

# The Antiphospholipid Syndrome

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1. Antiphospholipid syndrome (ASP) may be diagnosed when arterial or venous thrombosis, or recurrent miscarriage, occurs in a subject in whom laboratory tests for antiphospholipid antibody (ACA, LA or both) are positive. Because thrombotic disease, miscarriage and transient antiphospholipid antibody positivity are all common events, **persistence of the positive tests must be demonstrated and other causes and contributory factors considered.** Additional clinical and laboratory features are variably present in APS, particularly thrombocytopenia and livedo reticularis. Where the condition exists against a background of chronic inflammatory disease, especially systemic lupus erythematosus, it is referred to as secondary antiphospholipid syndrome to distinguish it from the primary syndrome, in which there is no evidence for another relevant underlying disease.

## 2. Antiphospholipid Reactivity

Although most early observations were on Lupus Anticoagulant (LA) and anticardiolipin (ACA) it is now clear that so-called antiphospholipid antibodies comprise a family of antibodies reactive with epitopes on proteins which are themselves complexed with antibodies require beta2-glycoprotein I (beta2 GP I), a phospholipid binding plasma protein with weak anticoagulant activity, for binding to acidic phospholipids such as phosphatidylserine and cardiolipin (Galli et al, 1990). The precise relationships between beta2 GP I, phospholipid and autoantibody are disputed. One possibility is that cryptic epitopes are exposed on beta2 GP I when it binds to phospholipid. Alternatively, binding of the glycoprotein to phospholipid may concentrate the antigenic sites and promote bivalent antibody binding. Whichever mechanism is active, it also applies when beta2 GP I interacts with other negatively charged surfaces, including the plastic of ELISA plates. This observation has allowed the development of new, possibly more specific assays for antiphospholipid antibodies which employ purified beta2 GP I. However, other proteins share this property of binding to phospholipid in a manner that promotes interaction with antiphospholipid antibodies. These included prothrombin, annexin V, protein C, protein S, thrombomodulin and high molecular weight kinninogen. The in vitro phenomenon known as LA can be due to antibodies reactive to beta2 GP I, phospholipid or to prothrombin/phospholipid. The beta2 GP I-dependant antibodies also bind in traditional anticardiolipin assays, as the glycoprotein is present in test serum and often in assay reagents. Despite this improved understanding of the true nature of APA, and because the clinical utility of the newer assays is incompletely evaluated, the laboratory diagnosis of the antiphospholipid syn-

drome still relies predominantly on coagulation-based assays for LA and solid-phase assays (ELISAs) employing cardiolipin.

## 3. Coagulation Assays (Lupus Anticoagulant Tests)

Criteria for the presence of LA are:

- I. Prolongation of a phospholipid dependent coagulation test.
- II. Evidence of an inhibitor demonstrated by mixing studies.
- III. Confirmation of the phospholipid dependent nature of the inhibitor.

Optional additional criteria are the ability to demonstrate any inhibitory activity directed against a specific, coagulation factor, and isolation of immunoglobulin with LA activity. These remain beyond the scope of most clinical haematology departments.

**In principle, the laboratory tests should employ a detection or screening stage and a confirmation stage, the latter often performed using different reagents in the same type of test.** The screening stage must be as sensitive as possible to eliminate false negative results, while the confirmation stage confers specificity to prevent false positive results, both of which may have important clinical consequences. No LA tests consistently shows 100% specificity and sensitivity, and because of the heterogeneous nature of APA, more than one test system should be used for detection of LA.

## 4. Solid Phase Assays (for ACA and beta2 GP I antibodies)

Solid phase assays for APA, such as the ACA ELISA test have been gradually refined. The ELISA format allows bulk testing and the results are not affected by factor deficiency or the use of anticoagulants.

### *Methodological considerations:*

The quality of the cardiolipin used, and the technique for coating the microtitre plates are important. The binding of most ACA requires the presence of the protein cofactor, beta2 GP I, which is essential for satisfactory and reproducible ACA results. This is provided by fetal calf serum or adult bovine serum in the blocking agent (used to reduce non-specific binding to the microplate) and/or sample diluent. The use of uncoated wells as sample blanks is essential to reduce the effect of non-specific binding due to interfering substances in the test sample (e.g. rheumatoid factor). ELISA assays based on the other single phospholipids have been described (e.g. phosphatidylserine), but these appear to have similar specificity to ACA assays, and

similar limitations.

It is recommended that the ACA test is standardised by the use of affinity purified standards (Harris et al 1987) or secondary standards derived from these. These allow the calculation of ACA results in IgG or IgM antiphospholipid units (GPLU and MPLU, respectively) related to a given concentration of affinity purified ACA immunoglobulin. Positive and negative QC sera should also be included in each batch of assays.

A variety of specific assays for beta2 GP I antibodies have been developed (Roubey et al 1996), and several commercial kits are available. Beta2 GP I antibody assays show higher precision and better correlation with the thromboembolic complications in APS and SLE than assays for ACA, and are less likely to show transient positive results in association with infection (McNally et al 1995). The problems of standardisation remain, however. Gamma irradiated (high antigen binding) polystyrene plates or other plates with a high density of surface electrostatic charge must be used to ensure efficient beta2 GP I binding. Also beta2 GP I preparations which are impure, or have undergone proteolytic cleavage should not be used.

Occasionally, sera are ACA negative, but beta2 GP I antibody positive. This is usually due to species specific antibodies which fail to bind to bovine beta2 GPI in ACA assays. These antibodies are nevertheless clinically significant because they may be associated with APS. ACA positive, beta2 GP I antibody negative samples may contain other autoantibodies, including those reactive with annexin V, protein C, protein S or prothrombin. These may be provided by the blocking agent, sample diluent, or by the test sample itself. Antiprothrombin antibodies generally exhibit poor specificity for venous thrombosis and recurrent fetal loss, and also may be found in patients with infection.

## References

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