Prevalence of Different Anti-Phospholipid Antibodies in Systemic Lupus Erythematosus and Their Relationship with the Antiphospholipid Syndrome

The antiphospholipid syndrome is a complex disorder (1) that is related to a set of unusual and fascinating antibodies. Recent research is defining these antibodies and their relationship to features of the syndrome.

The antiphospholipid syndrome is defined by the association of arterial and/or venous thrombosis, recurrent miscarriages, and anti-phospholipid (aPL) antibodies (1). The syndrome may be either idiopathic (primary) or may occur in the setting of an associated disease (secondary), most frequently systemic lupus erythematosus (SLE) or other autoimmune diseases (2, 3). Lupus anticoagulants and anti-cardiolipin antibodies at medium to high titers are laboratory indicators of the condition (4).

Lupus anticoagulants behave as acquired inhibitors of coagulation and prolong phospholipid-dependent in vitro coagulation tests (5), whereas anti-cardiolipin antibodies bind cardiolipin and other anionic phospholipids and are detected in solid-phase immunoassays (6). Despite their name, aPL antibodies recognize plasma proteins bound to suitable anionic (not necessarily phospholipid) surfaces rather than anionic phospholipids. Among them, β2-glycoprotein I (β2-GPI) (7, 8) and prothrombin (9) are the most common and investigated antigenic targets. β2-GPI is required by the majority of autoimmune anti-cardiolipin antibodies to react with cardiolipin in immunoassays (7, 8). Specific subgroups of anti-β2-GPI (10) and anti-prothrombin (11) antibodies are responsible for the lupus anticoagulant activity in phospholipid-dependent coagulation tests.

In addition to β2-GPI and prothrombin, (activated) protein C (12), protein S (12), annexin V (13), high- and low-molecular weight kininogens (14), factor XII (15), thrombomodulin (16), and tissue-type plasminogen activator (17) have been reported to be antigenic targets of aPL antibodies. Because all of these proteins are involved in the initiation and control of blood coagulation, it is conceivable that antibodies that reduce their availability or hamper their function may affect the pro- and anticoagulant balance. This might represent the pathophysiologic background underlying the increased thrombotic risk of aPL-positive patients. To date, limited and rather inconsistent information is available as to the prevalence and clinical significance of aPL antibodies other than lupus anticoagulants, anti-cardiolipin, anti-β2-GPI, and anti-prothrombin antibodies. There are multiple reasons for this inconsistency.

The laboratory methodology used for the detection of aPL antibodies is crucial. Both functional and immunoassays have been used. Enzyme-linked immunoassays are currently the most commonly used methods because they are easily performed, potentially may be automated, and allow the screening of large numbers of samples. Despite their widespread use, standardized assays for detection of the various aPL antibodies are still lacking. For anti-β2-GPI and anti-prothrombin antibodies, the mode of presentation of the antigens in immunoassays has long been known to greatly affect their recognition (18). In fact, the immune reaction occurs only when γ-irradiated polystyrene or high-density polyvinyl chloride plates are used, whereas no binding to proteins immobilized on plain polystyrene plates is observed (9, 11, 19). Under the former conditions, the prevalence of IgG and/or IgM anti-β2-GPI and anti-prothrombin antibodies is ~50% (9, 11, 19). This value is higher when the anionic phospholipid-protein complex represents the antigen (11).

Two hypotheses have been put forward to explain these findings:

- Anti-β2-GPI and anti-prothrombin antibodies are low-affinity antibodies whose binding is stabilized only at a high antigen surface density, such as that reached on an anionic (phospholipid) surface (20).
- Anti-β2-GPI and anti-prothrombin antibodies are directed against cryptic epitope(s), which are exposed only when the conformation of the antigen changes following its binding to an anionic surface (21).

Both hypotheses are sustained by experimental evidence and are not mutually exclusive.

This peculiar behavior in immunoassays is not confined to anti-β2-GPI and anti-prothrombin antibodies, but rather appears to be a common property of all aPL antibodies, as shown by Nojima et al. (22) in this issue of Clinical Chemistry. The authors measured anti-β2-GPI, anti-prothrombin, anti-protein C and S, and anti-annexin V IgG antibodies in 168 SLE patients. When plain polystyrene plates were used, virtually no binding was observed. Conversely, when the proteins were immobilized on γ-irradiated plates, the prevalence of the antibodies ranged from 21% to 56%. This finding strongly supports the use of high-binding or γ-irradiated plates for the detection of all types of aPL antibodies and may help assay standardization.

Possible cross-reactivity between aPL antibodies must be taken into account to avoid the useless and sometimes even dangerous multiplication of laboratory tests. The high degree of concordance between the ELISA for IgG anti-prothrombin and anti-protein C and S antibodies reported by Nojima et al. (22) and others (19) suggests that the same antibody may be measured by these assays. Because the N-terminal region of prothrombin shares homology with other vitamin K-dependent proteins, Pengo et al. (19) suggested that anti-prothrombin antibodies recognize a common epitope on this region of prothrombin as well as proteins C and S. Unfortunately, no inhibition studies were performed to sustain their
hypothesis. This possibility, however, seems unlikely in the light of experiments by Rao et al. (23), who analyzed the binding of 14 IgG fractions from lupus anticoagulant-positive patients to phosphatidylserine in the presence of prothrombin, protein S, or protein C: only prothrombin supported the binding of the IgG preparations to the anionic phospholipids. Puurunen et al. (24) elegantly demonstrated that anti-prothrombin antibodies cross-react with plasminogen in patients with myocardial infarction, possibly because of the homology between prothrombin kringle 2 and plasminogen kringle 5.

Nojima et al. (22) reported a higher prevalence of anti-β2-GPI and anti-prothrombin antibodies in lupus anticoagulant-positive than in lupus anticoagulant-negative patients. This is in line with the notion that lupus anticoagulant activity in plasma is caused by anti-β2-GPI and anti-prothrombin antibodies (10, 11), either alone or in combination (25). Conversely, no correlation was found for anti-protein C or S, or anti-annexin V antibodies, suggesting that these antibodies do not play a relevant role in the expression of lupus anticoagulant activity in phospholipid-dependent coagulation tests. Nojima et al. (22) reported that anti-β2-GPI and anti-prothrombin antibodies are risk factors for arterial, but not venous thrombosis in patients with SLE. These findings only partly confirm those already reported by other investigators [reviewed in Refs. (26, 27)]. This could depend, at least partially, on the different clinical settings (i.e., SLE vs non-SLE patients, or patients enrolled following myocardial infarction rather than deep vein thrombosis or because of other diseases), in which these antibodies have been measured.

Quantitative and/or qualitative protein S deficiencies are a known risk factor for venous thrombosis (28), which could represent the pathophysiologic rationale for the association between anti-protein S antibodies and venous thrombosis observed by Nojima et al. (22). However, this finding must be considered with caution, because the authors found no similar association with anti-protein C antibodies, despite the high degree of correlation between anti-protein C and S antibodies.

Anti-annexin V antibodies were the only significant risk factor for recurrent miscarriages in the SLE patients studied by Nojima et al. (22). Annexin V is a potent anticoagulant protein that binds to anionic phospholipid surfaces via calcium ions (29). Annexin V is necessary to maintain placentation integrity and may possibly have a thromboregulatory effect at the materno-fetal interface, as shown in a murine model (30). Interference of anti-annexin V antibodies with the annexin V shield on the placental villi has been hypothesized to lead to the exposure of anionic procoagulant phospholipids, which in turn favors blood coagulation processes in the placental vasculature (31).

The relevance of aPL antibodies to the thrombotic events associated with antiphospholipid syndrome must be viewed critically in the light of the design of the studies performed to date. The roles of lupus anticoagulants and anti-cardiolipin antibodies as risk factors for arterial and venous thrombosis have been evaluated by several prospective, cross-sectional, and case-control studies, which provided formal estimation of the strength of their association. Conversely, data on anti-β2-GPI, anti-prothrombin, anti-protein C and S, and anti-annexin V antibodies are derived mostly from retrospective studies on relatively small series of patients. More data, particularly from well-designed studies, are required to clearly establish which of the aPL antibodies are risk factors for antiphospholipid syndrome-related clinical complications. Until then, immunoassays for the detection of anti-β2-GPI, anti-prothrombin, anti-protein C and S, and anti-annexin V antibodies should be performed only in the setting of clinical studies and not in the routine evaluation of patients with clinical manifestations associated with antiphospholipid syndrome.

References
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