How I treat

How we diagnose the antiphospholipid syndrome

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The antiphospholipid syndrome (APS) is an acquired thrombophilia, characterized by the occurrence of venous and arterial events. This article examines the laboratory and key clinical aspects of APS. Particular focus is given to anti–beta 2-glycoprotein I (β2GPI) antibodies in view of their recent inclusion in the APS classification criteria. The clinical utility of using the β2GPI enzyme-linked immunosorbent assay, in conjunction with the established lupus anticoagulant assays and cardiolipin enzyme-linked immunosorbent assay, for diagnosing and risk stratifying patients suspected of having APS is discussed. The relative importance of the various assays in diagnosing obstetric APS (early and late gestation miscarriages) is explored. The implications of recent epidemiologic findings for possibly understanding the underlying pathophysiologic mechanisms of obstetric APS are highlighted. Insights into which patients with obstetric APS may be at most risk of thrombotic complications are presented. (Blood. 2009;113:985-994)

Introduction

The antiphospholipid syndrome (APS) is an important cause of acquired thrombophilia and recurrent miscarriages. This narrative style review discusses the key laboratory and clinical aspects of APS. Particular focus is given to antibodies against beta 2-glycoprotein I (β2GPI), in view of their recent inclusion in the APS laboratory classification criteria1 (Figure 1). The evidence for assessing antiprothrombin and antiphosphatidylethanolamine antibodies to diagnose APS is also examined.

The utility of the β2GPI enzyme-linked immunosorbent assay (ELISA), when used in conjunction with the cardiolipin (CL) ELISA and the lupus anticoagulant (LA) assays, in risk-stratifying APS patients is explored. Work undertaken by many groups over the years, ours included, in delineating the key characteristics of anti-β2GPI antibodies that associate with APS is presented.

Miscarriages are a major feature of APS. The relative importance of the LA assays, the CL-ELISA, and the β2GPI-ELISA in diagnosing APS in the context of early and late gestation miscarriages is assessed. The value of recent epidemiologic and basic science insights in refining our understanding of obstetric APS pathophysiology is examined, particularly with regards to considering the possibility that distinct mechanisms may be responsible for early and late miscarriages. The clinical implications arising from these observations are discussed.

Clarification of the nomenclature

Antiphospholipid antibodies is a term applied to antibodies detected traditionally by 2 types of assays, the CL-ELISA, which stems from the earlier work of Harris et al,2 and the LA assays.3 The first report of a cofactor requirement for antibodies to bind cardiolipin (an anionic phospholipid) from patients with APS was by McNiel et al in 1989.4 This was subsequently confirmed by Galli et al5 and Matsuura et al6 in 1990. Purification and sequencing of the cofactor as β2GPI was reported by McNiel et al in 1990.7 It was noted that anionic phospholipid is not an absolute requirement for antibodies to bind β2GPI8 negating the notion that β2GPI is a “cofactor” for antibody binding. Antibodies from these patients can bind to β2GPI immobilized on an irradiated plate in the absence of anionic phospholipids.9 A negatively charged surface serves a 2-fold role. It enables β2GPI clustering, allowing divalent binding by the low affinity antibodies.10,11 It also enables the β2GPI molecule to undergo a conformational change,9 exposing a cryptic epitope on the first domain.12 Positivity on the CL-ELISA is not directed against β2GPI in the context of a number of infections.13 However, this is not true for all types of infections, as elevated anti–β2GPI antibodies in patients with leishmaniasis, leptospirosis,14 and leprosy15 have been noted. A key distinguishing feature between anti–β2GPI antibodies occurring in the context of leprosy and those that strongly associate with thrombosis is that in the former instance they are directed against an epitope on domain V of β2GPI,15 whereas evidence generated by a number of independent groups suggests in the context of thrombosis they target a specific epitope on domain I16-20 (Figure 2). In addition, anti–β2GPI antibodies that associate with the APS clinical phenotype are predominantly the immunoglobulin G (IgG) isotype, particularly the IgG2 subclass,21 whereas those occurring in association with infections such as leprosy are of the IgM isotype.22 The LA assay detects either anti–β2GPI23-25 or antiprothrombin antibodies,26,27 with the latter also occurring in association with infections, such as leprosy.22 Hence, even though it is a misnomer, the term antiphospholipid antibody as a generic term persists when referring to antibodies that specifically target β2GPI and prothrombin.

Overview of the assays

Lupus anticoagulant

The LA is an assay that detects immunoglobulins that, although prevent coagulation in vitro, associate with thrombosis (Figure 3).28 It is an important tool for diagnosing APS.1 The LA assay system chosen has to comply with the 3-step strategy defined in the International Society of Thrombosis and Hemostasis criteria:26; (1) screening test: demonstration
of the prolongation of a phospholipid-dependent clotting time beyond
the upper limit of the reference interval; (2) mixing test: confirmation of
the presence of an inhibitor and the exclusion of a coagulation factor
deficiency; and (3) confirmation that the inhibitor is phospholipid-
dependent and not directed against a specific coagulation factor. Hence,
evidence for the presence of LA is provided by performing a sequential
series of investigations, which are demarcated by the terms screening
tests and confirmatory tests, the latter divided into mixing studies and
tests assessing for phospholipid dependence.

There are different assays that can be used to screen for LA, although
a number of surveys performed in different countries suggest that some of the commonly used ones are the activated
partial thromboplastin time (aPTT), the dilute Russell’s viper
venom time (dRVVT), and the kaolin clotting time. A description
of the various assays and the methodology for their performance
can be found in published guidelines. In view of the fact that no
additional screening test is recommended. One of the screening
tests undertaken should use activation of the intrinsic pathway of
coaulation (eg, aPTT or kaolin clotting time) and the other the
direct activation of factor X (eg, dRVVT). Local reference ranges
should be established for each LA method used and for each
clotting time.

The mixing studies involve combining the patient’s plasma with
normal plasma (1:1) and assessing the influence of this procedure on
clotting time, the theoretical underpinning being that if prolong-
ation of clotting time is the result of a coagulation factor
deficiency, it will correct to normal, whereas LA, correction
requires larger volumes of normal plasma. A number of methods
have been proposed by which this step can be interpreted, the
most robust being the calculation of the index of circulating
anticoagulant, initially proposed by Rosner et al.

To determine whether the inhibitor is phospholipid-dependent,
the platelet-neutralization procedure, which uses either washed
platelets activated with calcium ionophore or platelets lysed by
repeated freeze-thawing (leading to the exposure of anionic
phospholipid surfaces), is commonly used. Correction of the
clotting time will occur in the presence of LA. Alternate reagents to
assess for this property include a modified aPTT reagent, which
contains hexagonal (II) phase phospholipids, which specifically
bind LA. With regard to the dRVVT confirmatory tests, alterna-
tives to the platelet-neutralization procedure that can be considered
include reagents with a high concentration of phospholipid or an
LA-insensitive phospholipid.

It is pertinent to note that there is considerable interlabora-
tory variation with the performance of the LA assays. National
surveys carried out in Europe, the United States, and
Australia emphasize this issue. In recent surveys there was a
false-positive detection of LA in 24% of samples and a
false-negative result from 18.5% of participating centers. One
of the factors that might contribute to a false-positive result is
heparin contamination. This possibility may be assessed for by
measuring the thrombin time and, if prolonged, determining
anti-FXa activity. Other reasons for false-positive results
include the presence of specific anticoagulation factor antibod-
ies. The patient’s history, assessing for a bleeding diathesis,
is relevant in this setting. Preanalytical variables, such as
improper plasma preparation, may lead to false-negative results
because of contamination with platelets. The diluting effect of
mixing studies may also lead to false negatives in instances in
which the LA is weak. In one externally conducted quality
control study, half of the weak LAs were not detected. There
is evidence to suggest that adherence to published guidelines may
improve the performance of LA testing.

It is recommended that an assessment for LA not be undertaken
while the patient is anticoagulated. However, if it is necessary to do so,
LA can be detected by such methods as assessing the taipan snake
venom/ecarin clotting times because they are relatively insensitive to
oral anticoagulation. Alternatively, the performance of the dRVVT on
equal volume mixtures of normal and test plasma can be considered,
with a similar procedure for the confirmation steps. A positive result
is useful, although a negative result may be unreliable.

Regardless of the method chosen to test for LA, internal
laboratory quality controls are important, particularly the utiliza-
tion of known positive and negative LA controls with each batch of
testing. An attempt in the direction of standardization of the LA
test has also been made by the development of monoclonal
antibodies with LA activity for spiking normal plasma to overcome restrictions in the availability of patients samples. In addition, developing reference standards from lyophilized LA plasmas of known potency is being considered (International Society of Thrombosis and Hemostasis Scientific Subcommittee Session 2007). An update of the criteria for the optimal performance of LA is anticipated in the near future.

To refine the clinical utility of LA testing, investigators have been interested in differentiating the LA immunoglobulin according to target antigen given reports that 2GPI-dependent LA may more strongly associate with thrombosis than non–2GPI-dependent LA. These novel assays take advantage of the distinct properties of 2GPI-dependent LA to bind cardiolipin and to display enhanced phospholipid binding in the presence of calcium chloride, compared with non–2GPI-dependent LA. The value of these tests awaits further clinical trials.

The cardiolipin ELISA

This ELISA involves the assessment of diluted patient serum to binding a CL-coated plate in the presence of bovine serum. It will detect antibodies that bind CL alone and those that bind CL-bound bovine 2GPI. Both types are termed anti-CL antibodies (aCL; Figure 4). An inherent weakness of this assay is that there remains the potential to miss patients with antibodies that bind human but not bovine 2GPI. Hence, some assays now use human 2GPI in the CL-ELISA.

<table>
<thead>
<tr>
<th>Type of event</th>
<th>Anticardiolipin isotype</th>
</tr>
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<tbody>
<tr>
<td>Cerebral Stroke</td>
<td>Any</td>
</tr>
<tr>
<td>Deep Vein Thrombosis</td>
<td>First</td>
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<td></td>
<td>Recurrence</td>
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<td>Any Thrombosis</td>
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Figure 3. The lupus anticoagulant (LAC) correlates better with thrombosis than aCL. From Galli et al28 with permission.

Figure 4. Schematic representation of the cardiolipin ELISA, which detects a number of antibody specificities, including 2GPI. From Passam and Krilis108 with permission.
IgG and IgM αCL are expressed in international standardized GPL and MPL units, respectively. These units should be derived using standardized IgG and IgM αCL calibrators.52 GPL and MPL units are defined as binding observed with 1 μg of affinity-purified polyclonal IgG and IgM αCL that were distributed to participating laboratories.52 Secondary standards were ultimately calibrated against the primary standards. Hence, different batches of calibrators will contain heterogeneous polyclonal αCL from different patients. This has led to suggestions that these calibrators may not necessarily behave in a homogeneous fashion when assayed at different dilutions or using different kits.53 This is underlined by the observation of considerable interlaboratory variability,51 even when using the same batch of calibrators. There is also a high degree of variability between different commercial kits for αCL detection when assessed within the same laboratory, with lower variability seen with different commercial anti-β2-GPI kits.54 Methodologic insights pertaining to the optimal performance of the CL-ELISA have recently been published by Pierangetil and Harris.55

A positive result is defined as a medium or high titer (ie, either >99th percentile, or >40 GPL or MPL).1 To satisfy the APS laboratory classification criteria, a patient has to be persistently positive on either one of the assays (CL-ELISA, LA, or β2-GPI-ELISA) for at least 12 weeks.

The direct β2-GPI ELISA

This type of ELISA involves coating-purified, native β2-GPI directly onto an irradiated plate. In theory, this type of assay should detect a greater proportion of clinically relevant antibodies than the CL assay. However, observations that there exist “nonpathogenic” antibodies that bind β2-GPI within this assay12 suggest that human β2-GPI binding an artificial plastic surface may expose additional neoepitopes, thus reducing the specificity of this assay in detecting clinically relevant antibodies. This may in part explain why some studies have found no association of thrombosis with positivity on the direct β2-GPI-ELISA.56

There remains a lack of a formal, universally accepted method for performing this ELISA, coupled with a lack of standardized calibrators. The commercial kits are calibrated by the manufacturers’ own calibrators and expressed in arbitrary units. This has prompted calls to use universally standardized calibrators, such as the humanized monoclonal antibodies HC AL (IgG) and EY2C9 (IgM).57,58 Despite this, there generally seems to be better interlaboratory consensus with this ELISA compared with the CL assay.59,60

In the revised APS classification criteria, a positive result on the direct β2-GPI-ELISA is defined as a titer greater than the 99th percentile.1

Characteristics of anti-β2-GPI antibodies that associate with thrombosis

The β2-GPI molecule is divided into 5 domains (DI-DV).61 Anti-β2-GPI antibodies may predominantly target domain I in APS patients.16 There is some controversy, as a number of other epitopes on β2-GPI have been reported.62 This area has been extensively reviewed by Giles et al,63 who concluded that the major APS epitope is probably on domain I. The major β2-GPI domain I epitope includes the surface-exposed residues Gly40-Arg43.17,18 It is a complex conformational epitope, with evidence suggesting it may include Arg39, the domain I-II interlinker, and Asp8 and Asp9.20 Nondomain I anti-β2-GPI antibodies (which target domain V) may be detected in patients with leprosy15 and childhood atopic dermatitis.63

de Laat et al50 analyzed patients with thrombosis who were positive on the LA assay and satisfied the classification criteria for APS. The predominant autoantibodies were targeting β2-GPI,50 involving the domain I residues Gly40-Arg43.19 Non-β2-GPI antibodies with LA activity did not independently associate with thrombosis50 (Figure 5). The importance of anti-β2-GPI, relative to non-β2-GPI antibodies with LA activity, in the context of thrombosis has been suggested by others.64,65 Antiprothrombin and anti-β2-GPI antibodies may occur concurrently.66

Anti-β2-GPI antibodies may be divided into high and low avidity, and it is the former that tend to associate with thrombosis.67-71 The 2 types may be distinguished by their ability to dissociate from immobilized β2-GPI in the presence of increasing concentrations of urea67 or ionic buffer.70 Both types have been detected in patients with systemic lupus erythematosus (SLE).67,68

The epitopes to which the low- and high-avidity antibodies bind has not been determined. de Laat et al19 divided anti-β2-GPI antibodies into type A (those targeting the epitope on domain I) and type B (those targeting nondomain I epitopes) in patients with SLE, lupus-like illness, and APS. They found that it was the type A antibodies that associated with thrombosis. It should be noted this association has not been confirmed in rigorous prospective studies. It seems reasonable to hypothesize that high-avidity antibodies probably target the epitope containing the residues Gly40-Arg43, whereas the low-avidity antibodies may be representative of the type B, nondomain I antibodies.

The corollaries to these investigations would seem to be that (1) a patient who tests positive on the LA assay and the direct β2-GPI-ELISA may be likely to have a greater proportion of the high-avidity, domain I-targeting anti-β2-GPI antibodies than a
patient who tests positive on the direct β2GPI-ELISA alone, and (2) the LA in this context is likely to be related to anti-β2GPI antibodies, compared with a patient who tests positive on the LA assay alone. Whether these possibilities explain the greater association with thrombosis described in patients positive on the direct β2GPI-ELISA and the LA assay, compared with positivity on either assay alone,72 needs further analysis.

High-risk profile of patients who test positive on the CL-ELISA, the direct-β2GPI ELISA, and LA

A strong association in a number of retrospective analyses has been noted between positivity on multiple assays (LA, CL, and β2GPI-ELISA) and thrombosis and miscarriages, compared with patients positive on one or 2 assays72-76 (Figure 6). What do these findings imply? Patients positive on 3 assays (CL, direct β2GPI-ELISA, and LA) tend to have higher levels of anti-β2GPI antibodies than patients positive on fewer assays73 (Figure 7). Hence, this may be a factor. Potentially, they may also be the patients who have high-avidity, domain I binding anti-β2GPI antibodies. Certainly in the future, should assays that specifically detect antibodies with β2GPI-dependent LA activity become widely available,19,48,49 then these possibilities can be assessed.

There are other possibilities to be considered as to why testing positive on multiple assays may be associated with greater risk. There may be an additive pathogenic effect between multiple distinct antibodies. For this latter notion to be firmly supported, one of the requirements would be the demonstration of an association between the non–β2GPI-reactive autoantibodies detected by the relevant assays with thrombosis, independent of the presence of anti-β2GPI antibodies. Studies in this regard pertaining to antiprothrombin antibodies are not consistent, with some suggesting they may associate independently77 and others suggesting they do not.50,65

Antiprothrombin and other antibodies

It was noted by Galli et al,56 in their systematic review of the literature, that positivity on the prothrombin ELISA did not consistently associate with thrombosis. Furthermore, a recent study has suggested that this assay does not provide additional utility for diagnosing APS.78 However, it has been proposed that positivity on an ELISA that detects antibodies targeting the prothrombin-phosphatidylethanolamine complex may more consistently associate with thrombosis79; hence, this is an area of ongoing research. At the moment, testing positive for antiprothrombin and antiprothrombin/phosphatidylethanolamine antibodies is not a laboratory criterion for APS classification.1

In recent studies, it was noted that a positive result on an assay that specifically detects antibodies targeting phosphatidylethanolamine associates with thrombosis80 and recurrent miscarriages.81 However, the utility of this assay and other assays that detect antibodies that directly target various types of phospholipids needs further validation, as there are reports, assessing commercially available assays, which question their ability to improve the yield of APS diagnosis.82

Considerations arising at the laboratory-clinical interface of APS diagnosis

Venous thrombosis

There is evidence derived both from the SLE83,84 and the general population85,86 that LA associates with venous thrombosis. These observations support the notion that LA may be a potential risk factor for venous thromboembolism (VTE). As a point of contrast, in recent longitudinal prospective studies,87,88 which analyzed the general population, positivity on the CL-ELISA did not associate with the occurrence of a first VTE. There are data, however, to suggest that the CL-ELISA may have utility in the prognostic setting. Schulman et al89 noted that, on cessation of warfarin after 6 months of treatment for a VTE, patients who were positive on the CL-ELISA, compared with patients who were negative, had a higher risk of recurrence. The interplay between antibody profile, the type of initial VTE event (below knee, above knee, nonlower limb deep venous thrombosis, or pulmonary embolus), and the presence or absence of concurrent risk factors for VTE (transient, eg, pregnancy; or permanent,
eg, genetic thrombophilia) in modulating the risk of VTE recurrence is an area warranting further delineation in prospective studies. These types of studies may allow the development of a risk-stratified approach in guiding the optimal duration of anticoagulation in APS patients who have sustained a VTE.

**Arterial thrombosis**

LA has been found to associate with stroke in the SLE and the general population settings.\(^9\) The association may be greater in young adult stroke patients.\(^9\) In view of these findings, it is highly relevant for a physician who orders these tests after a patient has sustained an initial stroke, to know whether a patient’s risk for stroke recurrence or other thrombotic events is different relative to a patient who has had a stroke and does not have these antibodies. The importance of knowing this information is worth considering. If the risk for recurrence is the same, all else being equal (ie, the type of stroke: cardioembolic or noncardioembolic, the type of treatment and its duration), then the implication is that patients who are antibody positive do not have a worse prognosis and hence do not need to be treated differently to those who have had a stroke and do not have the antibodies. It also implies that information obtained from large prospective, randomized controlled trials assessing stroke treatment, which involve the general population, may be directly relevant to those who are antibody positive. On the other hand, if the risk for thrombotic recurrence is greater in positive patients, then the implication is that trial data from the general stroke population cannot be confidently extrapolated to antibody-positive stroke patients. The stroke population with antibody positivity would need to be assessed as an entity with distinct therapeutic implications.

The large, prospective, observational, Antiphospholipid Antibodies and Stroke Study, nested within a much larger randomized controlled trial involving the general stroke population,\(^3\) addressed this question. It concluded that patients from the general population who have sustained an initial noncardioembolic stroke and are positive for LA and/or the CL-ELISA (low, medium, and high titers included), within 30 days of the stroke (patients were tested once), do not have a different prognosis than those who test negative. This result was consistent within the aspirin and warfarin (low to moderate range international normalized ratio) arms of the trial.

It remains to be assessed in future prospective studies, whether testing positive on multiple assays (CL-ELISA, direct \(\beta_2\)-GPI-ELISA, and LA) is associated with a greater risk of stroke recurrence relative to the risk of thrombotic recurrence in patients who are antibody negative. The prognostic value of antibody persistence (at least 12 weeks apart) and medium or high titers on the ELISAs also needs to be assessed in the prospective setting.\(^1\)

**Distinct risk profile of persistent versus transient antibodies**

**Implications for the recruitment of APS patients for clinical trials**

Danowski et al\(^9\) noted, in a cohort of SLE patients who had persistent positivity on the direct \(\beta_2\)-GPI-ELISA, on at least 2 occasions, at least 12 weeks apart, an association with thrombosis to a greater extent than transient positivity. They also demonstrated that positivity on the direct \(\beta_2\)-GPI-ELISA correlates with LA positivity.\(^9\) Whether persistently elevated anti-\(\beta_2\)-GPI antibodies more strongly correlate with LA than transient ones was not addressed. This would seem a reasonable assumption, in view of the association between anti-\(\beta_2\)-GPI antibodies with LA activity and thrombosis.\(^5\) The implications of this, if verified, would be that persistent and transient anti-\(\beta_2\)-GPI antibodies may be distinguished by the presence or absence of LA activity, respectively.

The possibility that patients who initially test positive on multiple assays (LA, CL, and \(\beta_2\)-GPI-ELISA) are more likely to remain positive on repeat testing at a future time interval is also suggested by the study of Nash et al.\(^9\) They reported that the presence of persistently elevated IgG aCL, at levels more than 60 GPL, was a strong predictor of the presence of coexisting LA and positivity on the direct \(\beta_2\)-GPI-ELISA.\(^9\)

It was noted by Crowther et al,\(^94\) in their randomized controlled trial assessing secondary prophylaxis in APS patients, that one of its limitations was that the first 3-month period after the initial thrombotic event was not included in the analysis because patients had to satisfy the APS laboratory criterion for persistence. This is a legitimate concern because there is currently no validated manner to predict who will remain persistently positive. Trial organizers risk enrolling patients who do not satisfy the classification criteria for the syndrome if they randomize patients after they initially test positive. This possibility is emphasized by a recent study\(^95\) looking at young adults who had sustained a stroke and were noted to have a patent foramen ovale. Twelve had elevated titers (> 99th centile of normals) of IgG and IgM anti-\(\beta_2\)-GPI antibodies on initial testing, and yet on repeat testing at 12 weeks, only 1 of the 12 remained positive. Similar outcomes were seen using the CL-ELISA (IgG and IgM). None of the patients was initially positive for LA.

The importance of being able to analyze the first 3 months after an initial thrombotic event, in any future trials involving APS patients (perhaps when assessing the effectiveness of the newer generation of anticoagulants), can be inferred from the general population. It is not only a high-risk period during which thrombotic recurrences may occur,\(^8\) but it is also a period of increased bleeding risk associated with anticoagulation.\(^9\) Potentially, patients who initially test positive on all 3 assays (LA, CL, and \(\beta_2\)-GPI-ELISA) may enable the study of this critical period by allowing early randomization of patients who are likely, on subsequent testing, to satisfy the APS laboratory criterion of persistence.

**Obstetric considerations**

A meta-analysis has assessed the strength of association of positivity for LA, the \(\beta_2\)-GPI-ELISA, or the CL-ELISA, with recurrent miscarriages, focusing on women who did not have SLE (the period between 1975 and 2003 was analyzed).\(^9\) It was noted that a positive LA strongly associated with late gestation miscarriages (defined by the authors of the study as < 24 weeks), although its association with early miscarriages (defined by the authors as < 13 weeks) was not assessed.\(^8\) It should be noted that these definitions for early and late miscarriages are different from those in the APS classification criteria, in which early is defined as before 10 weeks’ gestation and late as miscarriage at or beyond 10 weeks.\(^\) In the same analysis,\(^8\) positivity on the \(\beta_2\)-GPI-ELISA did not associate with early recurrent miscarriage (< 13 weeks), although an assessment for an association with late miscarriages was not undertaken. In contrast, antibodies detected by the
CL-ELISA associate with both early (<13 weeks) and late (<24 weeks) recurrent miscarriages.98

Ruffatti et al75,76 have noted in 2 retrospective studies that there is a stronger association with recurrent fetal loss (>10 weeks gestation) and thromboembolic events in women who are positive on all 3 assays (CL, direct β2GPI-ELISA, and LA), compared with women who are either dual or single assay positive. Furthermore, a recent study has noted that positivity on the β2GPI-ELISA associates with a positive LA in women with miscarriages.99 Hence, one possibility warranting further consideration is whether anti-β2GPI antibodies with LA activity may specifically associate with late miscarriages.

It is relevant to note that in in vivo studies100-102, documenting the role of complement and inflammation in the pathogenesis of fetal loss, in which a murine model of APS was used, the specific human monoclonal antibody used (mAb 519) binds cardiolipin in the absence of β2GPI.103 Polyclonal antibodies from 3 patients diagnosed with APS were also used, although the antigen specificity of the antibody fraction responsible for the experimental murine fetal resorption via a complement-inflammatory-mediated mechanism was not determined.100 As a point of contrast, in a study conducted by Robertson et al,104 a spontaneously occurring murine monoclonal anti-β2GPI antibody directed against domain I, and patient-derived polyclonal antibodies with β2GPI reactivity did not cause murine fetal resorption, nor did they affect fecundity in all mice.

Hence, a conceptual synthesis of the current epidemiologic and in vivo observations raises the possibility that non-β2GPI antibodies detected by the CL-ELISA may be particularly relevant in early miscarriages, perhaps via the induction of an inflammatory mechanism. Anti-β2GPI antibodies with LA activity may mediate a more prominent effect in late gestation miscarriages via distinct mechanisms, perhaps by inducing intrauterine placental thrombosis (in view of the strong association of this class of antibodies with thrombosis19,50). The pathogenic mechanisms operational in association with late miscarriages in women who have anti-β2GPI antibodies may not be reflected in murine APS models, perhaps because of the differences between human and murine placental development.105 The development of obstetric APS animal models with closer homology to human placentation may be relevant to delineate the role of anti-β2GPI antibodies in the latter part of pregnancy.

It is important to confirm in future studies whether distinct antibodies are involved with different obstetric manifestations, as it may lay the foundation for exploring within clinical trials novel therapeutic approaches (eg, modulation of inflammation, such as targeting tumor necrosis factor-α,102 vs further modulation of coagulation, perhaps with novel anticoagulants), based on antibody profile. The possibility is also raised that the risk of maternal thromboembolic events occurring in a patient with obstetric APS may vary according to whether they have anti-β2GPI antibodies with LA activity (perhaps higher risk), or non-β2GPI-dependent CL antibodies (perhaps lower risk). This possibility, if verified in prospective studies, may have implications when considering in which obstetric APS patients (without a history of previous thrombotic events), the administration of postpartum thromboembolic prophylaxis treatment may be most warranted.

Some suggestions for future research

The relevant future research agenda, as can be inferred from this discussion, is broad and multifaceted. The development of novel assays that detect the clinically relevant anti-β2GPI antibodies and their subsequent validation will be of great utility. In addition, the validation of assays that assess for antibodies that specifically target prothrombin/phosphatidylserine and phosphatidylethanolamine is important.

Prospective, controlled epidemiologic analyses examining the relevance of issues, such as antibody profile in risk stratification, will be of value in informing decision making with regards to prognosis, laying the foundations for future therapeutic APS trials.

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Our perspective on diagnosing APS

When considering the importance of individual assays for diagnosing thrombotic APS, greater weighing would currently have to be given to a positive LA test, based on the systematic reviews of Galli et al.28,56 If a patient tests positive on all 3 assays (LA, β2GPI, and CL-ELISAs), confidence in the notion that the patient may have high titers of anti-β2GPI antibodies with LA activity is increased. The practical implication for initially testing with all 3 assays is as follows: Oral anticoagulants can potentially interfere with the performance of the LA assay,32 which has ramifications when considering repeating the LA test to assess for persistent positivity at 3 months to establish the diagnosis of APS. Knowing that the patient initially tested positive on all 3 assays suggests that the β2GPI and/or the CL-ELISAs can be performed as an alternative to LA at this later time point to enable a definitive diagnosis of APS to be made.

The type of thrombosis of particular relevance for considering APS, based on clinical association studies, appears to be venous thrombosis and perhaps stroke (particularly young adults). It is important when diagnosing thrombotic APS to also assess for concurrent prothrombotic factors, as they may be present in a significant proportion of patients, and some may be treatable in their own right (eg, traditional atherosclerotic risk factors).106 We incorporate investigation with a trans-esophageal cardiac echo to thoroughly assess for a possible cardiac source of the arterial thrombus (cerebral and noncerebral events) in view of the relevance of this mechanism, which has been highlighted by Fulham et al.107

In the obstetric APS setting, testing positive on all 3 assays (LA, β2GPI, and CL-ELISAs) seems to have implications for which patients may be at a greater risk for thrombosis.75,76 Hence, this may be one reason why it may be useful to perform all 3 assays when considering diagnosing obstetric APS, particularly in the context of late miscarriage (10 weeks and beyond). However, a case can also be made that, if a patient has only had recurrent early miscarriages (<10 weeks) without a history of thrombosis, then the performance of the β2GPI-ELISA and perhaps LA assays, on the basis of current evidence,99 cannot be confidently supported. In this situation, the CL-ELISA may have better utility in diagnosing APS.98 The exclusion of other causes of miscarriages is necessary.1

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How we diagnose the antiphospholipid syndrome

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