igarashi Y, Yasuda T, Triplett DA, Koike T. Anticardiolipin antibodies recognize beta 2-glycoprotein 1 structure altered by interacting with an oxygen modified solid phase surface. *J Exp Med* 1994; **179**:457–62.

115 de Groot PG, Urbanus RT. The significance of autoantibodies against beta2-glycoprotein I. *Blood* 2012; **120**:266–74.

116 de Laat B, Derksen RH, Urbanus RT, de Groot PG. IgG antibodies that recognize epitope Gly40-Arg43 in domain I of beta 2-glycoprotein I cause LAC, and their presence correlates strongly with thrombosis. *Blood* 2005; **105**:1540–5.

117 Rouby RA. Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulants and other "antiphospholipid" autoantibodies. *Blood* 1994; **84**:2854–67.

118 de Laat HB, Derksen RH, Urbanus RT, Roest M, de Groot PG. beta2-glycoprotein I-dependent lupus anticoagulant highly correlates with thrombosis in the antiphospholipid syndrome. *Blood* 2004; **104**:3598–602.

119 Pengo V, Del Ross T, Ruffatti A et al. Lupus anticoagulant identifies two distinct groups of patients with different antibody patterns. *Thromb Res* 2018; **172**:172–8.

120 Antovic A, Norberg EM, Berndtsson M et al. Effects of direct oral anticoagulants on lupus anticoagulant assays in a real-life setting. *Thromb Haemost* 2017; **117**:1700–4.

121 Dagenais P, Urowitz MB, Gladman DD, Norman CS. A family study of the antiphospholipid syndrome associated with other autoimmune diseases. *J Rheumatol* 1992; **19**:1393–6.

122 Sebastiani GD, Iuliano A, Cantarini L, Galeazzi M. Genetic aspects of the antiphospholipid syndrome: an update. *Autoimmun Rev* 2016; **15**:433–9.

123 Galeazzi M, Sebastiani GD, Tincani A et al. HLA class II alleles associations of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. *Lupus* 2000; **9**:47–55.

124 Lundstrom E, Gustafsson JT, Jonsen A et al. HLA-DRB1*04/*13 alleles are associated with vascular disease and antiphospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis* 2013; **72**:1018–25.

125 Wilson WA, Perez MC, Michaliszki JP, Armitis PE. Cardiolipin antibodies and null alleles of C-4 in black-americans with systemic lupus-erythematosus. *J Rheumatol* 1988; **15**:1768–72.

126 Islam MA, Khandker SS, Alam F, Kamal MA, Gan SH. Genetic risk factors in thrombotic primary antiphospholipid syndrome: A systematic review with bioinformatic analyses. *Autoimmun Rev* 2018; **17**:226–43.

127 Hirose N, Williams R, Alberts AR et al. A role for the polymorphism at position 247 of the beta2-glycoprotein I gene in the generation of anti-beta2-glycoprotein I antibodies in the antiphospholipid syndrome. *Arthritis Rheum* 1999; **42**:1655–61.

128 Yasuda S, Atsumi T, Matsuura E et al. Significance of valine/leucine247 polymorphism of beta2-glycoprotein I in antiphospholipid syndrome: increased reactivity of anti-beta2-glycoprotein I autoantibodies to the valine247 beta2-glycoprotein I variant. *Arthritis Rheum* 2003; **52**:212–8.

129 Svenungsson E, Gustafsson J, Leonard D et al. A STAT4 risk allele is associated with ischaemic cerebrovascular events and anti-phospholipid antibodies in systemic lupus erythematosus. *Ann Rheum Dis* 2010; **69**:834–40.

130 Horita T, Atsumi T, Yoshida N et al. STAT4 single nucleotide polymorphism, rs7574865 G/T, as a risk for antiphospholipid syndrome. *Ann Rheum Dis* 2009; **68**:1366–7.

131 Blank M, Faden D, Tincani A et al. Immunization with anticardiolipin cofactor (beta-2-glycoprotein I) induces experimental antiphospholipid syndrome in naive mice. *J Autoimmun* 1994; **7**:441–55.

132 Blank M, Krause I, Fridkin M et al. Bacterial induction of autoantibodies to beta2-glycoprotein-I accounts for the infectious etiology of antiphospholipid syndrome. *J Clin Invest* 2002; **109**:797–804.

133 Fickl H, Van Antwerpen VL, Richards GA et al. Increased levels of autoantibodies to cardiolipin and oxidised low density lipoprotein are inversely associated with plasma vitamin C status in cigarette smokers. *Atherosclerosis* 1996; **124**:75–81.

134 Gomez-Puerta JA, Cervera R, Espinosa G et al. Antiphospholipid antibodies associated with malignancies: clinical and pathological characteristics of 120 patients. *Semin Arthritis Rheum* 2006; **35**:322–32.

135 Branch DW, Dudley DJ, Mitchell MD et al. Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss. *Am J Obstet Gynecol* 1990; **163(1 Pt 1)**:210–6.

136 Pierangelii SS, Barker JH, Stilkovac D et al. Effect of human IgG antiphospholipid antibodies on an in vivo thrombosis model in mice. *Thromb Haemost* 1994; **71**:670–4.

137 Pierangelii SS, Liu XY, Barker JH, Anderson G, Harris EN. Induction of thrombosis in a mouse model by IgG, IgM and...
IgA immunoglobulins from patients with the antiphospholipid syndrome. *Thromb Haemost* 1995; 74: 1361–7.

138 Fischetti F, Durigutto P, Pellic V et al. Thrombus formation induced by antibodies to beta2-glycoprotein I is complement dependent and requires a priming factor. *Blood* 2005; 106: 2340–6.

139 Stoeeger ZM, Mozes E, Tartakovsky B. Anti-cardiolipin antibodies induce pregnancy failure by impairing embryonic implantation. *Proc Natl Acad Sci USA* 1993; 90: 6464–7.

140 Chamley LW, Allen JL, Johnson PM. Synthesis of beta2 glycoprotein I by the human placenta. *Placenta* 1997; 18: 403–10.

141 Mulla MJ, Myrtolli K, Brosens JJ et al. Antiphospholipid antibodies limit trophoblast migration by reducing IL-6 production and STAT3 activity. *Am J Reprod Immunol* 2010; 63: 339–48.

142 Alvarez AM, Mulla MJ, Chamley LW, Cadavid AP, Abrahams VM. Aspirin-triggered lipoxin prevents antiphospholipid antibody effects on human trophoblast migration and endothelial cell interactions. *Arthritis Rheumatol* 2015; 67: 488–97.

143 Kim MY, Guerra MM, Kapiwotz E et al. Complement activation predicts adverse pregnancy outcome in patients with systemic lupus erythematosus and/or antiphospholipid antibodies. *Ann Rheum Dis* 2018; 77: 549–55.

144 Girardi G, Berman J, Redecha P et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J Clin Invest* 2003; 112: 1644–54.

145 Ekdahl KN, Persson B, Mohlin C, Sandholm K, Skattum L, Nilsson B. Interpretation of serological complement biomarkers in disease. *Front Immunol* 2018; 9: 2237.

146 Holers VM, Girardi G, Mo L et al. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J Exp Med* 2002; 195: 211–20.

147 Davis WD, Brey RL. Antiphospholipid antibodies and complement activation in patients with cerebral-ischemia. *Clin Exp Rheumatol* 1992; 10: 455–60.

148 Oku K, Atsumi T, Bohgaki M et al. Complement activation in patients with primary antiphospholipid syndrome. *Ann Rheum Dis* 2009; 68: 1030–5.

149 Garabet L, Gilhoo IM, Mowinckel MC et al. Antiphospholipid antibodies are associated with low levels of complement C3 and C4 in patients with systemic lupus erythematosus. *Scand J Immunol* 2016; 84: 95–9.

150 Lood C, Tyden H, Gullstrand B et al. Platelet activation and anti-phospholipid antibodies collaborate in the activation of the complement system on platelets in systemic lupus erythematosus. *PLoS ONE* 2014; 9: e99386.

151 Mehta N, Uchino K, Fakhrran S et al. Platelet C4d is associated with acute ischemic stroke and stroke severity. *Stroke* 2008; 39: 3236–41.

152 Navratil JS, Manzi S, Kao AH et al. Platelet C4d is highly specific for systemic lupus erythematosus. *Arthritis Rheum* 2006; 54: 670–4.

153 Petri MA, Conklín J, O’Malley T, Derveux T. Platelet-bound C4d, low C3 and lupus anticoagulant associate with thrombosis in SLE. *Lupus Sci Med* 2019; 6: e000318.

154 Stachowicz A, Zabczyk M, Natorska J et al. Differences in plasma fibrin clot composition in patients with thrombotic antiphospholipid syndrome compared with venous thromboembolism. *Sci Rep* 2018; 8: 17301.
The antiphospholipid syndrome / E. Svenungsson and A. Antovic

171 Betapudi V, Lominadze G, Hsi L, Willard B, Wu M, McCrae KR. Anti-beta2GPI antibodies stimulate endothelial cell microparticle release via a nonmuscle myosin II motor protein-dependent pathway. Blood 2013; 122: 3808–17.

172 Moharrez F, Gunnarsson I, Svenungsson E. Altered beta2-glycoprotein I expression on microparticles in the presence of antiphospholipid antibodies. J Thromb Haemost 2017; 15: 1799–806.

173 Nielsen CT, Ostergaard O, Johnsen C, Jacobsen S, Heegaard NH. Distinct features of circulating microparticles and their relationship to clinical manifestations in systemic lupus erythematosus. Arthritis Rheum 2011; 63: 3067–77.

174 Celinska-Lowenhoff M, Iwaniec T, Padjas A, Musial J, Undas A. Altered fibrin clot structure/function in patients with antiphospholipid syndrome: association with thrombotic manifestation. Thromb Haemost 2014; 112: 287–96.

175 Vikerfors A, Svenungsson E et al. Studies of fibrin formation and fibrinolytic function in patients with the antiphospholipid syndrome. Thromb Res 2014; 133: 936–44.

176 Grosso G, Vikerfors A, Woodhams B et al. Thrombin activatable fibrinolysis inhibitor (TAFI) - A possible link between coagulation and complement activation in the antiphospholipid syndrome (APS). Thromb Res 2017; 158: 168–73.

177 Andreoli L, Bertssias GK, Agmon-Levin N et al. EULAR recommendations for women’s health and the management of family planning, assisted reproduction, pregnancy and menopause in patients with systemic lupus erythematosus and/or antiphospholipid syndrome. Ann Rheum Dis 2017; 76: 476–85.

178 Tektonidou MG, Andreoli L, Limper M et al. EULAR recommendations for the management of antiphospholipid syndrome in adults. Ann Rheum Dis 2019; 78: 1296–304.

179 Groot N, de Graeff N, Avinc T et al. European evidence-based recommendations for diagnosis and treatment of paediatric antiphospholipid syndrome: the SHARE initiative. Ann Rheum Dis 2017; 76: 1637–41.

180 Arnaud L, Mathian A, Devilliers H et al. Patient-level analysis of five international cohorts further confirms the efficacy of aspirin for the primary prevention of thrombosis in patients with antiphospholipid antibodies. Autoimmun Rev 2015; 14: 192–200.

181 Ruiz-Irastorza G, Cuadrado MJ, Ruiz-Arruza I et al. Evidence-based recommendations for the prevention and long-term management of thrombosis in antiphospholipid antibody-positive patients: report of a task force at the 13th International Congress on antiphospholipid antibodies. Lupus 2011; 20: 206–18.

182 Crowther MA, Ginsberg JS, Julian J et al. A comparison of two intensities of warfarin for the prevention of recurrent thrombosis in patients with the antiphospholipid antibody syndrome. N Engl J Med 2003; 349: 1133–8.

183 Finazzi G, Marchioli R, Brancaccio V et al. A randomized clinical trial of high-intensity warfarin vs. conventional antithrombotic therapy for the prevention of recurrent thrombosis in patients with the antiphospholipid syndrome (WAPS). J Thromb Haemost 2005; 3: 848–53.

184 Tektonidou MG, Ioannidis JP, Boki KA, Vlachoyiannopoulos PG, Moutsopoulos HM. Prognostic factors and clustering of serious clinical outcomes in antiphospholipid syndrome. QJM 2000; 93: 523–30.

185 Khamashta MA, Cuadrado MJ, Mujic F, Taub NA, Hunt BJ, Hughes GR. The management of thrombosis in the antiphospholipid-antibody syndrome. N Engl J Med 1995; 332: 993–7.

186 Rosove MH, Brewer PM. Antiphospholipid thrombosis: clinical course after the first thrombotic event in 70 patients. Ann Intern Med 1992; 117: 303–8.

187 Ohnishi N, Fujieda Y, Hisada R et al. Efficacy of dual antiplatelet therapy for preventing recurrence of arterial thrombosis in patients with antiphospholipid syndrome. Rheumatol (Oxford) 2019; 58: 969–74.

188 Dentali F, Manfredi E, Crowther M, Ageno W. Long-duration therapy with low molecular weight heparin in patients with antiphospholipid antibody syndrome resistant to warfarin therapy. J Thromb Haemost 2005; 3: 2121–3.

189 Glynn RJ, Danielson E, Fonseca FA et al. A randomizedtrial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med 2009; 360: 1851–61.

190 Jariaj P, Murthy V, Papalardo E, Romay-Penabad Z, Gleason C, Pierangeli SS. Statins for the treatment of antiphospholipid syndrome? Ann N Y Acad Sci 2009; 1173: 736–45.

191 Kumar D, Rouhey RA. Use of rituximab in the antiphospholipid syndrome. Curr Rheumatol Rep 2010; 12: 40–4.

192 Ruiz-Irastorza G, Ramos-Casals M, Brito-Zeron P, Khamashta MA. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: a systematic review. Ann Rheum Dis 2010; 69: 20–8.

193 Berman H, Rodriguez-Pinto I, Cervera R et al. Rituximab use in the catastrophic antiphospholipid syndrome: descriptive analysis of the CAPS registry patients receiving rituximab. Autoimmun Rev 2013; 12: 1085–90.

194 Ortel TL, Erkan D, Kitchens CS. How I treat catastrophic thrombotic syndromes. Blood 2015; 126: 1285–93.

195 Sukara G, Baresic M, Sentic M, Brcic L, Anic B. Catastrophic antiphospholipid syndrome associated with systemic lupus erythematosus treated with rituximab: case report and a review of the literature. Acta Reumatol Port 2015; 40: 169–75.

196 Rodriguez-Pinto I, Espinosa G, Cervera R. Catastrophic antiphospholipid syndrome: The current management approach. Best Pract Res Clin Rheumatol 2016; 30: 239–49.

197 Cohen H, Hunt BJ, Efthymiou M et al. Rivaroxaban versus warfarin to treat patients with thrombotic antiphospholipid syndrome, with or without systemic lupus erythematosus (RAPS): a randomised, controlled, open-label, phase 2/3, non-inferiority trial. Lancet Haematol 2016; 3: e426–36.

198 Pengo V, Denas G, Zoppellaro G et al. Rivaroxaban versus warfarin in high-risk patients with antiphospholipid syndrome. Blood 2018; 132: 1365–71.

199 Dufrost V, Risse J, Reshefian Y et al. Increased risk of thrombosis in antiphospholipid syndrome patients treated with direct oral anticoagulants. Results from an international patient-level data meta-analysis. Autoimmun Rev 2018; 17: 1011–21.

200 El-Husseini A, Hannan S, Awad A, Jennings S, Cornea V, Sawaya BP. Thrombotic microangiopathy in systemic lupus erythematosus: efficacy of eculizumab. Am J Kidney Dis 2015; 65: 127–30.

201 Kronischler A, Frank R, Kirschhink M et al. Efficacy of eculizumab in a patient with immunoadsorption-dependent
catastrophic antiphospholipid syndrome: a case report. Medicine (Baltimore) 2014; 93: e143.

202 Pickering MC, Ismajli M, Condon MB et al. Eculizumab as rescue therapy in severe resistant lupus nephritis. Rheumatology (Oxford) 2015; 54: 2286–8.

203 Shapira I, Andrade D, Allen SL, Salmon JE. Brief report: induction of sustained remission in recurrent catastrophic antiphospholipid syndrome via inhibition of terminal complement with eculizumab. Arthritis Rheum 2012; 64: 2719–23.

204 Strakhan M, Hurtado-Sbordoni M, Galeas N, Bakirhan K, Alexis K, Elrafei T. 36-year-old female with catastrophic antiphospholipid syndrome treated with eculizumab: a case report and review of literature. Case Rep Hematol 2014; 2014: 704371.

205 Kello N, Khoury LE, Marder G, Furie R, Zapantis E, Horowitz DL. Secondary thrombotic microangiopathy in systemic lupus erythematosus and antiphospholipid syndrome, the role of complement and use of eculizumab: Case series and review of literature. Semin Arthritis Rheum 2019; 49: 74–83.

206 Lockwood CJ, Romero R, Feinberg RF, Clyne LP, Coster B, Hobbs JC. The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. Am J Obstet Gynecol 1989; 161: 369–73.

207 Ruffatti A, Tonello M, Hoxha A et al. Effect of additional treatments combined with conventional therapies in pregnant patients with high-risk antiphospholipid syndrome: a multicentre study. Thromb Haemost 2018; 118: 639–46.

208 Idborg H, Zandian A, Sandberg AS et al. Two subgroups in systemic lupus erythematosus with features of antiphospholipid or Sjogren’s syndrome differ in molecular signatures and treatment perspectives. Arthritis Res Ther 2019; 21: 62.

Correspondence: Elisabet Svenungsson, Department of Medicine Solna, Division of Rheumatology, Karolinska Institutet, D2:00 Karolinska University Hospital, S-1717 6 Stockholm, Sweden. (fax: +46 8 300403; e-mail: elisabet.svenungsson@ki.se)