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MINIREVIEW

Testing for and Clinical Significance of Anticardiolipin Antibodies

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DEFINITION

The disputed nomenclature of the antibodies associated with the antiphospholipid syndrome (APS) can easily confuse the reader. In this paper we use the historical terms; thus, "antiphospholipid antibodies" (aPL) refers to antibodies associated with the clinical syndrome of venous and arterial thrombosis, recurrent fetal loss, livido reticularis, thrombocytopenia, and other, less common manifestations, termed APS. This syndrome may be primary or secondary in association with other autoimmune conditions such as systemic lupus erythaematosus (SLE). aPL can be divided into the lupus anticoagulants (LA), detected by in vitro clotting tests, and antibodies detected by solid-phase enzyme-linked immunosorbent assays (ELISA), which include anticardiolipin antibodies (aCL). This review covers the solid-phase assays for aPL, but not the LA tests usually performed in a hematology laboratory.

These terms are used to maintain historical and keyword search consistency. Clinically significant aCL detected in the aCL ELISA and also in some cases of LA are in fact largely directed to a plasma protein, β_2-glycoprotein I (B2GPI) (48, 50, 62), with the B2GPI itself binding to phospholipid in the assay. True aPL, although detected in the aCL ELISA, are false positive and are usually not associated with the APS. In addition, cardiolipin may not necessarily be the best phospholipid for detecting aCL, and phosphatidylserine, phosphatidylethanolamine, and phospholipid mixtures have also been used; some of these may also detect different antibodies (82, 86). The reader will see the terms "antiphospholipid/cofactors syndrome," "antiphospholipid-protein syndrome," and "Hughes' syndrome" used elsewhere referring to APS; no doubt, further terms will be introduced when the physiologic mechanism of the antibodies becomes clear.

CLINICAL UTILITY OF aCL ANTIBODIES

The aCL assay serves to assist in the diagnosis of APS. This diagnosis has important implications for the treatment and prediction of recurrent thrombosis and recurrent miscarriage. Anticoagulation with coumarins to an international normalized ratio (INR) of 2 to 3, standard after an episode of venous thromboembolism, appears inadequate in APS, and a target INR level of 2.5 to 4.0 (25) or >3.0 (52) has been suggested, although the risk of hemorrhage is increased. APS-associated recurrent pregnancy loss is improved by the addition of low-dose heparin and aspirin therapy (54, 70), although again there are risks, of bleeding and osteoporosis. Unfortunately, the prediction of further thrombosis or miscarriage after only the first episode (i.e., the confident diagnosis of APS) is difficult, as the aCL test is quite nonspecific and has a low positive predictive value, which improves somewhat if the LA test is also positive. This situation may improve with the use of the ELISA for B2GPI antibodies as a confirmatory, specific test after a positive screening result for aCL if the encouraging results in the early series hold true.

HISTORICAL PERSPECTIVE

aCL were initially recognized in the most basic sense as a test for syphilis using beef heart extract and subsequently were found to be directed against the cardiolipin in the mixture (68). Subsequently, a group of patients with false-positive syphilis test results were recognized (64): they had either other chronic infections such as leprosy or autoimmune disease such as SLE. Cardiolipin itself is a doubly anionic phospholipid consisting of 2 diacylglycerol groups covalently linked via phosphodiester bridges (each with a single negative charge at physiologic pH) to a central glycerol backbone (36). The double anionic charge of CL is, however, not necessary in that antibodies (and B2GPI) bind to phosphatidylserine, which has a single anionic charge, and some (with kininogens but not B2GPI) bind to phosphatidylethanolamine (82, 86), which is zwitterionic.

Although the association between LA and thrombosis in SLE was known, the description by Harris et al. (41) of the detection of aCL by radioimmunoassay led to the term "anticardiolipin syndrome" (46) and subsequently to the more inclusive (with LA) "APS" (42), both as part of a wider autoimmune disease such as SLE and in isolated or primary form. The situation has changed dramatically since the recognition that the clinically significant antibodies detected in the aCL assay are directed against B2GPI bound to cardiolipin (62). B2GPI is a phospholipid binding plasma glycoprotein, for which roles as a natural anticoagulant (16, 77) and in immune clearance (22, 74) have been suggested. Other antibodies detected in the LA test or the aCL ELISA may actually be directed against phospholipid itself or against other plasma proteins such as prothrombin (34), protein C, protein S (66), and annexin V (58), although these have not yet been demonstrated in multiple patient groups to have independent pathological significance. Research in the last decade focused on the interactions between antibodies, plasma proteins such as B2GPI and prothrombin, and binding surfaces such as phospholipid or irradiated, negatively charged polystyrene surfaces in "high-binding" ELISA plates.

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SENSITIVITY AND SPECIFICITY OF aPL

Assessment of true sensitivity and specificity requires a "gold standard" for comparison. No such gold standard test exists for APS. The generally used standard for comparison is the preliminary classification criteria of Alarcon-Segovia et al. (4). There are several potential causes of type I and type II errors in applying this method as a "gold standard" test.

Firstly, Alarcon-Segovia et al. (4), in a large group of patients already with a diagnosis of SLE, made an a priori requirement for positive aCL, before determining the clinical features that were most frequent in the subgroup with the positive aCL. Modified criteria including LA positivity as equivalent to a high aCL titer have been generally used, but the original data has not been republished with this inclusion. It is well established, however, that patients with primary or secondary APS may have a negative aCL result but may be positive for LA or occasionally anti-B2GPI in isolation (39), sometimes even with a nontreated polystyrene ELISA plate (17).

Secondly, the aCL assay does not have a standard distribution, with most normal-population sera having no detectable antibodies. The usual practice of defining a significant aCL result as one greater than 95 or 98% of the population has no inherent validity when the same result is used as an a priori requirement for presence of the disease; it simply states who one is allowing to have the disease. Although in APS the antibody titers do correlate with the severity of the disease in a patient group (28), it is established that in the low range of antibody titers some patients have significant clinical disease: as the patient's antibody level may fluctuate over time into the normal range and may also fall at the time of thrombosis (26), he or she may not be diagnosed with the syndrome. Recurrent thrombosis may even occur at the time of a normalized aCL level (78).

The combined aCL and LA tests, although assumed to be highly sensitive (but see above), are not specific. aCL are also found in infections (48) such as hepatitis C (20), malaria, lyme disease, syphilis, and HIV; leukemias and solid-organ malignancies; and frequently in alcoholic cirrhosis (21). LA are also found in children with infections such as varicella, in the elderly (49), and in cases of drug reactions (27, 67). Repeating the test in 6 weeks may help in acute infections, as in this instance the aPL are transient. In addition, elevated aCL levels are commonly found in the elderly, and 51% of a well, medication-free nursing home population with a mean age of 81 years was positive (56). In a blood donor study (80) the patients falling outside the normal range (mean ± 2 standard deviations [SD]) of the LA test were mostly young women, which is the typical population for APS in SLE, but there was no such association with aCL for age or isotype.

Thirdly, a positive test result (be it aCL, LA, or both) for a person without clinical features need not be a false-positive result. APS does not appear to be a uniform predilection to thrombosis but, rather, a spectrum of severity that may depend not only on antibody isotype and titer but also on antibody avidity (87) and other "hits" such as activated protein C resistance, smoking, oral contraceptive use, serum homocysteine level (the other prothrombotic state that can produce arterial, venous, and cerebral sinus thrombosis), and acute factors such as trauma or immobilization.

aCL ASSAY DATA ANALYSIS

The data set produced by the aCL ELISA can be described as a proportion of tests with a zero result (this proportion varies between groups being tested), the remainder of the results following an exponential curve with increasingly few data points at increasingly high test levels. This data therefore is not normal, and parametric tests such as defining simple SD from the mean are inappropriate. Most results are standardized by using GPL (IgG phospholipid binding units) controls supplied by the Anti-Phospholipid Standardization Laboratory, Louisville, Ky., giving a normal range of 0 to 20 ± 15 GPL units (depending on the lab; see footnote *b* in Table 1), with some abnormal results in APS patients over 100 GPL and very occasionally over 200 GPL; similar standards for immunoglobulin M (IgM), and in some laboratories IgA, are also used.

An appropriate means of dealing statistically with this data is to transform the data set to a log data set (3) after adding a value such as 1 to all data points. The data set then should approximate a log-linear set and can be treated normally. This approach is particularly useful for defining the normal range of a control group—hence what result range is abnormal—but is not terribly helpful at defining the likelihood of APS in the abnormal range when the tested population has a high proportion of results in the abnormal range; in a sample population of 500 SLE patients (3) 53.2% had results greater than 2 SD above control mean, and in the group with no manifestation of APS more than 30% were positive for aCL. The authors did find a better correlation between higher titers (i.e., the ranges of 5 to 10 SD and >10 SD) and clinical manifestations of disease, but at no point was the aCL titer essentially obligatory for disease. Interestingly, this was not the case for clinical disease, where the presence of four or more clinical manifestations was 99% specific for the aCL to be positive.

A second approach is to use receiver (or response) operator characteristic curves. These are generated by plotting sensitivity against (1 − specificity), the "falsely positive in health" rate (28, 75). Different points on this curve can be chosen with various sensitivities, at the price of false-positive results. Perhaps more generally applicable are stratum-specific likelihood ratios (28), the ratios between the pretest probability and the posttest probability for different strata of antibody titers (i.e., high, medium, low, and normal). Unfortunately, there is a tendency to give posttest probabilities in published series rather than likelihood ratios, but posttest probabilities are much more dependent upon the prevalence in the population being tested than is the case for likelihood ratios; fortunately, point-specific likelihood ratios can be calculated simply as sensitivity/(1 − specificity) (75) and, if required, at several cutoff points. The other major advantage of the likelihood ratio technique is that it provides clinicians with a direct numeric way of relating the estimated clinical probability of the patient having the condition (the pretest probability) with the outcome of the test. As an example, an elderly patient on tamoxifen gets a first case of deep venous thrombosis after an intercontinental flight. A clinical estimate of the probability of her having APS might be 50:1 against. The finding of a moderately positive aCL result (31 to 70 GPL in this instance) giving a stratum-specific likelihood ratio of 1.9 (28) shouldn't trouble the clinician when it becomes apparent that the posttest probability is still 26:1 against.

METHODOLOGY OF THE aCL ELISA

A large number of "homemade" protocols and commercial kits are now available to test for aCL, the radioimmunoassay having been superceded. The core features, however, are reasonably invariant. Despite this, a multicenter evaluation of nine commercial kits found an astounding discordance in outcomes, with simple positivity rates varying from 31 to 60% for IgG and from 6 to 50% for IgM for a selection of patient and

nonpatient sera; slopes of regression lines between the declared units of Anti-Phospholipid Standardization Laboratory standards and that assessed with kit calibrators ranged from 0.159 to 0.931 for IgG and from 0.236 to 0.836 for IgM (71).

(i) Cardiolipin diluted in ethanol with or without chloroform is coated onto standard, nonirradiated 96-well polystyrene plates by vacuum or overnight drying at 4°C, in some instances under nitrogen to prevent oxidation. The prevention of lipid oxidation, while sound general lab practice, may be of little benefit here, as the oxidation of cardiolipin may increase its net negative charge and hence binding efficiency. One important variation here is the use of phospholipids other than cardiolipin, including phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, and various mixtures of some or all. Although extensive research into phospholipid specificity took place in the previous decade (see the review by McNeil et al. [63]), it is likely that the results for APS patients reflect variable B2GPI binding, instead of specificity for particular phospholipids as previously argued. The exception to this may be the specific binding of aPL/anti-kininogen antibodies to phosphatidylethanolamine (13). The issue of which phospholipid(s) to use in the general aPL ELISA is still debated, with particularly good results obtained by a proprietary phospholipid mixture in the wet workshop of the 7th International Symposium on Antiphospholipid Antibodies, New Orleans, La., 1996 (69) using preselected test sera, although this performance was not repeated in a more recent prevalence study (24).

(ii) Blocking of nonspecific binding sites is generally done with diluted adult bovine serum in buffer, usually phosphate-buffered saline. This is of particular relevance, as in this instance the blocking of nonspecific binding sites actually provides the specific antigen B2GPI, which is absorbed mostly onto the bound phospholipids but possibly in part directly onto the plate itself. Any variation in the amount of adult bovine serum, the dilution and volume in the wells, and any change in cardiolipin (such as oxidation or stock differences) that affects the binding of B2GPI will influence the test. These are some of the reasons for such variation in the range of reported results even when standardization sera are used. Variations using highly purified bovine albumin or gelatin in the buffer are performed before and after the addition of exogenous B2GPI to obtain a measure of the B2GPI-dependent subfraction of the primary aCL versus the B2GPI-independent subfraction (59).

(iii) Application of the test serum usually takes place after dilution with more blocking buffer, again containing bovine B2GPI and other serum proteins. There is also human B2GPI in the test sample. Often the test is done in parallel wells, one of which does not contain phospholipids, and the no-phospholipid optical density is subtracted. The antibodies present in the test sera can have very diverse specificities, including not only phospholipids and B2GPI but also prothrombin, protein C, protein S (66) and kininogen (82); for these reasons and others the finding of low-range antibody binding is a very common problem and detracts significantly from the overall sensitivity of the test.

(iv) Application of second antibody and color development is by standard means. Immunoglobulin isotype-specific second antibody is used to assay aCL of IgG, IgM, and in some instances IgA, although IgA correlates poorly with disease for most groups (3). The IgM assay can be confounded by the presence of increased polyclonal IgM (23) or rheumatoid factor (2), which contributes to the problem of the frequency of low-titer IgM results.

(v) Heating of plasma or serum prior to testing converts negative samples to positive (43) including in normal individuals (18); this should therefore be avoided in a clinical assay setting.

(vi) Although it has been suggested that the use of the detergent Tween 20, 0.05% in buffer, may distinguish between B2GPI-dependent and -independent aCL (57), there is evidence that this may completely suppress the binding of B2GPI to cardiolipin (6) or simply detach cardiolipin from the plate (19); we therefore do not recommend the use of Tween 20.

ANTI-B2GPI ELISA METHODOLOGY AND TESTING: CLINICAL UTILITY OR RESEARCH USE ONLY?

One of the most difficult clinical issues in APS is the lack of specificity of the aCL for the diagnosis, leading to the possibility of false-positive diagnoses, both obscuring the true diagnosis and possibly leading to unnecessary anticoagulation. A clinically useful increase in specificity can be achieved with a phospholipid-free anti-B2GPI ELISA.

B2GPI appears necessary as a cofactor for the binding of aCL that are associated with APS but not aCL associated with infection (48) when purified patient antibody is used in a serum-free ELISA. Indeed, the binding of infectious aCL may be inhibited by B2GPI (48, 59). The amount of B2GPI required in the aCL ELISA for optimal binding is 8 μg/ml or more (at 50 μl/well), and binding disappears below 1 μg/ml (62). B2GPI is present at ≈200 μg/ml in human serum and two to three times greater levels in bovine serum (72). Most but not all aCL will also bind bovine B2GPI (8, 61). Antibody diluted in 10% fetal calf serum or adult bovine serum will therefore easily contain adequate B2GPI (assuming it is not a specifically anti-human-B2GPI serum that is being tested), but the binding of test serum diluted to 1:100 or greater in serum-free blocking solution without extrinsic B2GPI will be significantly impaired (72).

Attempts to turn this B2GPI dependency of the aCL ELISA into a quick and simple test as a direct anti-B2GPI ELISA have been less successful, and the test has not yet been standardized nor have accepted units been determined, even after Matsuura et al. (60) recognized the requirement for irradiated "high binding" plates in most but not all cases (17). These are commercially available and use significantly more irradiation than the level needed for sterilization.

The anti-B2GPI test using irradiated plates achieves good specificity but suboptimal (about 40 to 60%) sensitivity in testing the serum of typical primary or secondary APS patients (see Table 1) (24). The anti-B2GPI ELISA reported by Guerin et al. (37) was more successful, with high titers of anti-B2GPI antibodies in all patients with definite clinical APS and a non-overlapping distribution of titers in patients with definite APS and those with other known disorders (SLE, stroke, infectious mononucleosis). We believe the differences in findings between different groups may have resulted from crucial methodological variations rather than the sensitivity-specificity trade-off.

Firstly, some B2GPI preparations may be partially cleaved (47) at Lys317-Thr318, which is close to the phospholipid binding site at Cys281-Cys288, dramatically reducing phospholipid binding. Plasmin and to a lesser extent factor Xa may cleave B2GPI at this site (65). A commercially available source of B2GPI we considered was mostly cleaved at Lys317-Thr318 (47).

Secondly, the APS patient antibodies are mostly of low avidity and bind avidly to B2GPI only when bivalent binding is achieved (73, 88), although the antibody population is heterogenous and a small number of antibodies are of high avidity and can bind human or bovine B2GPI on untreated ELISA plates (9). This low-avidity binding requires adequate antigen

density: in the paper by Roubey et al. (73) human antibodies (but not high-affinity mouse monoclonal antibodies) began to bind only when the coating concentration reached 1 μg/ml, and from the provided data binding appeared to be still increasing at 5 μg/ml, on that particular plate. Guerin et al. (37) and Forastiero et al. (31) used B2GPI at 2 μg/well, which is up to 20 times greater than the concentrations used in other studies. It has been suggested by Koike et al. (53) (although Roubey [73] et al. differ) that a comparable amount of B2GPI is absorbed whether or not the plate is first irradiated as measured with high-affinity murine monoclonal antibodies; however, human anti-B2GPI antibodies do not bind well when B2GPI is coated on untreated microtiter plates.

The orientation and clustering of B2GPI may be important because as each single molecule of antibody is by definition monoclonal (or monogamous) in that each arm has the same idiotype, antigen that is arranged on the plate in such a way as to present each molecular epitope of B2GPI at the optimal distance and in the correct spatial orientation will be far more readily bound. It has been demonstrated that a dimer construct of B2GPI is bound more avidly than native B2GPI by polyclonal human anti-B2GPI (79). Fab′ fragments of anti-B2GPI antibodies do not bind native B2GPI (73) or dimerized B2GPI (79). An alternative, not incompatible, hypothesis is that cryptic epitopes on B2GPI may be revealed by binding-induced conformational changes (53).

Thirdly, the buffer used for coating the plate with the antigen varies; prevalence studies using carbonate buffer (37, 87) achieved higher sensitivity for APS than those with Tris-buffered saline. It is not clear on what basis coating buffer selections were made, and the ideal method often requires extensive empirical testing (83).

The fourth issue is of minimizing low-level and nonspecific binding. Antibodies from some patients not only bind phospholipid and B2GPI but may also be directed to prothrombin, annexin V, protein C, and protein S, as indicated above. These may be present in the serum tested and may bind to unblocked sites on the wells, although this is a lesser problem than with the aCL, where various serum proteins are also added in the bovine serum. Additionally, low-affinity antibodies may be redistributed significantly towards equilibrium with the fluid phase during each wash, cumulatively reducing the amount of bound antibody with each wash and thus reducing sensitivity. The duration of each wash before the buffer is discarded, and the number of washes may be of importance but has not been examined to our knowledge.

USE OF THE aCL AND ANTI-B2GPI ELISAs WITH PATIENT POPULATIONS

Table 1 summarizes the findings of some clinical series, focusing for simplicity's sake on the ability of a test to predict thrombosis in APS. Unfortunately, not all papers could be included for reasons of space or availability in a format suitable for comparison. The extensive studies by Alarcon-Segovia et al. (3, 4), although essential for an understanding of the subject, were conducted with the intention of determining the clinical features of APS and not the utility of the test for diagnosing APS. We do not believe that there is sufficient homogeneity to allow meta-analysis.

The findings listed in the table suggest that the aCL ELISA is neither very sensitive nor at all specific, whereas the anti-B2GPI ELISA, although not sensitive, is sufficiently specific (with positive results indicating sufficiently high relative risk for APS) to influence management decisions about prospective treatment.

LA

The recognition of the association between thrombosis and LA has a longer history than that for the aCL assay (14). The paradoxical nature of the apparent in vitro anticoagulation meant that it was some time before the isolated association of LA and thrombosis was treated with prolonged anticoagulation (55). LA reportedly have a better specificity for APS than do the aCL (44). Although it is not the intention of this review to address LA in detail (see the review by Exner [29]), we regard the LA as an obligate component of the testing process for aPL, and this includes rechecking for LA when repeating the aCL assay. Performing more than one LA screening test is important for optimal sensitivity (84); a common procedure is to perform both the kaolin clotting time (KCT) and the dilute Russell viper venom time (DRVVT) procedures. The KCT tests the clotting cascade more broadly, including contact activation, whereas the DRVVT, using limiting quantities of phospholipid and direct activation of factor X to X_a, focuses on the prothrombinase reaction. Careful checking for other causes of assay prolongation such as factor deficiencies, specific antifactor antibodies, or inhibitory anticoagulants such as heparin and confirmation of correction with excess phospholipid are essential (15).

PROTHROMBIN ANTIBODIES

The prolongation by LA of prothrombin-dependent clotting times and the occasional association of LA with hypoprothrombinemia and a bleeding tendency (10) suggested a role for antiprothrombin antibodies. Studies have confirmed immunoglobulin binding to human prothrombin absorbed on phospholipid (12) or on irradiated phospholipid-free plates (7) in some but not all cases of LA. Further studies, unlike those for anti-B2GPI, have not shown an association between these antibodies and a thrombotic tendency (31, 38, 44) other than that in the unusual association of APS with myocardial infarction (85) and that reported in one recent study not directly giving comparative data on anti-B2GPI (11). The links between a more abnormal KCT result and prothrombin-dependent aPL (32) and between a more abnormal DRVVT result and B2GPI-dependent aPL also have not been confirmed (45, 51). It appears that the antiprothrombin ELISA is of only investigational utility at this stage, but improvements in the technique (33, 34) may change this view.

TGT

We recently described a novel method (patent pending) of testing for aPL called "thrombin generation time" (TGT) (40). To a mixture of normal plasma, thromboplastin, and a chromogenic thrombin substrate in a standard 96-well plate, test plasma is added (mixture/test plasma ratio, 2:1) and then activated by the addition of calcium; the substrate cleavage curve is plotted by using a dynamic ELISA reader as a measure of thrombin generation. Test plasmas with LA activity or anti-B2GPI activity from APS patients and monoclonal anti-B2GPI antibodies prolong the TGT. The TGT test is more sensitive than LA, can be used with multiple samples, and does not require a coagulation laboratory.

CONCLUSION

The aCL assay is only one of the methods used to detect aPL, and the test should be administered with the LA and anti-B2GPI assays. The aCL assay is reasonably sensitive but

TABLE 1. Tests used to predict thrombosis[a]

Reference, patients, and test[b]	Sample size	Prev[c]	Positive test[d]	PPV[e]	Sens[e]	Spec[e]	Odds ratio[f]	Signif[g]
35,[h] DVT/PE patients: aCL > 33	90	1	0.21	NA	(0.21)	NA	5.3[i]	0.01
28,[j] any thrombosis, virtually all SLE patients								
aCL > 65.1	117	0.21	0.10	0.75	NA	NA	11.6	Yes
aCL 21.4–65.0	117	0.21	0.37	0.26	NA	NA	1.3	No
aCL < 21.3	117	0.21	0.53	0.06	NA	NA	0.3	Yes
1, any thrombosis, SLE patients								
aCL > 24	390	0.05	0.47	(0.07)	(0.65)	(0.54)	1.4[i]	0.114
aPTT > 35 s	390	0.05	0.17	(0.15)	(0.50)	(0.85)	3.38[i]	<0.001
30,[k] any thrombosis								
aCL > 40	252	0.10	0.28	(0.24)	(0.65)	(0.76)	3.66[l]	<0.01
Past thrombosis	360	0.09	0.325	(0.21)	(0.74)	(0.72)	4.90[l]	<0.005
5,[m] PAPS or SAPS vs SLE without APS								
aCL > 5	120	0.59	0.52	(0.81)	(0.70)	(0.76)	2.8	NA
Anti-B2GPI	120	0.59	0.33	(0.95)	(0.54)	(0.96)	13	NA
81,[n] any thrombosis: aCL > 20 or LA present	474	0.09	0.28	(0.22)	(0.64)	(0.76)	2.7	NA
44,[o] SLE with any thrombosis vs SLE without thrombosis								
LA	175	0.25	0.25	0.59	0.59	0.86	4.3	NA
aCL > 10	175	0.25	0.47	0.34	0.64	0.59	1.5	NA
Anti-B2GPI	175	0.25	0.17	0.47	0.32	0.88	2.6	NA
37,[p] definite or probable APS vs various others								
aCL > 30	280	0.08	0.23	0.31	0.87	0.83	5.1	NA
Anti-B2GPI	280	0.08	0.08	0.90	0.83	0.99	106	NA
78[q] DVT/PE patients								
Recurrence of VTE, aCL > 5	412	0.16	0.17	0.29	(0.30)	(0.86)	2.1[l]	Yes
Death, aCL > 5	412	0.07	0.17	0.15	(0.33)	(0.85)	1.8[l]	No
76, APS vs not APS in SLE patients with a positive aCL: anti-B2GPI	65	0.45	0.34	(0.82)	0.62	(0.89)	5.6	0.0001[r]

[a] Prev, prevalence; PPV, positive predictive value; Sens, sensitivity; Spec, specificity; Signif, significance; DVT/PE, deep venous thrombosis/pulmonary embolus; NA, not available; VTE, venous thromboembolism, aPTT, activated partial thromboplastin time; PAPS and SAPS, primary and secondary APS, respectively.

[b] The standard to which the test is compared is indicated. For APS this was definite clinical cases unless otherwise stated, for venous thromboembolism this was diagnosis by appropriate tests (i.e., Doppler ultrasound, high-probability ventilation perfusion scan, or in some cases pulmonary angiography), and for arterial thrombosis tests depended on the affected organ. All IgG aCL units are standardized to GPL sera as supplied by the Anti-Phospholipid Standardization Laboratory, unless otherwise indicated. Despite this, the normal range of healthy controls somehow varies tremendously, ranging from <5 U (78) to <24 U (1). The Physicians study (35) used a different reference standard, supplied by M. Lockshin. The 95th and 98th percentiles in that study were at 33 and 38 U, respectively. We suggest that their data in raw units should not be compared to data from other studies. Anti-B2GPI ELISAs are not standardized, so no units are given. Only IgG anti-B2GPI results have been cited in this paper, although IgM and IgA anti-B2GPI results may also be of relevance.

[c] Frequency of patients with the clinical condition.

[d] Frequency of patients with a positive test result.

[e] Data that were not given in the paper or that are of questionable relevance are in parentheses.

[f] Calculated point-specific likelihood ratio (not given in the paper), unless indicated otherwise.

[g] Significance of the odds ratio given in the paper. If a 95% confidence interval is given for a relative risk, a range is said to be significant if it is outside 1.

[h] Frozen serum samples of 90 patients were compared with the frozen sera of 90 age-, sex-, aspirin allocation-, and smoking-matched controls.

[i] Univariate analysis.

[j] The data are stratum-specific likelihood ratios, not point-specific likelihood ratios. Sensitivity and specificity do not fit the stratum concept well and are misleading if they include the higher strata in this context.

[k] Not all patients were tested for aCL. Three hundred twenty-six patients had LA, as an inclusion criterion. No aCL-positive, LA-negative patient had thrombosis. This was a prospective study.

[l] Multivariate analysis.

[m] Some patients in the SLE group had clinical features of APS but were repetitively negative for aPL. Thrombosis had occurred in 87% of the APS patients and 12% of the patients with SLE without APS.

[n] Numbers of patients with IgG aCL > 20 or who were LA positive were not given. The high-positive group had higher levels of preexisting thrombosis, although most patients in the study were being investigated for miscarriage. This was a prospective study.

[o] Multivariate odds ratios are given in the paper but only for venous and arterial thromboses separately. The raw data of these divisions was not given; therefore risk ratios have not been used.

[p] The manufacturer's preferred cutoff was 30 GPL; however, a cutoff at 15 GPL (mean + 5 SD) gives a positive predictive value of 0.16.

[q] Analysis of only the 6-month treatment subgroup; this was a prospective study.

[r] Chi-square test.

not at all specific, with additional significant variation among laboratories and among commercial kits; therefore, clinicians should treat the clinical state and not an incidentally found antibody. Although there is an association between antibody titer and risk of thrombosis, this is not a ground for ignoring or not reporting weakly positive results. False-positive results that are difficult to interpret are particularly likely to occur when there are other causes of thrombosis such as atherosclerosis in the elderly; therefore, screening widely should not be encouraged.

The predictive value of testing with the aCL ELISA for APS can be improved to an extent by concurrent LA testing, but often the appropriate duration and intensity of treatment after a first manifestation cannot be determined by these two tests. The anti-B2GPI ELISA offers an improved predictive value, but there is a need for an optimization or evaluation procedure to manipulate and assess the effects of the different variables on test performance. We suggest that laboratories interested in aPL evaluate the assay, both in an in-house form using an optimal buffer and a large quantity of B2GPI and in a commercial form when thoroughly tested and externally validated kits become available.

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