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ANTIPHOSPHOLIPID SYNDROME (S ZUILY, SECTION EDITOR)

Clinical Risk Assessment in the Antiphospholipid Syndrome: Current Landscape and Emerging Biomarkers

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Abstract
Purpose of Review Laboratory criteria for the classification of antiphospholipid syndrome include the detection of a lupus anticoagulant and/or anticardiolipin and anti-β2-glycoprotein I antibodies. However, the majority of patients who test positive in these assays do not have thrombosis. Current risk-stratification tools are largely limited to the antiphospholipid antibody profile and traditional thrombotic risk factors.
Recent Findings Novel biomarkers that correlate with disease activity and potentially provide insight into future clinical events include domain 1 specific anti-β_2GPI antibodies, antibodies to other phospholipids or phospholipid/protein antigens (such as anti-PS/PT), and functional/biological assays such as thrombin generation, complement activation, levels of circulating microparticles, and annexin A5 resistance. Clinical risk scores may also have value in predicting clinical events.
Summary Biomarkers that predict thrombosis risk in patients with antiphospholipid antibodies have been long sought, and several biomarkers have been proposed. Ultimately, integration of biomarkers with established assays and clinical characteristics may offer the best chance of identifying patients at highest risk of APS-related complications.

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Introduction

The antiphospholipid syndrome (APS) is one of the most common acquired thrombophilias, and is characterized by recurrent thrombosis and/or obstetrical morbidity in the presence of antiphospholipid antibodies (aPL), specifically lupus anticoagulant (LA), anti-β2-glycoprotein I (anti-β2GPI), and/or anti-cardiolipin (aCL) antibodies [1•]. Thrombi occur most commonly in the deep veins of the lower extremities and the cerebral arterial circulation [2]; however, patients may develop thromboses in more unusual locations such as the hepatic veins, visceral veins, or the cerebral venous circulation. Obstetrical criteria for APS include one or more miscarriages at or beyond the 10th week of gestation, severe pre-eclampsia or eclampsia causing premature birth of one or more morphologically normal neonates before the 34th week of gestation, and/or three or more consecutive, unexplained, spontaneous abortions before the 10th week of gestation (Table 1). Rare patients (<1%) develop catastrophic antiphospholipid syndrome (CAPS) [3, 4], which is diagnosed by the presence of small vessel thrombosis in three or more organs within a period of 1 week in the presence of aPL, and is associated with a high mortality rate (~50%) [1•, 4]. Other manifestations commonly seen in patients with aPL, such as thrombocytopenia, livedo reticularis, skin ulcers, transient ischemic attacks, seizures, and migraine [5, 6] are not included in the diagnostic classification, but should alert physicians to the possibility of APS, especially in patients who also have thrombosis or pregnancy loss.

Table 1 Summary of the Sydney Consensus Statement on Classification of APS [1]

Antiphospholipid antibody syndrome (APS) is present if at least one of the clinical criteria and one of the laboratory criteria are met.

Clinical criteria

1. Vascular thrombosis
   One or more documented episodes of arterial, venous, or small vessel thrombosis in any tissue. Thrombosis must be confirmed by objective validated criteria. For histologic confirmation, thrombosis should be present without significant vessel wall inflammation.
2. Pregnancy morbidity[a]
   i. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or direct examination of the fetus, or
   ii. One or more premature births of a morphologically normal neonate before the 34th week of gestation because of eclampsia or pre-eclampsia diagnosed by standard definitions, or recognized features of placental insufficiency, or
   iii. Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal or hormonal abnormalities, and maternal and paternal chromosomal causes excluded.

Laboratory criteria[b]

1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society of Thrombosis and Hemostasis
2. Anticardiolipin antibody (aCL) of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (>40 GPL or MPL, or > the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA
3. Anti-β_2 glycoprotein-I antibody (anti-β_2GPI) of IgG and/or IgM isotype in serum or plasma with a titer > the 99th percentile, on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA

[a] Investigators are advised to classify subjects with obstetrical morbidity according to groups a, b, and c in populations of patients with more than one type of pregnancy morbidity

[b] Investigators are urged to classify APS patients into one of the following categories: I—more than one laboratory criterion present (any combination), IIa—LA present alone, IIb—aCL present alone, and IIc—anti-β_2GPI present alone

Pathogenesis of APS

Interactions with Coagulation-Related Proteins and Inhibitors

One of most frequently identified prothrombotic mechanisms of aPL is inhibition of natural anticoagulant activities. APL have been reported to inhibit the activation of protein C [7–10] as well as the ability of active protein C to inactivate factors V and VIII [11, 12]. These activities are mediated by antibodies to β2GPI and/or prothrombin [13–16], and may require the presence of phosphatidylethanolamine [17]. In addition, aPL inhibit heparin binding and activation of antithrombin [18], as well as the activity of the tissue factor pathway inhibitor [19]. Antiphospholipid antibodies may also inhibit fibrinolysis, at least in part by neutralizing the ability of β2GPI to stimulate the activity of tissue-type plasminogen activator [20]. Finally, aPL may block the anticoagulant activity of annexin A5 by impairing its ability to form a lattice on procoagulant anionic phospholipids in a β2GPI-dependent manner [21, 22].

Activation of Vascular Cells

There is general consensus that aPL activate vascular cells, a property thought to contribute significantly to the pathogenesis of APS [23, 24]. aPL activate endothelial cells in a β2GPI-dependent manner [25–27]; activation of endothelial cells leads to disruption of the normally anticoagulant endothelial surface and transformation to a prothrombotic phenotype. Endothelial cells activated by aPL demonstrate increased expression of cell adhesion molecules (E-selectin, VCAM-1, ICAM-1) and tissue factor [25, 26, 28], and decreased elaboration of endothelial cell-derived nitric oxide [29]. The pathways and mechanism of cellular activation are not completely defined, and several receptor-mediated pathways have been suggested involving annexin A2, TLR4/NF-κB, LRP-8, TLR2, and TLR7, among others [30, 31, 32•]. Mice deficient in annexin A2, TLR4, or LRP-8, as well as those treated with an NF-κB inhibitor, are relatively protected from the enhanced thrombosis that occurs following passive infusion of aPL [33–36]. In addition to endothelial cells, monocytes are also activated by aPL in the presence of β2GPI; this occurs in lipid raft structures via annexin A2-mediated mechanisms [37], though recent studies have suggested, as with endothelial cells, important roles for several members of the TLR family including TLR2.

Though direct binding of β2GPI to unstimulated platelets has not been well characterized, platelets are activated in the presence of aPL/anti-β2GPI antibodies. In a non-flow system, aPL activate platelets in the presence of subthreshold concentrations of thrombin in a p38 MAP-kinase-dependent manner [38], while under flow, aPL enhance adhesion of platelets to collagen through a process dependent on platelet glycoprotein 1b and apoER2 [39, 40]. Several studies have also demonstrated that aPL interact with placental trophoblasts, leading to an inflammatory response that may underlie the pathogenesis of aPL-associated fetal loss [41].

Complement Activation

The role of complement activation in APS was first demonstrated in murine models of aPL-associated pregnancy loss [42, 43]. Complement products C3a and C5a were found to cause placental inflammation, and mice deficient in C3, C4, C5, or the C5a receptor were protected from fetal loss induced by passive infusion of aPL IgG [44]. Since then, it has been demonstrated that complement activation contributes to aPL-mediated thrombosis in mice as demonstrated by the ability of

C5 inhibition to prevent thrombosis in animals receiving passive infusion of anti-β_2GPI antibodies [45, 46, 47••]. Complement activation by aPL also generates the potent inflammatory mediator C5a, which recruits monocytes and neutrophils, activates endothelial cells, and induces expression of tissue factor [48, 49]. There is some evidence supporting activation of both the classical and alternative complement pathways in patients with catastrophic APS [50•], and several case reports document the successful use of eculizumab (humanized anti-C5a monoclonal antibody) in CAPS and in patients with APS complicating renal transplantation [51•, 52, 53••, 54, 55].

Diagnosis of APS

The classification of "definite APS" is based on the Sapporo criteria, which were first proposed in 1999 [56] and updated in 2006 [1•]. These include clinical and laboratory criteria (Table 1), and at least one of each must be present to make a diagnosis. Since the clinical criteria, thrombosis and pregnancy loss, are relatively prevalent in the general population and have many causes, laboratory investigations are central to the diagnosis of APS. These include the presence of a persistently positive lupus anticoagulant detected according to ISTH guidelines, and/or positive anticardiolipin (aCL) antibodies (IgG or IgM) exceeding 40 IgG or IgM antiphospholipid units, and/or anti-β_2GPI antibodies (IgG or IgM) at levels exceeding the 99th percenle in an enzyme-linked immunosorbent assay. To improve specificity, at least two assays should be performed to evaluate for each of the four ISTH criteria for detecting LA [57]. To minimize the risk of establishing a diagnosis based on transient aPL, recommendations suggest performing assays twice, with samples obtained at least 12 weeks apart [57, 58]. It is important to recognize that these criteria were initially proposed to standardize inclusion of patients into clinical studies. While they are widely applied as diagnostic tools, they were designed primarily for classification and have several shortcomings in clinical practice. For example, they do not account for patients who have persistent LA and/or aPL but have only non-criteria manifestations of APS, or for patients who have clinical criteria for APS but have only low to moderate titers of IgG/IgM aCL and anti-β2GPI. Occasional patients with clinical manifestations of APS lack positivity in any of the standard diagnostic laboratory studies, and are sometimes termed to have "seronegative APS" (Fig. 1), though the specificity of this term is uncertain. Some of these patients may have IgA antibodies against aCL or β_2GPI [59, 60•, 61, 62, 63•], or antibodies against other antigens such as phosphatidylserine, phosphatidylethanolamine, prothrombin, annexin A2 [64, 65], annexin A5 [66], or vimentin/cardiolipin complexes [62, 67].

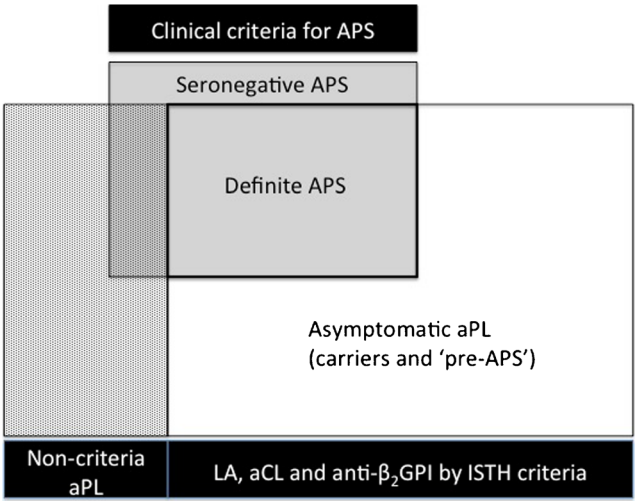

Fig. 1 Antiphospholipid antibodies and APS-related events: A proportion of patients with thrombosis and pregnancy morbidity meeting clinical criteria for APS are positive for aPL by standard diagnostic criteria and are diagnosed with definite APS. Other patients with clinical criteria of APS are negative by standard laboratory criteria, termed "seronegative APS." A large proportion of individuals with persistent aPL represent asymptomatic carriers, or individuals with "pre-APS" who may or may not develop APS-related clinical events in the future. Biomarkers of disease activity may have the greatest utility in the latter two categories

Thrombotic Risk Assessment in APS

The current classification criteria for APS (and aPL) provide relatively little information about the risk of recurrent thromboembolic events in an individual patient. This is an important clinical issue, since current guidelines recommend indefinite anticoagulation for patients with APS. Even more difficult is predicting the risk of thrombosis or obstetric morbidity in an individual with asymptomatic aPL, which may occur in a few percent of healthy individuals without a history of thrombotic events and in as many as 11% to 86% of individuals with systemic lupus erythematosus (SLE) [68].

The aPL profile, which refers to the type (LA, aCL, anti-β2GPI) and number of aPL (single, double, or triple positive), is the most extensively studied and validated risk stratification strategy in patients with aPL; however, it is a relatively insensitive marker for predicting thrombosis, its prognostic value is suboptimal, it may not consistently identify patients at greatest risk for obstetric morbidity, and it is likely to be significantly affected by poorly characterized factors underlying the lack of concordance in results of aPL assays performed in different clinical laboratories. Moreover, the aPL profile cannot be used to assess response to therapy or to identify patients with APS who may safely be treated with a shorter duration of anticoagulation. However, recent insights into the pathogenic mechanisms underlying APS have led to

the emergence of novel biomarkers that reflect disease activity and cellular activation.

Antiphospholipid Antibody Profile

The criteria tests for aPL (LA, aCL, and anti-β_2GPI) detect antibodies with overlapping, but not identical, specificities. Several retrospective and prospective studies have demonstrated that LA positivity is the strongest risk factor for both arterial and venous thrombosis in patients with and without SLE [69]. There is significant variation in strength of association in different studies that may reflect different methods used to detect LA, or the variable inclusion of LA that were not persistently positive. Retrospective and prospective studies have not shown a consistent association between aCL and thrombosis [70, 71]. In a systematic review of 25 observational studies including over 7000 patients, Galli et al. demonstrated that LA were associated with both venous (OR range, 4.09–16.2) and arterial (OR range, 8.65–10.84) thrombotic events; however, aCL were associated with thrombotic events in less than half of the studies [72]. In the Leiden Thrombophilia Study, LA was associated with a higher risk of thrombosis (OR 3.6, 95% CI 1.2–10.9) than anti-β_2GPI (OR 2.4, 95% CI 1.3–4.2) and antiprothrombin (anti-PT) antibodies (OR 1.4, 95% CI 1.0–2.1) [73]. Over the past decade, β_2GPI has been identified as a key antigen in APS, which has led to a focus on anti-β_2GPI as the more clinically significant and predictive aPL. Consistent with this hypothesis, several retrospective studies showed that anti-β_2GPI antibodies indeed correlate with thrombotic risk [73–75]; however, recent studies suggest that the thrombotic risk conferred by anti-β2GPI antibodies may be more modest, with odds ratios between 1.5 and 2.5 [74]. More recent data, discussed below, suggests that epitope specific domain1 anti-β_2GPI antibodies may be more predictive of clinical events. In the systematic review by Galli et al. that included 28 studies with 4394 patients and 1973 controls, only 57% of associations of anti-β2GPI antibodies with thrombosis were significant [72]. The proportion of significant associations increased to 71% when only studies of patients with SLE were considered [72]. However, most of these studies were retrospective, used different methods of measuring aPL, and did not control for other thrombotic risk factors. In the prospective Warfarin in APS (WAPS) study that included 462 patients with persistent LA, IgG anti-β2GPI antibodies were associated with both arterial and venous thrombosis [70]. A meta-analysis of 25 studies examining the association of different aPL with recurrent pregnancy loss demonstrated that LA was most strongly associated with late recurrent pregnancy loss (OR 7.79, 95% CI 2.30–26.45) followed by IgG aCL (OR 3.57, 95% CI 2.26–5.65) and IgM aCL (OR 5.61, 95% CI 1.26–25.03) [76]. This meta-analysis did not comment on the association of anti-β2GPI antibodies with recurrent pregnancy loss due to a lack of methodologically consistent studies evaluating this outcome [76]. Although LA is the single test most predictive of the thrombotic phenotype, current assays for LA such as the DRVVT, while sensitive, are difficult to quantify given the absence of a suitable standard of activity. Thus, LA may be false positive, and a weak LA alone may be an epiphenomenon rather than causative, especially when interpreted in light of the fact that thrombosis is a common occurrence in the general population. Another study has demonstrated that lupus anticoagulants that are dependent upon the presence of β2GPI for their activity may correlate more strongly with a history of thrombosis (OR 42.3; 95% CI 194.3–9.9) than β2GPI-independent LA [77]. These studies require confirmation.

Over the past decade, numerous studies have shown that the risk of thrombosis increases with the number of positive tests for aPL in APS patients as well as individuals with asymptomatic persistent aPL. For example, in the WAPS study, there was a significantly increased risk of thrombosis in patients with both LA and anti-β_2GPI antibodies (OR 4.1, 95% CI 1.3–13.5) [70]. In The Leiden Thrombophilia Study, LA positivity along with either anti-β_2GPI or antiprothrombin antibodies was associated with a significantly increased risk of thrombosis compared to LA alone (OR 10.1, 95% 1.3–79.8) [73]. Pengo et al. reported a cumulative incidence of recurrent thrombosis of 12.2, 26.1, and 44.2% after 1, 5, and 10 years of follow-up in a retrospective analysis of 160 APS patients positive for LA, aCL, and anti-β_2GPI—so-called "triple positive" patients, 123 of whom were on long-term anticoagulation [78]. In a prospective study of 104 triple positive aPL carriers, the rate of thromboembolism was 5.3% per year with a cumulative incidence rate of 37.1% over 10 years [79]. Other retrospective and prospective studies have confirmed the association of triple positivity with thrombosis in adults with APS (OR 5.24, 95% CI 1.5–18.3) [80] and asymptomatic aPL carriers [81•, 82•]. Triple positivity for LA, aCL, and anti-β_2GPI has also been associated with history of late pregnancy loss (OR 16.2, 95% CI 0.9–292) and unsuccessful subsequent pregnancy (OR 34.4, 95% CI 3.5–335.1) [83]. Based on these observations, the 2006 revision of the Sapporo criteria recommended that patients should be classified as those with only one positive aPL and those with two or three positive aPL [1•].

There is minimal debate regarding the association of clinical manifestations of APS with LA, IgG, and IgM isotypes of aCL and anti-β_2GPI antibodies that are included in the diagnostic criteria; however, the clinical importance of isolated IgA aCL and anti-β_2GPI antibodies remains controversial. IgA aPL have been shown to be thrombogenic in murine experiments [84]. Previous studies have also highlighted the high prevalence of IgA anti-β_2GPI antibodies in individuals with SLE, particularly in Afro-Caribbean populations [85, 86]. Others have reported an association between IgA anti-β_2GPI and thromboembo-lic events, especially in

patients with SLE [87, 88]. These antibodies usually occur in combination with other isotypes of anti-β_2GPI making it difficult to evaluate their independent contribution to thrombotic risk. There are several case reports of patients who meet clinical criteria for APS that are positive for IgA anti-β_2GPI antibodies in the absence of IgG or IgM antibodies ("seronegative-APS"). In the absence of standardized assays and well-designed prospective studies, we cannot recommend testing for IgA aPL in all patients with clinical manifestations of APS. IgA aPL testing may be useful in patients with "seronegative APS", especially those with SLE.

"Non-criteria" Antiphospholipid Antibodies

A large number of aPL directed against a variety of phospholipid-binding proteins have been identified [89]. The most promising of these in thrombotic APS recognize two major phospholipid-binding antigens—epitope specific (domain 1) anti-β2GP1 antibodies and antibodies to prothrombin (and phosphatidylserine/prothrombin complexes; Table 2). Others recognize vimentin/cardiolipin complexes, annexin A2 and annexin A5. The significance of these latter antibodies remains uncertain though it has been suggested that they may activate distinct intracellular signaling pathways leading to the pleomorphic manifestations of APS [90]. They may have particular relevance in the evaluation of patients who present with the classical clinical manifestations of APS with negative or subthreshold results on the standard diagnostic assays.

Anti-β_2GPI-domain1 Antibodies

Antibodies to β_2GPI can be directed against any of the five domains of β_2GPI. DeLaat et al. demonstrated that IgG antibodies that recognize the Gly40-Arg43 epitope in the first domain of β_2GPI, called anti-β_2GPI-domain 1 antibodies, are associated with LA activity and are more strongly associated with a history of thrombosis and obstetrical morbidity compared to antibodies directed against other regions of the protein [91, 92]. A prospective study reported that IgG anti-β_2GPI domain 1 antibodies were more often persistent at 12 weeks, associated with triple positivity, and correlated with thrombotic risk [93••]. In a recent study, anti-β_2GPI-domain 1 antibodies predicted clinical events with an OR of 17 (95% CI, 7.1–40.5) although they did not add to the diagnostic accuracy of the standard aPL panel since anti-β_2GPI antibodies were even more sensitive and almost as specific for patients with thrombosis [94]. However, this study also reported that β_2GPI-domain 1 antibodies identified triple positive patients and those with thrombosis and β_2GPI-dependent LA [94]. Mahler et al. detected anti-β_2GPI domain 1 antibodies in 122/144 patients with APS and 1/200 (0.5%) of controls without APS yielding 85% sensitivity and 99.5% specificity [95]. Assays for anti-β2GP1-domain 1 antibodies might be particularly useful in identifying asymptomatic carriers with clinically significant anti-β2GP1 antibodies that may lead to clinical complications. A commercial assay for anti-β2GP1-domain 1 antibodies has been developed (Quanta Flash β_2GPI-domain1, Inova Diagnostics); however, this is generally limited to the research setting.

Antiprothrombin and Antiphosphatidylserine/Prothrombin Antibodies

Anti-PT antibodies are detected in a 50–90% of LA-positive individuals [96]. To be antigenically recognized, prothrombin (PT) must either be coated on activated plates or combined with anionic phosphatidylserine (PS) to form PS/PT complexes. These antibodies are not associated with hypoprothrombinemia in the majority of cases [97]; however, in rare cases, LA-associated hypoprothrombinemia causes a significant bleeding diathesis [98]. Many anti-PT antibodies cause LA activity [97, 99–101]. Anti-PT and anti-PS/PT antibodies can co-exist and appear to represent distinct antibody populations [102]. The clinical significance of anti-PT antibodies, however, is still a matter of debate. While several studies have reported that anti-PT antibodies are associated with arterial or venous thrombosis [103–105], others have failed to demonstrate this association [106–108]. On the other hand, most studies evaluating the significance of aPS/PT antibodies have demonstrated an association with venous thrombosis [104, 105, 107–111]. Consistent with this, a systematic review of data from over 7000 individuals from 38 studies evaluating anti-PT and 10 studies evaluating anti-PS/PT as a marker of thrombosis noted that there was a stronger association of anti-PS/PT (OR 5.11, 95% CI 4.2–6.3) than of anti-PT with arterial or venous thrombotic events (OR 1.82, 95% CI 1.44–2.75) [112]. Of the seven studies that evaluated both anti-PS and anti-PS/PT, 90% identified an association of anti-PS/PT with thrombosis compared with only 45.5% that identified an association of anti-PS with thrombosis [112]. In another study evaluating 23 possible combinations of aPL specificities as a predictor of APS-related clinical events in 230 patients with SLE, a combination of LA, anti-PS/PT, and anti-β2GP1 had the best diagnostic accuracy for both thrombosis and pregnancy loss [113]. This has not yet been validated in patients with primary APS. Although well described, the association of aPT with thrombosis appears to be less strong than that of LA or anti-β2GP1 [114, 115]. While the current evidence is not enough to recommend routine testing for anti-PS/PT and anti-PT antibodies in patients with APS, this remains a promising area of investigation, and antibodies specific to

Table 2 Selected studies of associations of anti-β_2GPI-domain1 and anti-PS/PT antibodies with thrombosis in APS

Study	Population	Number of subjects	Prevalence	OR (95% CI)
Anti-β_2GPI-domain1				
De Laat et al. 2005 [91]	SLE, AI disease, primary APS	198	57.6% of anti-β_2GPI-positive samples	18.9 (53.2–6.8)
De Laat et al. 2009 [92]	Persistent anti-β_2GPI	477	55%	3.5 (2.3–5.4)
Pengo et al. 2015 [93]	APS with anti-β_2GPI	65	69.2%	5.43 (1.66–17.73)
De Craemer et al. 2016 [94]	APS and AI disease	426		29.2 (8.8–95.9)
Anti-PT antibodies				
Galli et al. 1997 [107]	aPL-positive	59	IgM 37.3%	No association
Atsumi et al. 2000 [108]	AI disease	265	IgG: PAPS 15%, SLE APS 42%, SLE no APS 20% IgM: PAPS 5%, SLE APS 4%, SLE no APS 6%	1.14 (0.54–2.43)
Bertolaccini et al. 1998 [103]	SLE	207	28% IgG 14% IgM 10%	2.49 (1.33–4.63)
Bertolaccini et al. 2005 [104]	SLE	212	IgG 24.5% IgM 5%	2.8 (1.5–5.3) 1.4 (0.5–4.2)
Tsutumi et al. 2006 [105]	SLE	139	25%	3.55 (1.22–10.35)
Pengo et al. 2010 [106]	LA positive	231	IgG 26% IgM 62%	1.4 (0.6–3.0)
Anti-PS/PT antibodies				
Galli et al. 1997 [107]	aPL-positive	59	IgM 66% IgG: PAPS 19%, SLE APS 63%, SLE-no APS 13%	No association
Atsumi et al. 2000 [108]	AI disease	265	IgM: PAPS 10%, SLE APS 29%, SLE-no APS 4%	2.92 (1.33–6.40) [APS manifestations except thrombocytopenia]
Bertolaccini et al. 2005 [104]	SLE	212	IgG 16% IgM 6%	3.5 (1.8–6.6) 5.3 (2.3–11.9)
Tsutumi et al. 2006 [105]	SLE	139	21%	4.59 (1.55–15.56)
Zigon et al. 2011 [109]	AI disease Healthy controls	203 222		–
Vlagea et al. [110]	aPL-positive	57	IgM 26.3 IgG 21.1	7.44 (3.97–13.92) 2.54 (1.35–4.77)
Pregnolato et al. [111]	APS	80	81.3	IgG: 4.77 (1.28–17.75)

PT, particularly anti-PS/PT, may prove useful risk stratification tools in APS.

Antiphosphatidylethanolamine Antibodies

Phosphatidylethanolamine (PE) is one of the primary lipid components of the cell membrane. While sera from APS patients usually react with negatively charged phospholipids and cofactors (e.g., β2GP1), sera reactive with PE, a neutral phospholipid, are less commonly observed. In several reports, aPE antibodies have been reported in patients with SLE and thrombosis in the absence of LA and aCL [116••, 117, 118], as well as in other patients with vasculopathy and livedo reticularis [119, 120]. In an analysis of 140 patients with thrombotic events and 136 controls, aPE was the only non-criteria aPL significantly more prevalent in patients than in controls (14.3 vs. 5.1%, $P = 0.014$) [121]. In another multicenter study, aPE were found in 15% of patients compared with 3% of controls [122]. Interestingly, 63% of the aPE-positive patients were negative for the standard serologic criteria for APS and the majority of them had venous thrombosis, half of which was recurrent VTE [122]. In contrast, a study by Bertolaccini et al. failed to demonstrate an association of aPE with thrombotic events in SLE [103]. Although plasma reactivity to PE is associated with LA activity and aCL, the direct relationship of aPE with LA, or with thrombotic mechanisms in APS, is not clear [123, 124]. Moreover, there have been

no standards developed for standardization of anti-PE measurements, and their associations with clinical events have been reported by only a limited number of laboratories. While there is currently insufficient data to recommend testing for aPE in patients with APS, this might be considered in patients with seronegative APS.

Antibodies to Annexin A2 and Annexin A5

APS is associated with resistance to the anticoagulant effect of annexin A5 [125]. This property has been proposed to distinguish patients with APS from asymptomatic individuals with aPL as well as patients with venous thromboembolism but no evidence of aPL [126]. Anti-annexin A5 antibodies have been described in APS [127], and have been associated with placental thrombosis and fetal absorption in a mouse model. However, clinical studies have failed to consistently demonstrate a strong association with thrombosis [127] or pregnancy complications [128, 129]. Annexin A2 is implicated in aPL medicated cellular activation [26]. Although anti-annexin A2 antibodies have been described in APS, their clinical significance is uncertain.

Antibodies Against Vimentin/Cardiolipin Complexes

Vimentin is a ubiquitous cytoskeletal protein. In patients with SLE, antivimentin antibodies have been described that correlate with aCL [130]. Ortona et al. identified vimentin-cardiolipin complexes as an antigenic target in APS and demonstrated antivimentin/cardiolipin antibodies in 92.5% (37/40) of patients with APS and 55.2% (16/29) with seronegative APS [67]. Antivimentin/cardiolipin antibodies induced interleukin receptor-associated kinase phosphorylation and nuclear factor-κB activation in endothelial cells suggesting a pathophysiologic role. However, it is not clear that these antibodies are an APS-specific biomarker since they were present in 16.7% and 6.7% of subjects with rheumatoid arthritis and non-APS-related thrombosis, respectively.

Clinical Risk Scores

In addition to the aPL profile, investigators have developed several risk scores combining clinical and/or laboratory findings in an attempt to better identify individuals at risk of APS-related thrombosis. The antiphospholipid score (aPL-S) includes LA, aCL, and anti-β_2GPI positivity and titers, and was developed to predict risk of APS-related clinical events in patients with autoimmune disorders [131] and subsequently validated in an independent cohort of patients with SLE [132]. The global APS score (GAPSS) was initially developed to predict both APS-related thrombosis and pregnancy loss in a cohort of patients with SLE [133]. In contrast to the aPL-S, the GAPSS included conventional cardiovascular risk factors in addition to aPL profiles; points assigned on the basis of a multivariable prediction model were 3 for hyperlipidemia, 1 for arterial hypertension, 5 for aCL IgG/IgM, 4 for anti-β2GPI IgG/IgM, 3 for antiphosphatidylserine/prothrombin IgG/IgM, and 4 for LA, and appeared to improve prediction of APS-related clinical events compared to aPL profile alone; a score GAPSS values ≥10 had the best diagnostic accuracy. The authors subsequently validated this score in a cohort of patients with primary APS [134•]. Independent validation of the GAPSS in a Japanese cohort of patients with autoimmune disease as well as primary APS also confirmed higher scores in patients with thrombosis, with maximum diagnostic accuracy for GAPSS >6; however, the predictive value of the GAPSS for pregnancy loss could not be validated [135]. Concerns about this scoring system include weighting aCL, for which the relationship to thrombosis is uncertain; greater than lupus anticoagulants; and the inclusion of antiphosphatidylserine/prothrombin IgG/IgM that are not routinely performed. Also, the optimal cutoff on the GAPSS score was different in all of these studies, which may be attributed to differences in the baseline characteristics of the cohort. While the GAPSS score may add to the utility of aPL in predicting thrombosis in APS, it still needs to be validated in patients with asymptomatic aPL and whether it will prove useful in clinical practice remains to be determined.

Other Risk Factors for APS-Related Clinical Events

While persistence and high levels of aPL, along with the aPL profile, are the major risk factors for thrombosis in APS, the presence of traditional risk factors such as inherited thrombophilia, systemic inflammatory disorders such as SLE, cancer, obesity, immobilization, smoking, pregnancy, the use of oral contraceptives, and a history of previous thrombosis also increase thrombotic risk [1•]. SLE, hypocomplementemia, decreased platelet counts, and a previous history of thrombosis and pregnancy failure are also additional risk factors for pregnancy failure [136]. The PROMISSE (Predictors of Pregnancy Outcome: Biomarkers in Antiphospholipid Antibody Syndrome and Systemic Lupus Erythematosus) study identified the presence of LA (OR 8.32, 95% CI 3.59–19.26), physician global assessment score >1 (OR 4.02, 95% CI 1.84–8.82), and low platelet count (OR 1.33, 95% CI 1.09–1.63 per 50×10^9/L) as predictors of adverse pregnancy outcomes [137]. Uterine Doppler ultrasound parameters can also identify women with SLE or APS at risk for obstetric complications [138, 139].

Emerging Biologic and Functional Biomarkers

Recognizing the limitations of current diagnostic and risk stratification tools in APS, there has been increasing interest in novel biomarkers based on recent insights into

pathophysiologic mechanisms underlying APS that may correlate better with disease activity and could help in evaluating response to anticoagulation and other therapies.

The thrombin generation assay, a global coagulation assay that evaluates the generation of thrombin under in vitro conditions that attempt to approximate in vivo conditions, is one of the earliest markers to be evaluated as a measure of "clotting potential" [140, 141]. There has been limited evaluation of thrombin generation assays in APS; however, early studies demonstrated that anti-β_2GPI antibodies with LA activity cause prolongation of the lag time similar to the prolongation in clotting times in the DRVVT and aPTT assays [142]. Moreover, in patients with LA, there is a marked inability of activated protein C to diminish peak thrombin generation indicating acquired resistance to protein C [143–145]. Devreese et al. demonstrated that the ratio of peak height (of thrombin generation) and lag time correlated reliably with LA activity detected in standard mixing tests [144]. They supplemented this approach with measurement of P-selectin and factor VII, markers of hypercoagulability, to develop a layered strategy with sensitivity and specificity for future thrombotic events [146]. More recently, Efthymiou et al. used thrombin generation assays to evaluate intensity of anticoagulation in thrombotic APS and non-APS patients [147••]. Endogenous thrombin potential and peak thrombin generation correlated inversely with INR; however, 20% of patients with APS had increased peak thrombin generation that exceeded the expected relative to the intensity of anticoagulation assessed by INR, suggesting that thrombin generation may be a useful tool for monitoring ongoing hypercoagulable states in patients with APS on anticoagulation [147••]. Although thrombin generation assays are largely limited to the research setting, they may prove useful for monitoring anticoagulation efficacy in thrombotic APS, particularly for those who develop recurrent thromboses despite anticoagulation.

Activation of vascular cells by aPL effects is central to the pathogenesis of APS. Microparticles, submicron particles released from all cells in response to stimuli such as cellular activation and/or apoptosis [148], have been evaluated as a biomarker in APS. Several studies have reported elevated numbers of circulating endothelial cell- and platelet-derived microparticles in patients with APS [149–155]. MP from APS plasma also demonstrate elevated TF activity [156]. While some studies noted a correlation between levels of MP and thrombotic complications [150, 153], others have failed to demonstrate this association [149, 154, 155]. In patients with aPL, elevated levels of MP are present remote from the time of thrombotic events, and anticoagulant therapy does not reduce MP levels, indicating that anticoagulation masks but does not address the chronic pro-inflammatory and prothrombotic state underlying APS. Microparticles are an attractive candidate biomarker in APS, both to predict thrombotic risk and to monitor efficacy of therapy. However, MP measurements have been plagued by a lack of standardized methodology for isolation, quantification, and functional analyses, as well as lack of reproducibility of measurements in individual patients on repeated testing. Concerted efforts to address these issues are needed.

Given the role of complement in the pathogenesis of APS-related complications, complement markers have been evaluated as biomarkers in APS. In primary APS, hypocomplementemia may be associated with LA, as well as livedo reticularis and thrombocytopenia [157]. While increased levels of complement activation products, indicating complement activation, have been reported in patients with primary APS, data regarding their correlation with thrombosis are conflicting [158, 159]. Complement activation (elevated alternate pathway convertase C3bBbP terminal complement components sC5b-9) has been demonstrated and is likely involved in the pathogenesis of catastrophic APS [157]. Developing validated assays for complement activation through measuring complement activation products or using novel functional assays [160] could potentially aid in the diagnosis of catastrophic APS, and also might predict responses to eculizumab in this disorder. Others have evaluated gene expression signatures and proteomic approaches to predicting the risk of clinical events [161]. These novel approaches may yield clinically useful markers and insights into pathophysiology.

A relatively unexplored area of research is the role of genetic predisposition in APS-mediated clinical events. It is possible that polymorphisms in key proteins involved in anti-β2GPI antibody-mediated signaling or effector pathways may render certain individuals more susceptible to thrombosis or pregnancy loss induced by these antibodies. For example, one study demonstrated that mice in which the inflammatory response to LPS was absent due to a missense point mutation in the cytoplasmic tail of TLR4 did not display enhanced thrombosis after passive infusion of human aPL [33], while those with wild-type TLR4 did. Moreover, co-segregating TLR4 Asp299Gly and Thr399Ile polymorphisms were found to occur with lower frequency in patients with APS vs. healthy controls; however, the frequency in patients with aPL without thrombosis was not determined.

Conclusions

Tools to individualize thrombotic risk assessment are critical for the optimal management of individuals with persistent aPL, with or without APS-related complications. Current approaches are mostly limited to the aPL profile and traditional thrombotic risk factors. Recent developments include domain 1 specific anti-β_2GPI antibodies, other aPL, clinical risk calculators, and "biologic" assays based on pathophysiology such as thrombin generation and complement activation. Prospective studies will be needed to design and validate

layered approaches that integrate standard diagnostic criteria with newer analytic assays to improve APS diagnosis. In addition, laboratory strategies to identify patients that can safely be treated with a shorter duration of anticoagulation, and those with persistent hypercoagulable states on anticoagulation, would be useful. Concerted efforts are required to validate, standardize, and implement these promising new strategies for patients with APS.

Compliance with Ethical Standards

Conflict of Interest Shruti Chaturvedi and Keith R. McCrae declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance,
- •• Of major importance

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