

Laboratory Advisor

University of Louisville School of Medicine

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A Review of Anti-Beta 2 Glycoprotein I for Clinicians

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INTRODUCTION

The University of Louisville Hospital clinical laboratory is pleased to announce the addition of anti β_2 -glycoprotein I (anti- β_2 GPI) to our inhouse test menu. This Laboratory Advisor will give information regarding the clinical utility of this assay.

The antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by the persistent presence of a heterogeneous group of autoantibodies with specificity for phospholipid- protein complexes. APS is among the commonly *acquired* blood protein alterations associated with thrombosis, and is highly related to other autoimmune diseases, particularly systemic lupus erythematosus (SLE). Although the clinical conditions that characterize the disease occur frequently, the incidence of APS is low (1). Two different laboratory assays are used to support the diagnosis of APS: 1) Lupus anticoagulants (LACs) and 2) anticardiolipin antibodies (ACAs) (2). LA is a paradoxical phenomenon in which one or more *in-vitro* screening clotting tests are prolonged, suggesting an anticoagulant, but clinically, causing *in-vivo* hypercoagulability and thrombosis. The name lupus anticoagulant comes from the first discovered association with patients having SLE, however, it is not confined to this patient population.

ACAs are detected using an ELISA method, rather than a clotting method as used for LACs. Clinically, APS may be manifested by venous thrombosis, arterial thrombosis, or both, as well as recurrent fetal loss (3).

LABORATORY DIAGNOSIS OF APS:

The clinical criteria of APS are clear and can be detected objectively. In contrast, the laboratory criteria initially seem to be clear, but in practice the assays used to detect antiphospholipid antibodies (APAs) are not standardized, and outcome depends on the laboratory in which the assay is performed. Despite attempts to increase the specificity of the laboratory testing, and the establishment of consensus criteria for interpretation of the tests, a number of patients are still inaccurately diagnosed when the revised Sapporo classification criteria for APS are followed (Table 1). In this classification, only one clinical and one laboratory criterion are required to establish the diagnosis of APS (4). Hence the importance of correlation of the laboratory studies with the clinical presentation. Demonstration of persistence of APAs by repeat laboratory testing is important in helping to make the testing more specific and the diagnosis more reliable.

The International Society on Thrombosis and Haemostasis has identified the following criteria to be used by laboratories of offering testing for LACs (5). Two or more screening tests must be performed before reporting a sample as negative for LACs.

1. Prolongation of a phospholipid-dependent clotting assay (such as aPTT with "LA sensitive reagent", dilute prothrombin time, dilute Russell's viper venom time)
2. Evidence of an inhibitor demonstrated by mixing studies (patient's plasma mixed 1:1 with normal plasma)
3. Confirmation of the phospholipid-dependent nature of the inhibitor, by repeating the clotting assay after addition of a high concentration of phospholipids to neutralize the antibody, or repeating after adding altered phospholipids to enhance specificity of testing
4. Exclusion of a specific coagulation factor inhibitor or deficiency.

Additional laboratory tests that are used to detect LACs are: platelet neutralization procedure, kaolin clotting time, tissue thromboplastin inhibition test, and hexagonal phase phospholipid assay (Staclot® LA). Specific coagulation factor assays may also be used to help determine a specific coagulation factor deficiency or inhibitor. Correlating the laboratory findings with the clinical history of bleeding versus thrombosis is critical for proper interpretation of the laboratory findings APAs can develop in response to bacterial, viral, fungal, or parasitic infections and disappear within 12 weeks. These transient antibodies have no clinical consequences, but if persistent, become a risk factor for thrombosis. (3) Thus positive tests must be repeated after 12 weeks to confirm persistence of the APA.

ACAs and anti- β_2 GPI arise as IgM, IgG, or IgA subtypes. Initially these antibodies were thought to bind directly to phospholipids, but it is now recognized that the target antigens are a combination of proteins complexed with phospholipids. The variety of possible protein-phospholipid complexes makes for numerous different types of antibodies to these complexes. The most common plasma protein bound to APA is β_2 GPI, as well as prothrombin and annexin-V. The detection of ACAs is straightforward, with solid-phase ELISA being the method of choice. In the past, only IgG and IgA idiotypes were assayed.

Table 1**Revised classification criteria for the antiphospholipid syndrome**

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

Clinical criteria1. Vascular thrombosis[†]

One or more clinical episodes[‡] of arterial, venous, or small vessel thrombosis[§], in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.

2. Pregnancy morbidity

- (a) 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 by direct examination of the fetus, or
- (b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe pre-eclampsia defined according to standard definitions [11], or (ii) recognized features of placental insufficiency[¶], or
- (c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

In studies of populations of patients who have more than one type of pregnancy morbidity, investigators are strongly encouraged to stratify groups of subjects according to a, b, or c above.

Laboratory criteria**

1. Lupus anticoagulant (LAC) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies) [82,83].
2. Anticardiolipin (ACA) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA [100,129,130].
3. Anti- β_2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures [112].

*Classification of APS should be avoided if less than 12 weeks or more than 5 years separate the positive aPL test and the clinical manifestation.

[†]Coexisting inherited or acquired factors for thrombosis are not reasons for excluding patients from APS trials. However, two subgroups of APS patients should be recognized, according to: (a) the presence, and (b) the absence of additional risk factors for thrombosis. Indicative (but not exhaustive) such cases include: age (>55 in men, and >65 in women), and the presence of any of the established risk factors for cardiovascular disease (hypertension, diabetes mellitus, elevated LDL or low HDL cholesterol, cigarette smoking, family history of premature cardiovascular disease, body mass index $\geq 30 \text{ kg m}^{-2}$, microalbuminuria, estimated GFR $< 60 \text{ mL min}^{-1}$), inherited thrombophilias, oral contraceptives, nephrotic syndrome, malignancy, immobilization, and surgery. Thus, patients who fulfil criteria should be stratified according to contributing causes of thrombosis.

[‡]A thrombotic episode in the past could be considered as a clinical criterion, provided that thrombosis is proved by appropriate diagnostic means and that no alternative diagnosis or cause of thrombosis is found. [§]Superficial venous thrombosis is not included in the clinical criteria. [¶]Generally accepted features of placental insufficiency include: (i) abnormal or non-reassuring fetal surveillance test(s), e.g. a non-reactive non-stress test, suggestive of fetal hypoxemia, (ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxemia, e.g. absent end-diastolic flow in the umbilical artery, (iii) oligohydramnios, e.g. an amniotic fluid index of 5 cm or less, or (iv) a postnatal birth weight less than the 10th percentile for the gestational age.

**Investigators are strongly advised to classify APS patients in studies into one of the following categories: I, more than one laboratory criteria present (any combination); IIa, LA present alone; IIb, aCL antibody present alone; IIc, anti- β_2 glycoprotein-I antibody present alone.

Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, DE Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost.* 2006 Feb;4(2):295-306.

However, currently it is recognized that IgM idiotypes, whether primary or secondary (especially drug-induced), are also associated with thrombosis, and this has led to all three idiotypes being assayed. The high sensitivity of the ACA ELISA for infection-related antibodies generates significant numbers of false-positive results, which makes the assay nonspecific for the detection of patients at risk for thrombosis. Thus it is recommended that this test be followed by the anti- β_2 GPI test to add specificity to testing. In these assays the microtiter plates are coated with β_2 GPI. The anti- β_2 GPI in patient serum bind to β_2 GPI. An enzyme-labeled IgG, IgM, or IgA conjugate is added, and a substrate detects the presence of bound antibody. The results are given in G, A or M Units. Each laboratory establishes its own reference limits. Also when tests for ACAs or LACs are negative, and a high clinical suspicion for APS exists, testing for β_2 GPI is recommended. Again, each laboratory establishes its own reference limits. At University of Louisville Hospital, the values for negative results are as follows: IgG<20 G Units, IgA<20 A Units, and IgM<20M Units.

Figure 1 gives a recommended testing algorithm for inclusion of β_2 GPI in testing for APS. Figure 2 shows the correlation of tests for APS with specificity for thrombosis. Table 2 lists antiphospholipids antibodies that have been associated with thrombosis.

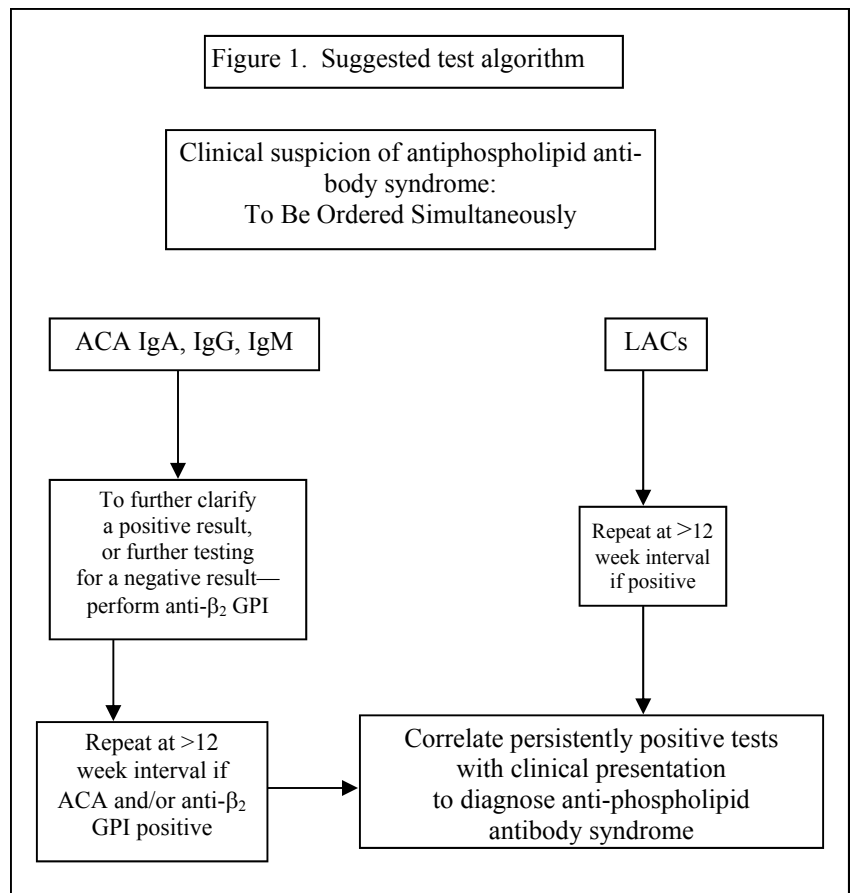
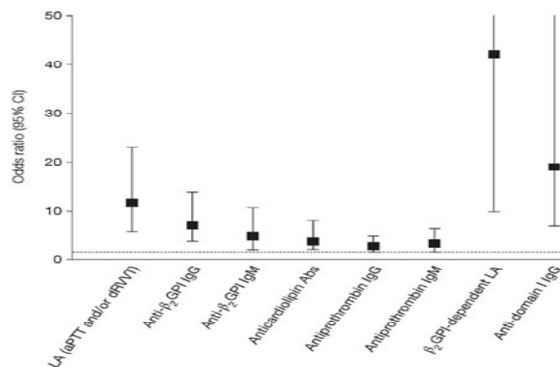


Figure 2 Correlation between antibody specificity and thrombosis



De Laat B *et al.* (2008) Mechanisms of Disease: antiphospholipid antibodies—from clinical association to pathologic mechanism
Nat Clin Pract Rheumatol doi:10.1038/ncprheum0740

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WHY Anti- β_2 GPI?

Although antibodies to many phospholipid-protein complexes have been found to be involved in APS, antibodies against β_2 GPI are thought to be the those with most clinical significance. Bas de Laat et al. found, in a cohort of 198 patients, almost all of whom were diagnosed with SLE, that the odds ratio for vascular thrombosis from a positive assay result increased from 10.2 for the classic LA assay and 6.8 for the classic anti- β_2 GPI antibody ELISA to 42.3 for LA caused by anti- β_2 GPI antibody activity (1). Pengo *et al.* also showed that the combination of positive LA with a positive anti- β_2 GPI antibody ELISA dramatically increased the correlation with thrombosis compared with only one of these assays being positive (6).

Furthermore, in a population of 198 patients suffering from a variety of autoimmune diseases, antibodies that recognized domain I of β_2 GPI were detected most commonly in those patients with a history of thrombosis (1). Antibodies that recognized other parts of β_2 GPI (mostly domain V) were predominantly found in patients with no history of thrombosis. By discriminating between these antibodies, the odds ratio for thrombosis could increase from 6.7 for a positive classic anti- β_2 GPI antibody ELISA to 18.9 for the anti-domain I ELISA. In the same study, only antibodies with affinity towards domain I showed a coexistent LA (1). Whether the anti-domain I ELISA would be a legitimate addition to the diagnostic criteria for APS or not is still being investigated.

Summary

At University Hospital testing for both LACs and ACAs should be ordered when evaluating a patient with possible APS. The aPTT, including "sensitive reagents," is unreliable as a screening assay in testing for LACs (prolonged in less than half of patients), and is usually not prolonged in patients who have ACAs. Therefore the laboratory work up for APS should not be done just in patients who have thrombosis and a prolonged aPTT. Testing for LACs and ACAs should also be accompanied by anti- β_2 GPI when results are inconclusive, or when ACAs are present, but not LACs.

Laboratory testing is a critical part of the diagnosis of APS. Anti- β_2 GPI represents an independent risk factor for thrombosis and pregnancy complications (7), and offers improved specificity and sensitivity in testing for APS over ACA or LA alone. In 3%–10% of APS patients, anti- β_2 GPI may be the only test positive (4). The association of anti- β_2 GPI with pre-eclampsia and/or eclampsia in unselected pregnant women who tested negative for ACA implies that the inclusion of anti- β_2 GPI may also help to assess this condition that leads to pregnancy complications. High titers of anti- β_2 GPI antibodies are associated with a high risk of thrombosis, but it is difficult to define diagnostic interpretations for medium and high titers at this stage. Currently, the international consensus group proposes a threshold for positive anti- β_2 GPI antibodies as any value above the reference range. (4) The possible interference of cryoglobulins and rheumatoid factors should be considered in the interpreta-

tion of IgM anti- β_2 GPI. Data are inadequate for establishing IgA anti- β_2 GPI as an independent risk factor for APS in the absence of other anti- β_2 GPI isotypes.

Table 2

Important antiphospholipid antibodies in thrombosis

Lupus anticoagulant
Anticardiolipin antibodies
Beta-2-Glycoprotein I
antibodies to Hexagonal phase phospholipid
Anti-phosphatidylserine
Anti-phosphatidylethanolamine
Anti-phosphatidylinositol
Anti-phosphatidylcholine
Anti-phosphatidylglycerol
Anti-phosphatidic acid
Anti-Annexin-V antibodies

Hoppensteadt DA, Fabbrini N, Bick RL, Messmore HL, Adiguzel C, Fareed J. Laboratory evaluation of the antiphospholipid syndrome. *Hematol Oncol Clin North Am.* 2008 Feb;22(1):19-32.

Case History Sample: anti-Beta 2 glycoprotein I

A 29 year-old female was seen for complaints of decreased vision in her right eye. She had been given a diagnosis of systemic lupus erythematosus with lupus dermatitis two years previously. An exam by ophthalmology revealed changes to be most consistent with lupus vasculitis. The patient was admitted to the hospital where a MRI of the brain was performed with impression of ischemic changes consistent with lupus vasculitis. A 2D echocardiogram revealed endocarditis, and a follow up transesophageal echocardiogram showed a thrombus attached to the posterior apical left ventricular wall. These thrombi were present despite a moderate thrombocytopenia (platelet count=90,000) on complete blood count.

In assessment of the hypercoagulable state, and due to history of SLE, anticardiolipin and anti-Beta 2 glycoprotein I antibodies were obtained from the laboratory. The assays revealed the presence of a high titer anticardiolipin antibody of IgG type at 83.7 GPL units (reference=0-22 GPL units), and a high titer anti-Beta 2 glycoprotein I of IgG type at >100 U/mL (<20 U/ml). These results were considered significant in that the presence of an antibody to B2GPI added specificity to the finding of the ACA, and that both were high titer and of the IgG antibody type, making them likely pathological. A diagnosis of anti-phospholipid antibody syndrome was favored as a way to explain the patient's clinical findings, and anticoagulation therapy was added to the treatment plan. Repeat testing to determine persistence of the antibodies is warranted, although high titer antibodies of the IgG type, in the proper clinical setting, can be diagnostic.

Laboratory Information:

Testing for Anti- β_2 glycoprotein-I antibody
will be performed by Serology on
Monday of each week.

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After attending U of L School of Medicine, Sandra C. Hollensead, M.D. completed residency training at UofL in AP/CP, and then performed a hemepathology fellowship at Ball Memorial Hospital in Muncie Indiana. Dr. Hollensead has been practicing pathology with an interest in fluid hematology since 1988. She is the medical director of the hematology and coagulation laboratory at the University of Louisville Hospital, and provides clinical pathology consultations for the laboratory diagnosis of bleeding and clotting disorders.



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