27 Antiphospholipid Syndrome in the Absence of Standard Antiphospholipid Antibodies: Associations with Other Autoantibodies

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Introduction

Although the presence of antiphospholipid antibodies (aPL) is, by definition, a sine qua non of antiphospholipid syndrome (APS), the spectrum of autoantibodies associated with APS is likely to extend beyond standard anticardiolipin and lupus anticoagulant assays. These include autoantibodies to phospholipid-binding proteins, such as β_2-glycoprotein I (β_2-GPI) and prothrombin, as well as antibodies detected in immunoassays using phospholipids other than cardiolipin. These antibodies are present in some patients with clinical manifestations of APS, that is, thrombosis, pregnancy loss, who have negative tests for anticardiolipin antibodies (aCL) and lupus anticoagulant (LA). At the present time such patients would not be classified as having definite APS by international criteria [1], but fall within a broader concept of APS or APS-like conditions. If these other autoantibodies are shown to be associated with the same risks of thrombosis, pregnancy loss, etc., as conventional aPL as research proceeds, then it is likely that consensus serological classification criteria for APS will expand.

This broader serologic view of APS highlights the unusual relationship of conventional aPL tests to the syndrome. APS is currently defined as the association of certain clinical events, for example, thrombosis or recurrent fetal loss, with a persistently positive aCL or LA test. Positivity in an aPL test is, therefore, an essential element of the syndrome, rather than a diagnostic test for the syndrome. There is no independent gold standard for APS by which one can assess the diagnostic sensitivity or specificity of aPL tests. Positive aPL assays are probably best thought of as risk factors for the clinical manifestations of APS. A highly positive aPL assay should not be considered a false positive if the patient does not have a history of thrombosis or another clinical feature of APS. Further, there are no established exclusion criteria for APS. A patient with thrombosis and a positive aPL test would be considered to have APS whether or not other important risk factors for thrombosis were present. In fact, other factors may be critical in determining which individuals with aPL will have a thrombotic event or miscarriage.

Autoantibodies Associated with APS

In considering "aPL-negative" APS, it should be keep in mind that conventional aCL ELISAs and LA assays were developed based on an inaccurate or incomplete understanding of the specificities of the antibodies detected in these tests. Elucidation of these specificities and the discovery of additional autoantibodies associated with thrombosis and/or fetal loss are a work in progress.

Antibodies Detected in Conventional aPL Assays

Before discussing APS-associated antibodies that may not be detected in conventional aPL assays, it is necessary to understand the antibodies that are detected in these tests. The specificities of aPL are reviewed in detail elsewhere in this volume. Briefly, in APS patient sera, the large majority of antibodies detected in aCL ELISAs recognize β_2-GPI, not cardiolipin. β_2-GPI in these assays derives from bovine serum, a common component of the blocking buffer/sample diluent. Antibodies directed against cardiolipin may also be detected in aCL assays, but do not appear to be associated with the clinical manifestations of APS. LA activity in most APS patient plasmas is due to autoantibodies directed against β_2-GPI and/or autoantibodies to prothrombin. Although LA activity is commonly thought of as an intrinsic property of certain antibodies, differences in the various assays used to detect LA may be critically important in determining whether a patient sample will exhibit LA in a particular assay. For example, the LA activity of certain anti–β_2-GPI monoclonal antibodies derived from APS patients was found to be dependent on the concentration of β_2-GPI in the LA assay [2]. Certain antibodies may be detected in one type of LA assay but not another. Galli and colleagues found that the dilute Russell viper venom time was more sensitive to prolongation by anti–β_2-GPI antibodies, whereas the kaolin clotting time was more sensitive to prolongation by anti-prothrombin antibodies [3].

Antibodies Detected in Immunoassays Using Other Anionic Phospholipids

Early in the development of aCL immunoassays, it was observed that most APS-associated "anticardiolipin" antibodies appeared to be broadly cross-reactive with other anionic phospholipids, for example, phosphatidylserine, phosphatidylinositol, phosphatidic acid, and phosphatidylglycerol. With the discovery that bovine β_2-GPI was the key antigen in aCL ELISAs, this apparent cross-reactivity is currently best understood in terms of β_2-GPI binding similarly to the various anionic phospholipids. Bovine serum is commonly used in the assays with other anionic phospholipids and β_2-GPI binds reasonably well to micro-titer plates coated with any negatively charged phospholipid [4]. Accordingly, most anti–β_2-GPI antibodies can be detected in any of the anionic phospholipids ELISAs. The generally good correlation among ELISAs using different anionic phospholipids has led many investigators to conclude that the aCL assay, along with LA testing, are adequate for