Antiphospholipid syndrome: advances in diagnosis, pathogenesis, and management

Jason S Knight,1 D Ware Branch,2 Thomas L Ortel3

1Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA
2James R. and Jo Scott Research Chair, Department of Obstetrics and Gynecology, University of Utah Health and Intermountain Healthcare, Salt Lake City, Utah, USA
3Division of Hematology, Departments of Medicine and Pathology, Duke University, Durham, North Carolina, USA

Correspondence to: TL Ortel
thomas.ortel@duke.edu

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Abstract

Antiphospholipid syndrome (APS) is a thrombo-inflammatory disease propelled by circulating autoantibodies that recognize cell surface phospholipids and phospholipid binding proteins. The result is an increased risk of thrombotic events, pregnancy morbidity, and various other autoimmune and inflammatory complications. Although antiphospholipid syndrome was first recognized in patients with lupus, the stand alone presentation of antiphospholipid syndrome is at least equally common. Overall, the diagnosis appears to affect at least one in 2000 people. Studies of antiphospholipid syndrome pathogenesis have long focused on logical candidates such as coagulation factors, endothelial cells, and platelets. Recent work has shed light on additional potential therapeutic targets within the innate immune system, including the complement system and neutrophil extracellular traps. Vitamin K antagonists remain the mainstay of treatment for most patients with thrombotic antiphospholipid syndrome and, based on current data, appear superior to the more targeted direct oral anticoagulants. The potential role of immunomodulatory treatments in antiphospholipid syndrome management is receiving increased attention. As for many systemic autoimmune diseases, the most important future direction is to more precisely identify mechanistic drivers of disease heterogeneity in pursuit of unlocking personalized and proactive treatments for patients.

Introduction

Antiphospholipid syndrome (APS) is a thrombo-inflammatory disease that complicates up to one third of cases of systemic lupus erythematosus, where it portends more organ damage over time.1-4 Primary antiphospholipid syndrome can also occur in the absence of other systemic autoimmune disorders. Antiphospholipid syndrome is propelled by circulating antiphospholipid antibodies that cause vascular thrombosis and obstetrical complications.5 Antiphospholipid antibodies engage phospholipids and phospholipid binding proteins at cell surfaces to activate the endothelium, platelets, and leukocytes—thereby tipping the circulating intravascular milieu toward in situ thrombosis while also promoting other autoimmune and inflammatory complications.6-7 Beyond a history of at least one thrombotic or obstetric morbidity, the most recent antiphospholipid syndrome classification criteria (2006, table 1) seek the persistent presence of anticardiolipin antibodies or anti-beta-2 glycoprotein 1 antibodies (αβ2GPI). Further, the lupus anticoagulant test—a panel of functional assays that screens for antiphospholipid antibodies based on paradoxical prolongation of phospholipid dependent clotting times—is also part of the classification criteria. This review endeavors to tackle the current landscape of antiphospholipid syndrome diagnosis, pathogenesis, and management with a particular focus on updates from the past five years. The intended audience is individuals interested in learning more about the clinical care and research of patients with antiphospholipid syndrome.

Epidemiology

The estimated population prevalence of antiphospholipid syndrome is 40 to 50 cases per 100,000, with an annual incidence of 1 to 2 per 100,000.8-9 Antiphospholipid syndrome is typically diagnosed in relatively younger individuals with just 12.7% of patients diagnosed after the age of 50 in one study of 1000 patients.10 The epidemiology of antiphospholipid syndrome remains understudied, and population based studies of patients with diverse ages and ethnic backgrounds are needed. The population prevalence of persistently positive antiphospholipid antibodies among healthy individuals has yet to be rigorously determined. Although pediatric antiphospholipid syndrome is thought of as rare, it could plausibly be
underdiagnosed for a variety of reasons, including that pregnancy (and associated morbidity) will be less common in this group. One registry of 121 children reported a mean age at onset of antiphospholipid syndrome of 10.7 years. Non-thrombotic manifestations of antiphospholipid syndrome such as thrombocytopenia and autoimmune hemolytic anemia might be more common in the pediatric population.

Sources and selection criteria
PubMed was searched using the terms “antiphospholipid syndrome”, “antiphospholipid antibodies”, “anticardiolipin”, “lupus anticoagulant”, and “beta-2 glycoprotein I”. All English language studies published between 1 January 2017 and 15 June 2022, were considered. Relevant publications outside this timeline were selected based on review of article bibliographies. Studies were prioritized for discussion based on their level of evidence (eg, randomized controlled trials and meta-analyses preferred), their pursuit of mechanistic insights with rigorously defined reagents, and their time of publication (more recent studies preferred).

Clinical manifestations
Thrombotic manifestations of antiphospholip syndrome
Deep veins and cerebral arteries of the lower extremities are the most frequent sites of venous and arterial thrombosis in APS, respectively. Thrombi can also form in sites which do not commonly form thrombi in the general population, including the arteries that supply the intestinal viscera and the dural venous sinuses of the brain. Patients with antiphospholip syndrome are also at risk for microvascular thrombosis in the skin, eyes, heart, lungs, kidneys, and other organs.

A meta-analysis reported that lupus anticoagulant test positivity (odds ratio >10) carries more risk for thromboembolism than do anticardiolipin antibodies. A well characterized cohort of patients with triple positive antiphospholipid syndrome (ie, positivity for anticardiolipin antibodies, aβ2GPI, and the lupus anticoagulant test) has shown the high risk associated with that profile, including a 44.2% (95% confidence interval 38.6 to 49.8) prevalence of thrombosis by 10 years; this risk was more than twice as high (hazard ratio 2.4, 95% confidence interval 1.3 to 4.1) in the subset of patients not taking anticoagulants. In another report, the site of recurrence was arterial in >90% of those patients with an initial arterial thrombotic event, and venous in 76% of those patients with an initial venous thrombotic event. Whether this seeming predilection for particular vascular beds is attributable to fine autoantibody specificity not revealed by current clinical testing, or other factors inherent to each individual, is an area deserving of further exploration.

Obstetric manifestations of antiphospholipid syndrome
When considering fetal death at ≥10 weeks’ gestation, a meta-analysis of publications through 2009 concluded that the presence of a lupus anticoagulant and anticardiolipin antibodies were significantly associated with fetal death, but found “insufficient evidence to establish a significant link between [aβ2GPI] antibodies and pregnancy morbidity.” Two more recent systematic reviews and meta-analyses also found a lack of evidence associating aβ2GPI with fetal death, whereas they found that lupus anticoagulant test positivity was strongly associated with fetal death. Investigating the association from a different perspective, a multicenter, population based case-control study comparing 582 stillbirths with 1547 live births found positive tests for antiphospholipid antibodies (anticardiolipin antibodies or aβ2GPI) in 9.6% (56/582) of fetal deaths at ≥20 weeks’ gestation, and therefore threefold to fivefold increased odds of stillbirth. In patients with known antiphospholipid syndrome, the likelihood of fetal death remains higher even when treated with heparins and low dose aspirin; two prospective, observational studies

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Table 1 | Classification criteria for antiphospholipid syndrome

<table>
<thead>
<tr>
<th>Clinical criteria*</th>
<th>Vascular thrombosis</th>
<th>≥1 clinical episode of arterial, venous, or small vessel thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy morbidity</td>
<td>a) ≥1 unexplained death of a morphologically normal fetus at ≥10 weeks’ gestation</td>
<td></td>
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<tr>
<td></td>
<td>b) ≥1 premature delivery of a morphologically normal fetus at &lt;34 weeks’ gestation because of:</td>
<td></td>
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<tr>
<td></td>
<td>i) Severe pre-eclampsia or eclampsia defined according to standard definition</td>
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<tr>
<td></td>
<td>ii) Recognized features of placental insufficiency</td>
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<tr>
<td></td>
<td>c) ≥3 unexplained consecutive miscarriages at ≥10 weeks’ gestation, with maternal and paternal factors (anatomic, hormonal, or chromosomal abnormalities) excluded</td>
<td></td>
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<tr>
<td>Laboratory criteria</td>
<td>The presence of antiphospholip antibodies on ≥2 occasions ≥12 weeks apart, identified as one or more of the following:</td>
<td></td>
</tr>
<tr>
<td>a) Lupus anticoagulant, defined as:</td>
<td></td>
<td></td>
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<tr>
<td>i) Prolongation of a phospholipid dependent clotting test (eg, aPTT or dilute Russell viper venom test);</td>
<td></td>
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<tr>
<td>ii) Evidence for an inhibitory effect in patient plasma (eg, mixing with normal plasma does not correct the prolonged clotting time as would be expected for a simple factor deficiency); and</td>
<td></td>
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<tr>
<td>iii) Evidence that the inhibitory activity can be quenched via the addition of excess phospholipids</td>
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<tr>
<td>b) Moderate to high titer anticardiolipin antibodies of IgG or IgM isofoms, defined as &gt;40 (moderate) to &gt;80 (high) GPL or MPL units, or greater than the 99th centile, determined by the laboratory providing the test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c) Moderate to high titer anti-β2GPI antibodies of IgG or IgM isofoms, defined as greater than the 99th centile, determined by the laboratory providing the test</td>
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</tr>
</tbody>
</table>

aPTT=activated partial thromboplastin time; β2GPI=beta-2 glycoprotein I
*Antiphospholipid syndrome is present if at least one of the clinical criteria and one of the laboratory criteria are met.
of patients with antiphospholipid syndrome found fetal death rates of 10-12% despite the use of the standard treatments.23 24

Preterm delivery for pre-eclampsia with severe features or placental insufficiency (ie, fetal growth restriction) is likely the most specific obstetric criterion for antiphospholipid syndrome. A 2011 meta-analysis of case-control studies found that the presence of a lupus anticoagulant and anticardiolipin antibodies were significantly associated with pre-eclampsia, with odds ratios of 2.34 (95% confidence interval 1.18 to 4.64) and 1.52 (95% confidence interval 1.05 to 2.20), respectively.23; fewer data were available for anti-β2GPI, although a potential association with pre-eclampsia was noted based on two cohort studies.23 In the same meta-analysis, lupus anticoagulant test positivity was associated with fetal growth restriction with an odds ratio of 4.65 (95% confidence interval 1.29 to 16.71).23 A meta-analysis performed in 2022 found fetal growth restriction associated with anticardiolipin antibodies (odds ratio 2.25, 95% confidence interval 1.55 to 2.94) and anti-β2GPI (odds ratio 1.31, 95% confidence interval 1.12 to 1.49), but not with lupus anticoagulant test positivity.25 A single center prospective study of pre-eclampsia with severe features or placental insufficiency in consecutive general obstetric patients who delivered for medical indications before 36 weeks of gestation found that 11.5% of cases were positive for antiphospholipid antibodies (LA, anticardiolipin antibodies, or anti-β2GPI), compared with 1.4% of matched controls.26 Prospective observational studies have found that 9-10% of pregnant women with antiphospholipid syndrome develop pre-eclampsia with severe features despite standard treatments.22 24

Recurrent early miscarriage (<10 weeks’ gestation) is the obstetric complication for which a diagnosis of antiphospholipid syndrome is most often considered, but for which the role of antiphospholipid antibodies is least clearly defined. Limitations of the literature include lack of discrimination between losses before and after 10 weeks’ gestation, incomplete evaluation for other causes of pregnancy loss, inclusion of minimally positive antiphospholipid antibodies, and absence of confirmatory autoantibody testing. A review of studies through 2011 concluded that the median frequencies of lupus anticoagulant, anticardiolipin antibodies, and anti-β2GPI in such patients were 0%, 2%, and 4%, respectively.27 A subsequent meta-analysis of studies published before 2014 found that the frequencies of lupus anticoagulant test positivity and anticardiolipin antibodies in patients with recurrent early miscarriage ranged widely from 1.8% to 36.5% and 2% to 88.1%, respectively.28 A recent single center retrospective cohort study and systematic analysis concluded that the prevalence of antiphospholipid antibodies in patients with recurrent early miscarriage is similar to that of the general population.29 Well designed studies to better define the association between antiphospholipid antibodies and early miscarriage are needed.30

**Catastrophic antiphospholipid syndrome**

Catastrophic antiphospholipid syndrome is characterized by the rapid onset of thrombosis in multiple vascular beds leading to multiorgan failure and a high risk of mortality.31 32 Fortunately, this complication only develops in a small subgroup of patients with antiphospholipid syndrome. Catastrophic antiphospholipid syndrome is a systemic thrombo-inflammatory state, and must be distinguished from other systemic thrombotic disorders such as thrombotic thrombocytopenic purpura, hemolytic-uremic syndromes, and
Other manifestations sometimes associated with APS

Cardiovascular
- Accelerated atherosclerosis
- Aortic or mitral valve thickening
- Cardiomyopathy
- Noninfectious vegetations

Neurologic
- Chorea/athetosis
- Cognitive decline
- Longitudinal myelitis
- Migraine headache
- Retinal ischemia
- Seizures
- White matter hyperintensities

Pulmonary
- Diffuse alveolar hemorrhage
- Pulmonary hypertension

Hematologic
- Autoimmune hemolytic anemia
- Bleeding (hypoprothrombinemia)
- Evans syndrome
- Thrombocytopenia

Musculoskeletal
- Arthralgia/arthritis
- Avascular necrosis

Dermatologic
- Digital ischemia
- Livedo racemosa
- Livedo reticularis
- Livedoid ulcers
- Raynaud’s phenomenon
- Splinter hemorrhages

Other manifestations of antiphospholipid syndrome

Antiphospholipid syndrome is associated with a variety of autoimmune and inflammatory manifestations (fig 1). These features are sometimes referred to as “non-criteria” manifestations as they were not deemed specific enough for inclusion in the 2006 classification criteria despite being regularly appreciated in clinical practice. Common non-criteria features include thrombocytopenia and livedoid reticularis or racemosa. Less common manifestations such as cardiac valve damage, diffuse alveolar hemorrhage, and antiphospholipid antibody associated nephropathy are also associated with organ threatening morbidity. From the patient’s perspective, physical and emotional quality of life are negatively affected by the long term obligation to take medications such as vitamin K antagonists, as well as the burden of non-criteria manifestations like fatigue, pain, and premature cognitive dysfunction. Indeed, one study administered the Short Form 36 to 270 people living with antiphospholipid syndrome (either with or without lupus) and identified pain and fatigue, lack of healthcare professional/public awareness, and medication unpredictability as major
Factors detracting from quality of life. In addition, recent studies have shown an association between damage accrual (as measured by the damage index for antiphospholipid syndrome) and quality of life. An international effort to update the classification criteria for antiphospholipid syndrome is currently under way, and is likely to better represent the full disease spectrum of antiphospholipid syndrome.

Clinical laboratory identification of antiphospholipid antibodies

In addition to a history of at least one clinical event, antiphospholipid syndrome classification criteria (table 1) require the stable presence of one or more of the following autoantibodies: anticardiolipin antibodies, αβ2GPI, or a lupus anticoagulant. The three tests should be performed concurrently and be repeated at least once after a minimum interval of 12 weeks, although in some situations clinical decisions need not wait for this second round of testing. Antibodies of low positivity are most likely to disappear over 12 weeks.

Anticardiolipin antibodies

Antiphospholipid syndrome classification criteria are fulfilled by moderate to high titer anticardiolipin antibodies of the IgG or IgM isotype, although the clinical importance of IgM antibodies is debated. Assays for anticardiolipin antibodies are solid phase
Box 2: Disruptions in coagulation and fibrinolysis that might contribute to antiphospholipid syndrome

Coagulation
- Anti-β2GPI antibodies (in complex with βGPI) disrupt the annexin A5 “anticoagulant shield” that neutralizes procoagulant phospholipids on cell surfaces.
- Antibodies against protein C (and potentially other targets) contribute to an acquired “activated protein C resistance” whereby protein C does not efficiently inactivate coagulation factors Va and VIIIa.
- Antibodies associated with antiphospholipid syndrome limit the interaction between antithrombin and the heparan pentasaccharides that license antithrombin’s full activation.
- Autoantibodies target factor II, factor IX, and factor X, preventing their negative regulation by antithrombin.

Fibrinolysis
- Autoantibodies disrupt the function of fibrinolytic mediators such as annexin A2, tissue type plasminogen activator (tPA), and plasmin itself.
- Anti-β2GPI antibodies inhibit fibrinolysis by disrupting the ability of βGPI to potentiate cleavage and activation of plasminogen by tPA.
- Upregulation of anti-fibrinolytic factors such as plasminogen activator inhibitor-1 through unknown mechanisms.

and optimized to recognize αβ2GPI antibodies, as phospholipid binding βGPI protein present in patient serum or sample diluent provides a bridge between antibody and cardiolipin. Indeed, guidance recommends that laboratories test for βGPI dependent antibody cardiolipin antibodies to increase specificity of the result. Results are typically expressed as IgG and IgM phospholipid units, but one must be cautious assigning definitive status to a particular number, given the imperfect correlation between the values obtained from assays provided by different manufacturers. In practice, the clinician could be confronted with values above the manufacturer’s threshold but less than moderately positive (>40 units by enzyme linked immunosorbent assay (ELISA)); the clinical significance of these low positive results is unclear.

Anti-β2GPI antibodies
αβ2GPI of the IgG and IgM isotypes, present at moderate to high titer and detected by solid phase assays, are used for diagnosis and classification of antiphospholipid syndrome. Again, IgG antibodies have more clinical relevance than IgM. Units are typically defined by the manufacturer, and antiphospholipid syndrome classification criteria recommend only considering antibodies positive at the 99th centile or greater. While a strong mechanistic case can be made for the importance of αβ2GPI in antiphospholipid syndrome pathogenesis, it should be noted that a recent systematic review found few prospective data to support added diagnostic value beyond testing for anticardiolipin antibodies and lupus anticoagulant. This study was conducted in the preceding section. Furthermore, at least some αβ2GPI have lupus anticoagulant activity. IgA anticardiolipin antibodies as well as αβ2GPI can also be detected in antiphospholipid syndrome, commonly in combination with other isotypes. While one retrospective study found isolated IgA αβ2GPI to be an independent risk factor for thrombosis and also showed potential pathogenicity in a preclinical model, no recommendations currently support of testing for IgA anticardiolipin antibodies or αβ2GPI in the diagnostic workup for antiphospholipid syndrome.

Lupus anticoagulant
Although the association between lupus anticoagulant test positivity and thrombotic complications has been appreciated since the 1960s, the assay was not formalized until the early 1990s. Conceptually, a lupus anticoagulant test should have three characteristics: (a) prolongation of a phospholipid dependent clotting test, (b) evidence for an inhibitory effect in patient plasma, and (c) evidence that the inhibitory effect can be quenched by excess phospholipids (table 1). Samples for lupus anticoagulant testing are ideally collected from patients not receiving anticoagulant treatment. Vitamin K antagonists have been associated with both false positive and false negative results, and successful interpretation of results requires experienced laboratory staff. More problematic are direct oral anticoagulants such as rivaroxaban, which cause false positive results even at low concentrations. While absorbents have been developed that can remove the interference of direct oral anticoagulants, such agents are not widely available. Emerging strategies for lupus anticoagulant testing, such as use of the Taipan venom time, could eventually help resolve concerns about concomitant anticoagulant use. Testing for a lupus anticoagulant should also be interpreted with caution in the acute clinical setting, as elevated levels of acute phase reactants can interfere with test results.

Antiphospholipid profile
A persistently positive lupus anticoagulant test is the single most powerful predictor of thrombotic risk, as well as pregnancy morbidity. Patients who are “triple positive” for all three standard assays are at highest risk for development of thrombotic and obstetric complications. As will be discussed in more detail below, triple positive patients also appear to be more likely to develop thrombotic events while under treatment with direct oral anticoagulants (compared with vitamin K antagonists).
Box 3: Mechanisms of (and possible approaches to blocking) the release of neutrophil extracellular traps (NETs) in antiphospholipid syndrome

Mechanisms of NET release in antiphospholipid syndrome
- Triggered by anti-β2GPI antibodies
- Requires TLR4 signaling and downstream generation of reactive oxygen species by NADPH oxidase
- Mac-1 mediated adhesion also required for efficient NET release

Strategies that reduce antiphospholipid syndrome mediated NET release and thrombosis in mice
- Neutrophil depletion
- Deoxyribonuclease administration
- PSGL-1 inhibition
- Activation of neutrophil surface adenosine receptors
- Administration of phosphodiesterase inhibitors

New assays
While risk stratification is somewhat informed by an individual’s antiphospholipid antibody profile, these profiles themselves are not proven to allow for strategic or pre-emptive therapeutic interventions. Emerging autoantibody classes associated with antiphospholipid syndrome include anti-β2GPI domain I antibodies, antiphosphatidylserine/prothrombin antibodies (anti-PS/PT), antilysoosphosphatidic/endothelial protein C receptor antibodies (anti-LBPA/EPCR), anti-β2GPI/HLA class II complex antibodies, and antineutrophil extracellular trap antibodies. More research is needed and none of these antibodies currently have a defined role in the clinic. An open question is whether the answer to disease heterogeneity will eventually be unlocked by comprehensively defining each individual’s circulating autoantigenome. Alternatively, it could be that the same antibodies drive distinct disease manifestations based on the genetic, epigenetic, and metabolic landscapes of various circulating and tissue resident cells.

Timing of antiphospholipid antibodies testing in relation to thrombotic events and pregnancy
For practical reasons, initial testing is usually done shortly after a clinical event, followed by confirmatory testing at least 12 weeks later. Some nuances should be discussed though. At the time of an acute thromboembolic event, acute phase reactants such as C reactive protein, factor VIII, and fibrinogen might be markedly increased, altering coagulation test results (either increasing or decreasing times) and making it particularly important that lupus anticoagulant testing is repeated once the patient is stable. In addition, acute events have long been known to trigger the appearance of transient anticardiolipin antibodies, especially of low titer. Because of reports that antiphospholipid antibodies levels can fluctuate during pregnancy, either decreasing or increasing, current International Society on Thrombosis and Haemostasis guidelines recommend that antiphospholipid antibodies results obtained during pregnancy be interpreted with caution. Results of a prospective observational study of 152 pregnant patients positive for antiphospholipid antibodies enrolled in the late first or early second trimesters found that 25% of lupus anticoagulant positive patients became negative in the second or third trimesters. IgG antiphospholipid antibodies levels were also significantly lower during the second and third trimesters, but among patients testing positive, IgG anticardiolipin antibodies and αβ2GPI results remained in the positive range through pregnancy in 93% and 85% of patients, respectively. Guidelines recommend that confirmation or exclusion of antiphospholipid antibodies positivity be confirmed after the postpartum period. Although the optimal timing of such testing is not established, one study found that lupus anticoagulant test results returned to baseline status three months after delivery.

Pathogenesis of antiphospholipid syndrome
The evidence that IgG antibodies targeting domain I of β2GPI contribute to the prothrombotic state of antiphospholipid syndrome is convincing. Meanwhile, data also support roles for other isotypes of αβ2GPI, antibodies that target heterotypic complexes of phospholipids and phospholipid binding proteins, and antibodies that target phospholipids directly. Given that most mechanistic studies have focused on either polyclonal IgG fractions isolated from patients with antiphospholipid syndrome or IgG anti-β2GPI, we unfortunately could not build a nuanced model of how diverse species of antiphospholipid antibodies conspire to cause various manifestations of antiphospholipid syndrome. Some potential mechanisms downstream of individual autoantibodies are highlighted in fig 2.

Since circulating antiphospholipid antibodies are typically unaffected by immunomodulatory treatments, the antibody producing cells of antiphospholipid syndrome likely reside among long lived plasma cells. Meanwhile, evidence suggests that some antiphospholipid antibodies have undergone extensive affinity maturation from original germline sequences, indicating a role for helper T cells in shaping the antiphospholipid syndrome antibody repertoire. The best defined B cell epitope relevant to antiphospholipid syndrome is in domain I of β2GPI, while the best defined T cell epitope is in domain V of the same protein. As has been recently reviewed, several reports have found genetic associations with HLA class II alleles in antiphospholipid syndrome (again supporting a role for helper T cells in pathophysiology); only two genetic loci outside of the HLA region (STAT4 and CX3D) have reached the threshold for significance at the genome wide level. Larger collaborative studies are needed.

Thrombotic events
It has been posited that “two hits” are necessary to trigger a thrombotic event in antiphospholipid syndrome. This hypothesis argues that antiphospholipid antibodies provide the first hit by
creating a generalized procoagulant state, which conspires with a second hit (often cryptic and potentially the result of subclinical vascular injury, stasis, or inflammation) that triggers thrombosis.

Inappropriate activation of hemostatic factors (clotting cascade, endothelium, and platelets)

Breakdowns in natural anticoagulant systems were among the first prothrombotic consequences of antiphospholipid antibodies to be reported (box 2); however, as is true for many of the mechanisms that follow, the weight that should be attributed to each concept in a particular vascular bed remains mostly unknown. A recent report found that β2GPI deficiency in mice creates a prothrombotic environment independent of antiphospholipid antibodies,99 an observation that has the potential to shine new light on the interplay between this abundant plasma protein and other clotting regulators in antiphospholipid syndrome.

In vitro, antiphospholipid antibodies appear to activate endothelial cells by co-opting pathways normally associated with non-autoimmune inflammatory stimuli.100 Potential surface receptors for the complex of β2GPI and aβ GPI include apolipoprotein E receptor 2 (apoER2),101 and the complex of annexin A2 and TLR4.102 Implicated pathways downstream of antiphospholipid antibodies include the activation of p38 MAPK and NF-κB and the suppression of homeostatic Krüppel-like factors.103-109 Modulation of these pathways by antiphospholipid antibodies leads to a reduction in nitric oxide synthesis and increased expression of tissue factor and cell adhesion molecules.106-109 Concordantly, mice can be protected from antiphospholipid antibodies mediated thrombosis by disrupting the function of various selectins, selectin ligands, and integrins.110-113 Endothelial derived microparticles can be detected at increased levels in the circulation of patients with antiphospholipid syndrome, indicating that the endothelium is activated and primed for interactions with platelets and leukocytes.114-116 All the above phenotypes have either been attributed to aβ2GPI or have been elicited with polyclonal IgG fractions isolated from patients with antiphospholipid syndrome.

When studied in vitro, unstimulated platelets resist binding by β2GPI protein; however, when platelets are exposed to either shear stress or thrombin, β2GPI complexes with apoER2 and platelet glycoprotein Ib, enabling aβ2GPI to trigger platelet activation.117-118 Antiphospholipid syndrome patient derived antiprothrombin antibodies have also recently been shown to activate healthy platelets in the presence of calcium and prothrombin119; the authors proposed that this might explain why vitamin K mediated depletion of prothrombin (in contrast with anticoagulation with factor Xa targeting direct oral anticoagulants) might be especially effective in antiphospholipid syndrome.119

In a mouse model of antiphospholipid syndrome, platelets are recruited to the injured endothelium in exuberant fashion where they support fibrin generation in the thrombus.120 While there have been only a few direct characterizations of antiphospholipid syndrome patient platelets,121,122 it has also been shown that circulating platelet leukocyte aggregates are present at increased levels in patients with antiphospholipid syndrome.123

Immunothrombosis (complement, monocytes, and neutrophils)

After early work showed a requirement for complement in animal models of antiphospholipid antibodies mediated pregnancy loss,124 attention turned to its potential role in thrombosis. In a femoral vein model, antagonizing any of complement C3, C5, or C6 protects against thrombosis.125-128

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Table 2 | Randomized clinical trials for the treatment of patients with thrombotic antiphospholipid syndrome*

<table>
<thead>
<tr>
<th>Study</th>
<th>No subjects</th>
<th>Previous TE</th>
<th>AT treatment</th>
<th>Primary outcome</th>
<th>Results</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crowther, 2003</td>
<td>56</td>
<td>45 VTE (75%)</td>
<td>warfarin, INR 2.3</td>
<td>Recurrent TE</td>
<td>2 TE (3.4%)</td>
<td>HR 3.1 (95% CI, 0.6 to 15.0); P=0.15</td>
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<tr>
<td></td>
<td>56</td>
<td>42 VTE (75%)</td>
<td>warfarin, INR 3.4</td>
<td>Composite outcome of vascular death, non-fatal major VTE and/or ATE</td>
<td>3 TE (5.5%)</td>
<td>HR 1.63 (95% CI, 0.39 to 6.83); P=0.5043</td>
</tr>
<tr>
<td>Finazzi, 2005</td>
<td>55</td>
<td>38 VTE, 23 ATE</td>
<td>warfarin, INR 2.3</td>
<td>No ATE in either group at day 21†</td>
<td>5 TE (9.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>37 VTE, 21 ATE</td>
<td>warfarin, INR 3.4</td>
<td>No ATE in either group at day 21†</td>
<td>5 TE (9.3%)</td>
<td></td>
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<tr>
<td>Cohen, 2016</td>
<td>57</td>
<td>32 DVT, 25 PE</td>
<td>rivaroxaban, 20 mg/day*</td>
<td>Percentage change in ETP from randomization to day 42†</td>
<td>1086 nmol/L per min (95% CI, 0.6 to 15.0); P=0.01 [as treated analysis]</td>
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<tr>
<td></td>
<td>59</td>
<td>37 DVT, 22 PE</td>
<td>warfarin, INR 3.4</td>
<td>Treatment effect 2 (95% CI 1.7 to 2.4); P&lt;0.0001</td>
<td></td>
<td></td>
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<tr>
<td>Pengo, 2018</td>
<td>59</td>
<td>11 ATE, 38 VTE</td>
<td>rivaroxaban, 20 mg/day</td>
<td>Composite outcome of TE, MB, and vascular death</td>
<td>7 ATE, 4 MB</td>
<td>HR (all events) 6.7 (95% CI 1.5 to 30.5); P&lt;0.01 [as treated analysis]</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>14 ATE, 39 VTE</td>
<td>warfarin, INR 3.4</td>
<td>No ATE in either group at day 21†</td>
<td>2 MB</td>
<td></td>
</tr>
<tr>
<td>Ordi-Ros, 2019</td>
<td>95</td>
<td>37 ATE, 69 VTE</td>
<td>rivaroxaban, 20 mg/day</td>
<td>New TE</td>
<td>9 ATE, 1 VTE, 1 ATE+VTE</td>
<td>HR (all events) 1.94 (95% CI 0.72 to 5.24); P=0.190 [as treated analysis]</td>
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<tr>
<td></td>
<td>95</td>
<td>34 ATE, 70 VTE</td>
<td>warfarin, INR 3.4</td>
<td>No ATE in either group at day 21†</td>
<td>3 ATE, 3 VTE</td>
<td></td>
</tr>
<tr>
<td>Wolfer, 2022</td>
<td>25</td>
<td>11 ATE, 20 VTE</td>
<td>apixaban, 2.5 mg twice daily‡</td>
<td>Combined rate of ATE, VTE, and vascular death</td>
<td>6 ATE, all strokes; no VTE, no deaths</td>
<td>1 MB in the warfarin arm; no MB in the apixaban arm</td>
</tr>
</tbody>
</table>

AT=anti-thrombotic; ATE=arterial thromboembolism; CI=confidence interval; DVT=deep vein thrombosis; ETP=endogenous thrombin potential; HR=hazard ratio; INR=international normalized ratio; MB=major bleeding; PE=pulmonary embolism; TE=thromboembolic events; VKA=vitamin K antagonist; VTE=venous thromboembolism.

*Secondary outcomes included the occurrence of thromboembolism up to 210 days after randomization.

†Composite outcome of TE, MB, and vascular death.

‡Dose of apixaban increased to 5 mg twice daily after the first 25 patients were enrolled. No patients with ATE were randomized to the study after the first 30 patients were enrolled.
Blocking either C5 or C6 is protective in a model of antiphospholipid antibodies mediated mesenteric thrombosis.129 Studies in patients found evidence of smoldering complement activation,130 via both the classical pathway and alternative pathway.131-133 Another mechanism of complement activation in antiphospholipid syndrome could require mannose binding lectin bound to β2GPI.134 A notable recent study used a “modified Ham test” to show complement activity in antiphospholipid syndrome sera as measured by C5b-9 deposition and complement mediated cell death135; complement hyperactivity associated with both triple positive status and recurrent thrombosis.

In vitro, αβ2GPI engage TLR4 signaling and trigger monocytes to express tissue factor and proinflammatory cytokines such as TNF-α and IL-1β,136 via signaling that depends on p38 MAPK and NfκB.137 Cofactor independent antiphospholipid antibodies such as anti-LBPA/EPCR are also able to activate monocytes via endosomal NADPH oxidase.71 138 139 Patients with antiphospholipid syndrome show enhanced monocyte production of tissue factor as well as VEGF and its receptor Flt-1.140-142 Unbiased transcriptomic profiling has found upregulation of proinflammatory genes including TLR8, CD14, and others associated with inflammation and oxidative stress.143-145 Microparticles derived from monocytes are detected at increased levels in antiphospholipid syndrome blood,146-147 where they present tissue factor.148

Although αβ2GPI were shown to activate neutrophils many years ago,149 interest in this concept intensified recently based on emerging connections between neutrophil extracellular traps (NETs) and thrombosis. NETs are extracellular web-like scaffolds of decondensed chromatin decorated with microbicidal proteins,150 which trigger thrombosis in the original place through multiple mechanisms including activation of coagulation factors, the endothelium, platelets, and the complement system.151 Relevant mechanisms and inhibitors of NET release are discussed in box 3. High levels of NET remnants are found in the circulation of patients with antiphospholipid syndrome even in the absence of active thrombosis.152-154 The prothrombotic and complement activating properties of NETs might also be further amplified by anti-NET antibodies.73 158

Catastrophic antiphospholipid syndrome

Most of what is known about catastrophic antiphospholipid syndrome has been inferred through collaborative international cohort studies.155 One small study found high levels of von Willebrand factor and P-selectin in patients with active disease, consistent with endothelial/platelet activation.156 A role for complement activation has been indirectly suggested by complement gene variants in 60% of patients who experience a catastrophic antiphospholipid syndrome event.157 The likely role of complement in catastrophic antiphospholipid syndrome is also supported by reports of successful use of eculizumab in some, but not all, patients refractory to standard treatment.160 161

Obstetric morbidity

A meta-analysis of placental histopathology found that thrombosis is actually a rare feature of antiphospholipid syndrome pregnancies; rather, common abnormalities included decidua inflammation, deposition of complement split product C4d, impaired spiral artery remodeling by extravillous trophoblasts, and decreased vasculosyncytial membranes.162 Unlike most other cells in the body, trophoblasts and decidual endothelial cells constitutively display β2GPI on their surfaces,163-164 making the placenta a target for binding of pathogenic αβ2GPI.

In vitro, αβ2GPI interfere with extravillous trophoblast proliferation and invasion.165 166 Like endothelial cells and platelets, the low density lipoprotein receptor apoER2 has been implicated in antiphospholipid antibodies mediated trophoblast dysfunction.167 β2GPI and apoER2 are clustered by αβ2GPI on the cell surface, which triggers protein phosphatase 2A (PP2A) activation.168 Notably inhibition/deletion of either apoER2 or PP2A protects antiphospholipid syndrome mice from maternal hypertension, proteinuria, and fetal growth restriction.169 Beyond apoER2, in vitro studies have also implicated the TLR4/MyD88 pathway in anti-β2GPI mediated trophoblast dysfunction.170 Downstream of TLR4, endogenous uric acid contributes to inflammation.171 While miR-146a-3p activates TLR8,172 the extent to which other autoantibodies associated with antiphospholipid syndrome beyond αβ2GPI contribute to trophoblast dysfunction has not been rigorously investigated.

In addition to trophoblast dysfunction, antiphospholipid antibodies also promote complement activation in the placenta, which could explain the effectiveness of heparins in reducing antiphospholipid syndrome obstetric morbidity.173 Notably, mice deficient in C3 are almost completely protected from fetal injury when they receive IgG from patients with antiphospholipid syndrome.174 Subsequent studies implicated both C4 (classical pathway) and factor B (alternative pathway) in mediating the full antiphospholipid syndrome phenotype in mice.174 175 Downstream of complement, neutrophils are activated in C5a receptor dependent fashion with inflammation further amplified by TNF-α and tissue factor.174 176 Studies in patient cohorts have shown that blood markers of complement activation (Bb, soluble C5b-9) predict pregnancy morbidity in patients positive for antiphospholipid antibodies.177 Notably, NETs have also been found in the intervillous space of antiphospholipid syndrome placentas where they could contribute to reduced trophoblast function.178
Occlusive vasculopathy

Although this antiphospholipid antibodies related pathology was initially reported and is still best defined in the renal microvasculature,\textsuperscript{179,180} occlusive lesions are likely under-recognized in tissues that are not commonly biopsied such as brain, heart, and mesentery.\textsuperscript{181-183} The chronic vasculopathy of antiphospholipid syndrome is characterized by progressive expansion of the intima,\textsuperscript{181-183} which could be partially attributable to endothelial cell proliferation, but might also require infiltration of vascular smooth muscle cells and deposition of proteoglycan rich extracellular matrix. The molecular pathways that propel these vascular lesions have not been studied in detail, although one report posited that the mTOR/Akt pathway is an important mediator of antiphospholipid syndrome nephropathy and therefore a potential pharmacological target.\textsuperscript{184}

Management of thrombotic antiphospholipid syndrome

Risk factor reduction

Given that many patients with persistent antiphospholipid antibodies will also have other cardiovascular risk factors such as hypertension, dyslipidemia, and obesity,\textsuperscript{185} attention should be given to correcting reversible predispositions whenever possible. The importance of such factors is emphasized by an easy-to-use thrombosis risk prediction tool called the adjusted Global Antiphospholipid Syndrome Score (aGAPSS), which includes hypertension and hyperlipidemia in addition to the traditional antiphospholipid antibody tests discussed above.\textsuperscript{186}

Primary prevention

Antiphospholipid antibodies can be detected during evaluations of patients with rheumatic disease, obstetric morbidity, thrombocytopenia, or livedo reticularis/racemosa, or during further assessment for a false positive syphilis test or prolonged activated partial thromboplastin time. Data to guide management of such patients are limited.\textsuperscript{187-189} A prospective observational study of 258 individuals with persistently positive antiphospholipid antibodies (54.3% using primary prophylaxis) showed an annual thrombotic incidence rate of 1.86%.\textsuperscript{190} By contrast, when 104 carriers of triple positive antiphospholipid antibodies were followed for an average of 4.5 years, annual thrombosis incidence was 5.3%.\textsuperscript{191}; in this cohort, use of low dose aspirin was not associated with a reduced risk of thrombosis.

One randomized controlled trial has evaluated the effectiveness of aspirin (n=48) versus placebo (n=50) for prevention of first thrombotic event in individuals persistently positive for antiphospholipid antibodies\textsuperscript{192}; the trial found no difference between groups, albeit with a low event rate as a clear limitation.\textsuperscript{192} A recent Cochrane systematic review found insufficient evidence to support the use of aspirin for primary thrombosis prevention in asymptomatic carriers of antiphospholipid antibodies.\textsuperscript{193} Some observational studies have suggested a protective effect of aspirin,\textsuperscript{194-196} which might be most relevant in patients with lupus or thrombocytopenia.\textsuperscript{196} Such data informed the recommendations by EULAR\textsuperscript{197} and the 16th International Congress on Antiphospholipid Antibodies Task Force on APS Treatment Trends\textsuperscript{198} to provide low dose aspirin prophylaxis in subjects with a high risk profile, defined as persistently positive for lupus anticoagulant or with triple positive status, or both. At this point, the decision regarding primary prophylaxis remains individualized and should consider other thrombosis risk factors.

Treatment and secondary prevention

Key trials that have informed the approach to treatment and secondary prevention of thromboembolism are highlighted in table 2. Vitamin K antagonists are the recommended treatment for thrombotic antiphospholipid syndrome. Several early observational studies suggested that optimal anticoagulation regimens were those that maintained the INR between 3.0 and 4.0 (so called high intensity anticoagulation).\textsuperscript{18,199,200} Conversely, two randomized controlled trials in 2003 and 2005 found no difference between INR goals of 2.0-3.0 and 3.1-4.0,\textsuperscript{201,202} other than a higher risk of hemorrhagic complications with the high intensity regimen.\textsuperscript{202} Many patients in the high intensity arms did not routinely achieve the higher INR target, and a minority of patients with a history of arterial thrombosis were included; some experts therefore continue to endorse the use of high intensity anticoagulation, especially for patients with arterial thrombosis. Such an approach can be supported by a systematic review that found only seven of 180 recurrent thrombotic events occurred in patients when the INR was >3.0,\textsuperscript{203} while noting that recent EULAR guidelines did not reach a consensus on this topic.\textsuperscript{197} Whether a higher target INR is better for certain subsets of patients with recurrent venous thromboembolism or an initial arterial event remains controversial.\textsuperscript{203,205} Rather than targeting a higher goal INR, some favor the combination of aspirin and standard intensity anticoagulation for secondary prevention of arterial thrombosis, which can be supported by observational data.\textsuperscript{206}

Several randomized controlled trials have investigated the use of direct oral anticoagulants in patients with thrombotic antiphospholipid syndrome. One randomized controlled trial of 116 patients with antiphospholipid syndrome (28% triple positive) compared rivaroxaban with the vitamin K antagonist warfarin.\textsuperscript{207} While the study did not meet its primary endpoint (change in endogenous thrombin potential), no thrombosis or major bleeding was observed in either group over seven months.\textsuperscript{207} Another trial evaluated rivaroxaban compared with warfarin in 120 triple positive patients with antiphospholipid syndrome.\textsuperscript{208} The composite primary outcome of thrombotic events, major bleeding, and vascular mortality
was higher in the rivaroxaban group (hazard ratio 7.4, 95% confidence interval 1.7 to 32.9). A second study also failed to show non-inferiority of rivaroxaban compared with warfarin as a secondary thromboprophylaxis agent. Finally, a randomized controlled trial of apixaban versus warfarin was stopped early after the primary outcome, stroke, occurred in six of 23 patients randomized to apixaban (albeit three receiving prophylactic rather than therapeutic dosing), as compared with 0 of 25 patients randomized to warfarin. A recent meta-analysis found no evidence of a higher risk of recurrent venous thromboembolism in patients with antiphospholipid syndrome treated with direct oral anticoagulants compared with those treated with warfarin; this was in contrast to a significantly increased risk of recurrent arterial thrombosis. Moreover, risk of recurrent arterial thrombosis tended to be more frequent in patients with a history of arterial thrombosis. These results are in line with international guidelines which recommend not to use direct oral anticoagulants in patients with antiphospholipid syndrome with a history of arterial thrombosis, but raise the question of the efficacy of direct oral anticoagulants to prevent venous thrombosis in a subset of patients with antiphospholipid syndrome. At present, our opinion is that direct oral anticoagulants should be avoided when possible in patients with a history of arterial thrombosis or with triple positive antiphospholipid syndrome, regardless of whether the initial thromboembolic event was venous or arterial, pending further well designed clinical trials.

Treatment with long term anticoagulation unfortunately brings with it an increased risk of bleeding complications. At a traditional INR target (eg, 2.0-3.0), the risk of major bleeding in patients with antiphospholipid syndrome is similar to that observed in patients receiving vitamin K antagonists because of inherited thrombophilia or prosthetic mitral valve replacement. The risk of bleeding is higher when vitamin K antagonists are either combined with aspirin, or administered at a higher intensity in pursuit of an INR target such as 3.0-4.0. The risk of bleeding is increased in patients with poor anticoagulant control, and optimally, these patients would be managed by experienced providers to minimize the bleeding risk. Novel approaches to anticoagulation in antiphospholipid syndrome that might reduce bleeding risk, such as factor XI inhibitors, are currently being studied in different patient populations. We also need to identify patients with antiphospholipid syndrome who might be able to eventually discontinue anticoagulation altogether, such as in individuals with marginally positive antibodies at baseline that eventually disappear.

If a patient develops a thrombotic event while on a vitamin K antagonist, alternative treatments, such as the addition of aspirin, a switch to low molecular weight heparin, or initiation of high intensity anticoagulation (eg, vitamin K antagonist with a target INR>3.0), would typically be considered first.

**Catastrophic antiphospholipid syndrome**

In addition to recognizing and treating any underlying triggers (eg, infection), so called “triple therapy” with anticoagulation (typically unfractionated heparin), corticosteroids, and plasmapheresis or intravenous immunoglobulin (or both) is typically utilized in patients with catastrophic antiphospholipid syndrome. This approach has improved survival as evidenced by a large retrospective case series (471 patients). Specifically, triple therapy was positively associated with a higher chance of survival when compared with non-treatment (odds ratio 9.7, 95% confidence interval 2.3 to 40.6) or treatment with other combinations of drugs included in the triple therapy (odds ratio 1.7, 95% confidence interval 1.2 to 2.6). Rituximab is sometimes used as adjuvant treatment in catastrophic antiphospholipid syndrome. Furthermore, while it seems likely that complement inhibiting treatments would benefit a subset of patients with catastrophic antiphospholipid syndrome who present with features of a complement mediated thrombotic microangiopathy, real world experience has not found such treatment to be universally effective. More research is therefore needed to identify the patients most likely to benefit.

**Management of obstetric antiphospholipid syndrome**

Patients positive for antiphospholipid antibodies without history of thrombotic events or obstetric morbidity

Two randomized controlled trials and one retrospective study of pregnant individuals with positive antiphospholipid antibodies (but without systemic lupus erythematosus) did not show a difference in live birth rate with low dose aspirin. A large randomized controlled trial of patients with pregnancies at risk for pre-eclampsia (n=1176), including a minority with antiphospholipid syndrome, found a significant decrease in the rate of preterm pre-eclampsia with low dose aspirin at a dose of 150 mg daily started at 11-14 weeks’ gestation. Various professional organizations recommend aspirin doses of 75 to 150 mg daily beginning ≥12 weeks’ gestation and ideally by 16-22 weeks’ gestation to reduce the rate of pre-eclampsia in at risk patients. The optimal low dose aspirin dose is uncertain because dose comparison trials are lacking. Specifically regarding patients with antiphospholipid antibodies without antiphospholipid syndrome, American expert guidelines call for prophylactic low dose aspirin (81 or 100 mg daily) during pregnancy beginning before 16 weeks’ gestation. European guidelines recommend low dose aspirin (75-100 mg daily) for patients with lupus anticoagulant or who are double positive for antiphospholipid antibodies.
Individuals with antiphospholipid syndrome based on recurrent early miscarriage (no thrombosis history)

While some trials have found improved live birth rates with the combination of low dose aspirin and prophylactic dose heparin, other studies have not shown a benefit. A systematic review highlighted the many limitations of the literature characterizing antiphospholipid antibodies in the setting of recurrent early miscarriage. A Cochrane systematic review including 1295 pregnancies from five qualifying studies concluded that treatment with low dose aspirin and prophylactic dose heparin might increase the number of live births (relative risk 1.27, 95% confidence interval 1.09 to 1.49), but the characteristics of participants and adverse events were not uniformly reported and the evidence was judged to be of low certainty. In most cases, combination treatment with low dose aspirin and prophylactic dose heparin is ultimately recommended in the clinic.

Individuals with antiphospholipid syndrome based on history of thrombosis or of second trimester or third trimester morbidity

Well designed trials to determine the efficacy of treatments (including low dose aspirin and heparin) and to improve obstetric outcomes in patients diagnosed with antiphospholipid syndrome are lacking. Against a background of increased maternal risk for thrombosis owing to antiphospholipid antibodies positivity and results from retrospective case series, current European and American guidelines recommend patients with antiphospholipid syndrome based on a history of second trimester or third trimester morbidity and without a history of thrombosis be treated during pregnancy with low dose aspirin and prophylactic dose low molecular weight heparin, while those with a history of thrombosis should be treated with low dose aspirin and therapeutic dose low molecular weight heparin. American guidelines also conditionally recommend treatment during pregnancy with hydroxychloroquine. Clinicians should recognize that despite treatment with low dose aspirin and heparin, patients with lupus anticoagulant, and perhaps particularly those that are triple positive, have a 30% or higher risk of adverse pregnancy outcomes. Given the risks of pre-eclampsia and placental insufficiency in these patients, experts recommend careful monitoring of fetus and mother.

Other considerations

Given that the postpartum period represents the highest risk for pregnancy related thrombosis, antiphospholipid antibodies-positive individuals who have never had thrombosis should receive 6-12 weeks of prophylactic low molecular weight heparin, while participants with antiphospholipid syndrome and history of thrombosis should resume full anticoagulation. Beyond hydroxychloroquine, other treatments such as intravenous immunoglobulin and corticosteroids are sometimes trialed in refractory cases, albeit without compelling evidence from the literature.

Considerations regarding covid-19

Critical and severe cases of covid-19 are associated with an increased risk of thrombosis in arterial, microcirculatory, and venous vascular beds. Reports from early in the pandemic found antiphospholipid antibodies at high titers in a small number of patients with covid-19 who experienced macrovascular thrombotic events. Studies of hospitalized covid-19 patients have detected both traditional antiphospholipid antibodies (such as IgG and IgM isotypes of anticardiolipin antibodies) and non-criteria antiphospholipid antibodies (including IgG and IgM isotypes of anti-PS/PT, as well as IgA isotypes of anticardiolipin antibodies and αβ-GPI). Studies show significant heterogeneity in terms of both overall prevalence of antiphospholipid antibodies (some as high as 50%) and which antiphospholipid antibodies species are most commonly detected. While most studies have not found a clear link between circulating antiphospholipid antibodies and large vessel thrombosis, some evidence has been found that IgG fractions isolated from patients with covid-19 have prothrombotic properties in vitro and in mice. Future studies are required to further clarify whether antiphospholipid antibodies found in patients with severe covid-19 are similar to antiphospholipid antibodies seen in patients with antiphospholipid syndrome.

Vaccination with adenovirus based covid-19 vaccines has been associated with rare complications of thrombocytopenia and thrombosis, likely attributable to antiplatelet factor 4 antibodies; this complication has not been seen with other types of covid-19 immunizations, including mRNA vaccines. Nevertheless, both patients and providers have questioned the extent to which patients with antiphospholipid syndrome might be at increased risk for vaccine associated complications. Fortunately, this does not appear to be the case with multiple studies showing no thrombosis or serious adverse events in 102, 146, 249 and 4450 vaccinated patients positive for antiphospholipid antibodies.

Emerging treatments

Hydroxychloroquine and statins

Both hydroxychloroquine and statins were highlighted as emerging treatments by recent International Congress on Antiphospholipid Antibodies task forces on APS treatment trends. Hydroxychloroquine appears to be protective against thrombosis in people with lupus, whether characterized by positive antiphospholipid antibodies or not.
In animal models of antiphospholipid syndrome, hydroxychloroquine reduces thrombus size. In vitro, hydroxychloroquine prevents antiphospholipid antibodies mediated disruption of the annexin A5 shield that coats phospholipid bilayers. In patients, a reduced type I interferon signature is observed in patients with antiphospholipid syndrome taking hydroxychloroquine, as compared with those who are not. Based on these mechanistic data and its excellent safety profile, hydroxychloroquine is regularly deployed in patients with antiphospholipid syndrome with high risk antibody profiles, breakthrough thrombotic events, or other disease manifestations that are progressing despite anticoagulation.

HMG-CoA reductase inhibitors, known as statins, have been widely used for primary and secondary cardiovascular disease prevention, and clearly have a role in patients with antiphospholipid syndrome who also have hypercholesterolemia. Beyond their impact on cholesterol, administration of fluvastatin to mice with antiphospholipid syndrome reduces leukocyte adhesion to endothelial cells as well as thrombus size. Small mechanistic clinical trials of fluvastatin in patients with antiphospholipid syndrome have shown reduced tissue factor expression by monocytes, and modulation of proinflammatory and prothrombotic biomarkers. Like hydroxychloroquine, statins are sometimes prescribed to patients with antiphospholipid syndrome with breakthrough manifestations or at high risk of recurrent events.

Other less common agents
Case reports have described the successful use of rituximab in patients with antiphospholipid syndrome with severe thrombocytopenia, autoimmune hemolytic anemia, skin ulcers, and antiphospholipid antibodies associated nephropathy. A pilot open label phase II clinical trial found rituximab to exhibit potential efficacy for non-criteria manifestations of antiphospholipid syndrome. Eculizumab is a humanized monoclonal antibody that prevents complement C5 cleavage and is approved for treatment of atypical hemolytic uremic syndrome and paroxysmal nocturnal hematuria. Eculizumab is a humanized monoclonal antibody that prevents complement C5 cleavage and is approved for treatment of atypical hemolytic uremic syndrome and paroxysmal nocturnal hematuria. Case reports and series have suggested efficacy of eculizumab in treating refractory antiphospholipid syndrome especially in the setting of catastrophic antiphospholipid syndrome. Regarding pregnancy morbidity, one ongoing study aims to determine whether the addition of tumor necrosis factor alpha blockade to standard of care treatment can improve outcomes as compared with historical controls (NCT03152058); to date (December 2022), this study has recruited 43 patients with a pre-prescribed target of 50.

Other agents that might warrant further study include sirolimus, adenosine receptor agonists such as diprydramol, and plasma cell depleting agents such as daratumumab. Patients are also interested in further study of supplements such as CoQ10, ginger, and vitamin D that have shown promise in preclinical and mechanistic studies. A review of clinicaltrials.gov in September 2022 identified antiphospholipid syndrome trials recruiting subjects for interventions targeting B cells (belimumab, telitacicept, zanubrutinib), tumor necrosis factor (certolizumab), and innate immunity (hydroxychloroquine).

Guidelines
Currently, researchers rely on an international consensus statement for classification of definite antiphospholipid syndrome published in 2006. An international effort to update these criteria is under way and is likely to take into account features of antiphospholipid syndrome beyond thrombosis and pregnancy morbidity, such as thrombocytopenia, heart valve damage, and livedo racemosa. Other risk factors surrounding venous (malignancy, major surgery) and arterial (hypertension, hyperlipidemia) thromboemboli are also likely to be better accounted for in the new criteria. Other notable guidelines include expert guidance from the scientific and standardization committee for lupus anticoagulant/antiphospholipid antibodies of the International Society on Thrombosis and Haemostasis, and EULAR recommendations for the management of antiphospholipid syndrome in adults, both of which are referenced above. Areas to watch in future include the development of pediatric specific criteria, continued refinements for best laboratory practices in patients receiving anticoagulant therapy, and more nuanced recommendations for which patients are most likely to benefit from medications beyond warfarin.

Conclusion
In summary, antiphospholipid syndrome is a thrombo-inflammatory autoimmune disease driven by autoantibodies that recognize cell surface phospholipids and phospholipid binding proteins. The result is an increased risk of thrombotic events, pregnancy morbidity, and other autoimmune or inflammatory complications. Vitamin K antagonists remain the most appropriate treatment for most patients with thrombotic antiphospholipid syndrome and, based on current data, appear superior to direct oral anticoagulants. Beyond anticoagulants and anti-aggregants, recent research has highlighted additional potential therapeutic targets within the innate immune system, including the complement system and NETs. The potential role of immunomodulatory treatments in antiphospholipid syndrome management is rightly receiving increased attention.

Some of the most pertinent and persistent questions for the antiphospholipid syndrome field are highlighted in the Questions for future research box. While mechanistic in vitro and in vivo studies will likely continue to prove valuable, we are fortunate that recent advances in biomedical and clinical sciences have created unprecedented
research opportunities to understand disease causes more deeply, and ultimately, improve outcomes through an approach personalized to the care of each individual living with antiphospholipid syndrome. It will be imperative in the coming decade to introduce individual and multimomics approaches to the study of carefully phenotyped patient cohorts, including during interventional clinical trials. Profiling circulating and tissue resident cells, as well as cytokines, metabolism, microbiome, and other areas is likely to be necessary to identify the next generation of safer and more effective treatments for patients.

Provenance and peer review: Commissioned; externally peer reviewed.

Questions for future research

- What adjuvant agents can best suppress breakthrough antiphospholipid syndrome manifestations, especially those that target the microvasculature and thereby threaten organ function?
- Will agents that decrease antibody titers (neonatal Fc receptor inhibitors, plasma cell depleting agents) find a role in the management of antiphospholipid syndrome?
- Can lupus anticoagulant functional panels eventually be replaced with a combination of solid phase assays? If so, what will be the relative roles of IgG and IgM autoantibodies in antiphospholipid syndrome?
- How can we explain the heterogeneity of antiphospholipid syndrome? Will it be defined by a comprehensive understanding of autoantibodies or by other patient-specific factors?
- Anti-β2GPI antibodies clearly contribute to antiphospholipid syndrome pathogenesis; how might these antibodies conspire with autoantibodies such as anti-PS/PT and anti-LPBA/EPCR?

Patient involvement

A draft of the manuscript was shared with three patients who are living with antiphospholipid syndrome. Their feedback led to numerous improvements to the manuscript, especially in the sections that describe non-criteria manifestations of antiphospholipid syndrome, the impact of antiphospholipid syndrome on quality of life, the risks and benefits of currently available treatments, and future directions for antiphospholipid syndrome treatment.

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Contributors: All authors substantially contributed to conception and analysis of the manuscript; drafted the manuscript and revised it critically for important intellectual content; approved the final version to be published; and take responsibility for the accuracy and integrity of the manuscript.


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Circulation patients activate endothelial cells in vitro and in vivo.

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state of Antiphospholipid Syndrome.

In conclusion, the combination of antiphospholipid antibodies and inflammatory cytokines plays a crucial role in the pathogenesis of atherosclerosis. However, more research is needed to fully understand the mechanisms involved and to develop effective therapeutic strategies.

Conflict of Interest: None.

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