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A Practical Algorithm for the Laboratory Evaluation of  
Antiphospholipid Antibodies

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The antiphospholipid syndrome (APS) is currently recognized as a common risk factor for arterial or venous thromboembolic disease. The presence of high serum levels of antiphospholipid (aPL) antibodies in patients with APS has been strongly associated with thrombosis, and experimental evidence suggests that these antibodies play an important pathogenic role in this disease. To assist the laboratory in the routine evaluation of the heterogeneous group of aPL antibodies detected by ELISA, we have developed a practical algorithm for the clinical interpretation of the results. This algorithm takes into consideration the antibody titer and isotype as well as the patient's clinical information as laboratory results should always be interpreted in the context of clinical findings. Due to their high sensitivity but low specificity, initial screening should start with either aCL and/or aPS ELISAs in order to test for a wide range of aPL antibodies. A negative screening result in the absence of clinical findings of thrombosis would not require further testing. However, a negative screen with clinical findings suggestive of thrombosis would require additional testing with a more specific assay such as an anti-cofactor ELISA (i.e. anti-B2GPI and anti-prothrombin). An anti-cofactor ELISA positive result would strongly point towards APS. A positive aCL and/or aPS ELISA would also require follow up testing for anti-cofactor antibodies. A negative anti-cofactor result may suggest the presence of "infectious" aPL antibodies which are usually present in low titers and transient. Repeat testing is recommended as a serologic diagnostic criteria for APS requires the demonstration of persistently high serum levels of aPL antibodies. A positive anti-B2GPI result would strongly suggest APS and/or increased risk of thrombosis. LA has not been included in this algorithm, however, it must be kept in mind that the presence of LA has been shown to be more specific for thrombosis than aPL antibodies detected by ELISA and adds valuable information to the serologic diagnosis of APS.

Introduction

The evaluation of antiphospholipid antibodies has been surrounded by confusion in many clinical laboratories, and with the introduction of new technologies and serologic markers, it is likely that some confusion will continue. ELISA's for aCL and aPS antibodies can detect different populations of antiphospholipid antibodies, including some not associated with thrombosis or APS i.e. "infectious" antibodies, or to "other" bovine proteins present in blocking reagents or sample diluent. These assays may be more useful as a preliminary screening method to test for antiphospholipid antibodies since they are sensitive, but lack a high level of specificity. Patients that are found positive with one or both of these methods should be further tested with a more specific assay (i.e. anti-B2GPI). Specimens found only positive with the screening test(s) would be suggestive of "infectious disease" related antibodies, which are usually present in low levels and transient. Patients that are confirmed positive with a more specific follow-up test may have an increase risk of thrombosis and antiphospholipid syndrome.

We have reviewed both in-house and current published research in this area to develop a practical algorithm for the evaluation of aPL antibodies. This algorithm takes into consideration the antibody titer and isotype as well as the patient's clinical information. This should facilitate the decision making process for subsequent testing and the final interpretation of the results. LA testing, though not shown in this algorithm, should be included when testing for thrombotic risk or serologic diagnosis of APS.

Objective

- Simplify ELISA antiphospholipid testing (algorithm) allowing laboratories to easily determine appropriate additional testing and properly interpret results.

Material and Methods

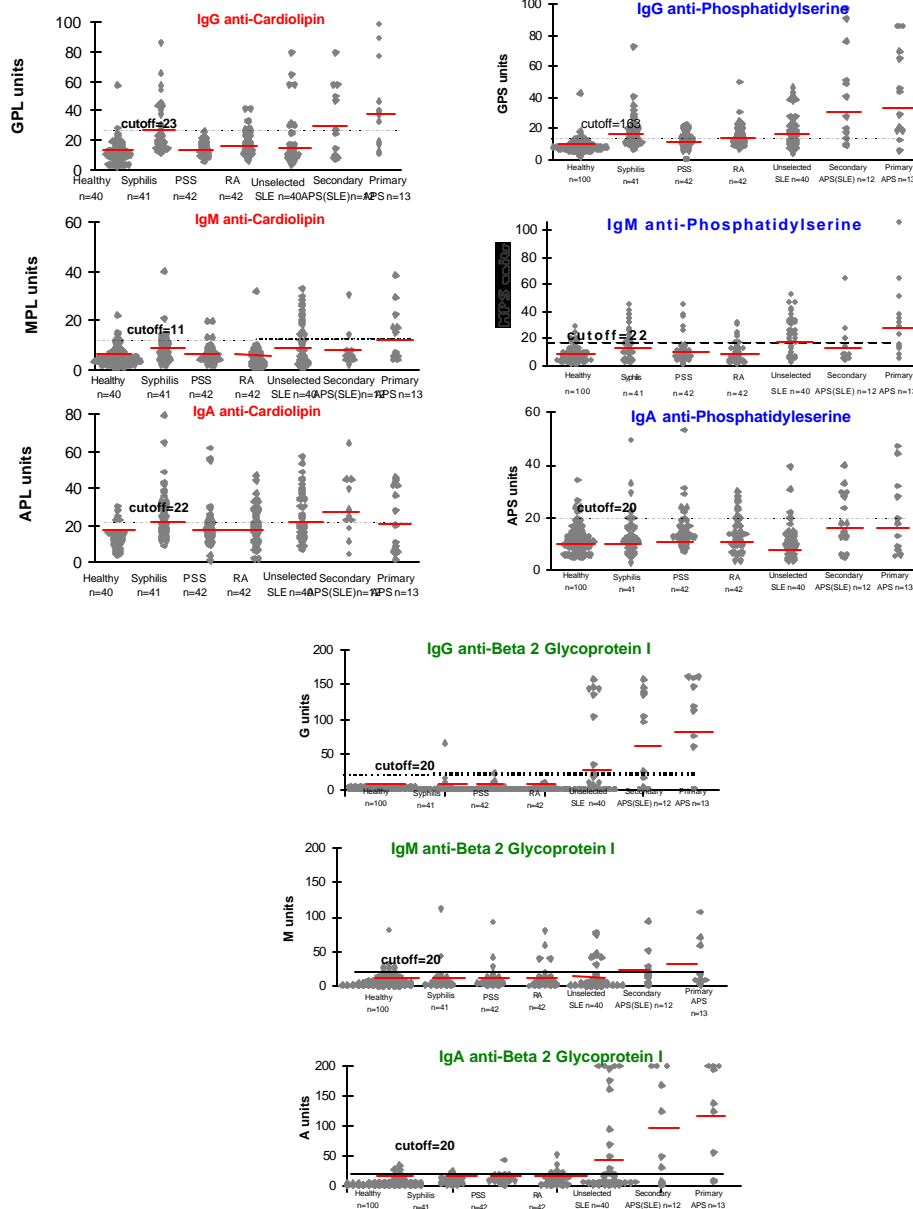
**anti-Cardiolipin ELISA:** Purified bovine cardiolipin was coated onto 96-microwell plates, blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG, IgM, or IgA was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and absorbance read at 450/650 nm.

**anti-Phosphatidylserine ELISA:** Purified bovine phosphatidylserine was coated onto 96-microwell plates, blocked and stabilized. 100uL of diluted patient serum (1:50) in sample diluent containing bovine B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG, IgM, or IgA was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and absorbance read at 450/650 nm.

**anti-Beta 2 Glycoprotein I ELISA:** Purified human B2GPI (purity > 95% SDS-PAGE) was coated onto 96 well micro-plates, blocked, and stabilized in the absence of exogenous B2GPI. 100uL of diluted patient serum (1:50) in sample diluent containing no B2GPI was incubated in coated microwells for 15 minutes at room temperature. After washing, 100uL of HRP conjugated anti-human antibody heavy chain specific for IgG, IgM, or IgA was added for another 15 minute incubation, followed by TMB substrate. The reaction was stopped with 0.36N H2SO4 and optical density read at 450/650 nm.

Serum Samples:

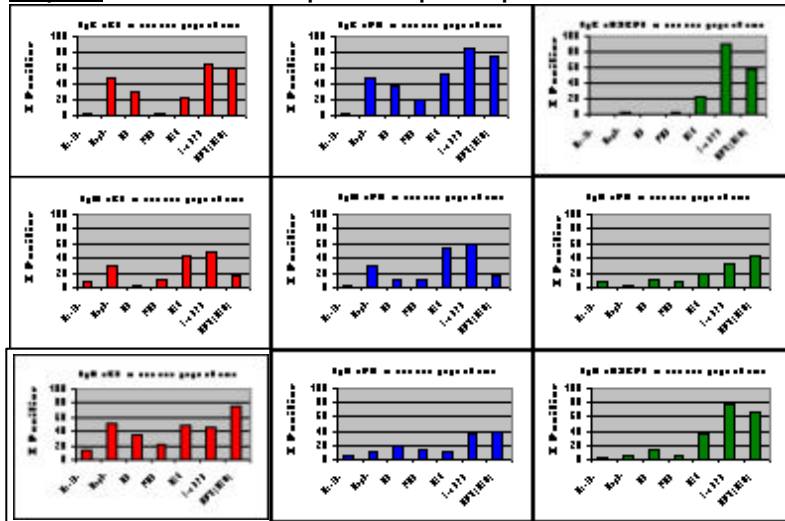
- Healthy Blood Donors
- Infectious Disease (syphilis)
- Progressive Systemic Sclerosis (PSS)
- Rheumatoid Arthritis (RA)
- Unselected Systemic Lupus Erythematosus (SLE)
- Secondary Antiphospholipid Syndrome



Summary Distribution of aCL, aPS, and anti-B2GPI antibodies in various populations

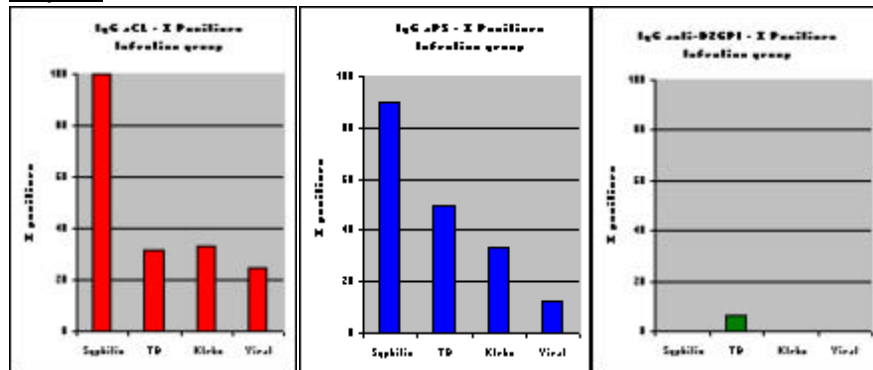
Graph #4

Results expressed in percent positive



Prevalence of IgG aCL, aPS, and anti-B2GPI Antibodies in Various Infectious Disease Patients

Graph #5



Simultaneous Determination of IgG aCL, aPS, and anti-B2GPI Antibodies in Various Populations

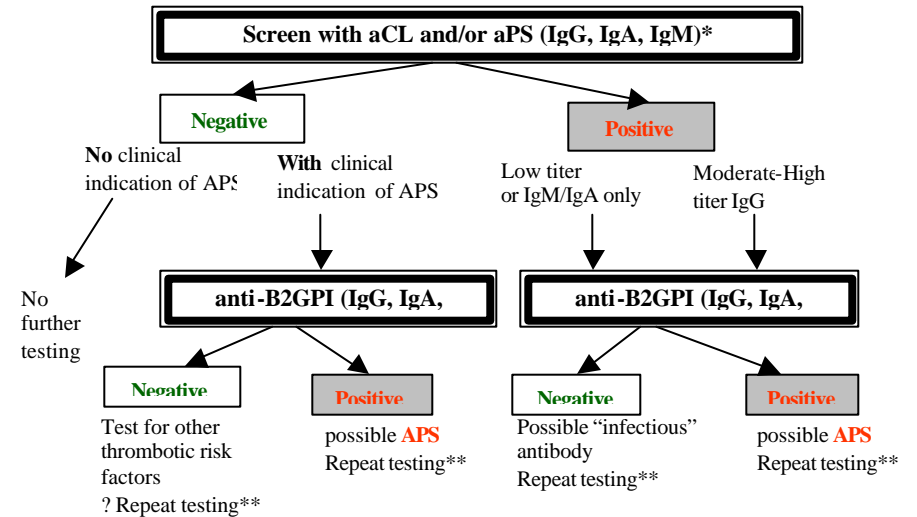
Table #1

Correlation with the Antiphospholipid Syndrome (APS)

Patient Population (n=)	% with 3 positive*	% with 2 positive*	% with 1 positive*
Healthy (40)	0	0	7%
Syphilis (41)	5%	15%	29%
PSS (42)	0	5%	12%
RA (42)	0	12%	26%
Autoimmune (49)	22%	45%	14%
Unselected SLE (40)	15%	13%	13%
Control SLE (16)	0	33%	50%
Secondary APS (SLE) (12)	58%	0	17%
Primary APS (9)	56%	44%	0

\* When tested for IgG aCL, aPS, and anti-B2GPI

Antiphospholipid Antibody ELISA Screening Procedure



APS = anti-phospholipid syndrome B2GPI = beta 2 glycoprotein I  
aCL = anti-cardiolipin \*Screen for each antibody isotype. Polyvalent screen not recommended  
aPS = anti-phosphatidylserine \*\* Repeat testing in 8-10 weeks for seroconversion or Ab persistence

Summary

- Both aCL and aPS antibody testing resulted in positive values with the syphilis, PSS, and RA patient groups for all three isotypes. These groups had a much lower prevalence of anti-B2GPI antibodies.
- High prevalence and titers of anti-B2GPI antibodies were found mostly in clinically relevant patients compared to aCL or aPS. However, none of the anti-B2GPI isotypes demonstrated 100% sensitivity.
- aPS antibody testing demonstrated a higher prevalence and values than aCL antibodies with the clinically relevant patients (unselected SLE, secondary APS, primary APS).
- Although aCL and aPS antibodies were detected in infectious disease patients, anti-B2GPI antibodies were virtually absent.
- Samples that were positive for two or three different assays (aCL, aPS, or anti-B2GPI) were more prevalent in patients with SLE or APS compared to control groups.

Conclusions

- Test results should be interpreted in the context of clinical manifestations.
- Antibody level and isotype (IgG, IgM, IgA) determination provides valuable information.
- Both aCL and aPS assays detect "infectious" as well as "autoimmune" aPL antibodies.
- More than one assay may be required for diagnosis of antiphospholipid syndrome: antiphospholipid assay(s) for screening and anti-cofactor assay(s) for confirmation.
- The use of an algorithm in antiphospholipid detection could not only assist the laboratory and physicians in determining what additional testing is appropriate, but can also help minimize the cost related to this testing.