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REVIEW

Antiprothrombin antibody: why do we need more assays?

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Anticardiolipin antibodies (aCL), anti-β_2-glycoprotein I (β_2GPI) antibodies and lupus anticoagulant (LA) are the only laboratory tests considered within the revised criteria for the classification of the antiphospholipid syndrome (APS). Recently, the significance to assay the antibodies against phosphatidylserine–prothrombin complex (aPS/PT) has been discussed, and these antibodies, rather than antibodies against prothrombin alone, are closely associated with APS and LA. The sensitivity and specificity of aPS/PT for the diagnosis of APS were assessed in a population of patients with a variety of autoimmune disorders. The aCL and aPS/PT have similar diagnostic value for APS, and most of APS patients with aPS/PT had positive LA. Therefore, aPS/PT should be further explored, not only for research purposes, but also as a candidate for one of the enzyme-linked immunosorbent assay (ELISA)-based confirmatory test for APS associated LA. *Lupus* (2010) **19,** 436–439.

Key words: antiprothrombin antibody; phosphatidylserine; thrombosis; lupus anticoagulant; antiphospholipid syndrome

Introduction

Although the original concept of antiphospholipid antibodies (aPL) considers that those antibodies were directed against anionic phospholipids, evidence has shown that phospholipid-binding plasma proteins such as β_2-glycoprotein I (β_2GPI) and prothrombin are the dominant antigenic targets recognized by aPL in patients with the antiphospholipid syndrome (APS).

Anticardiolipin antibodies (aCL), anti-β_2GPI antibodies and lupus anticoagulant (LA) are the laboratory tests considered in the revised criteria for the classification of the APS. However, a number of issues regarding the definition of 'aPL positive' are in discussion. For example, there would be many *in vitro* false positives in LA in daily practice, despite enormous efforts for its standardization by many investigators (laboratorical false positive). In addition, LA was found in patients with a variety of diseases, such as infectious, malignant or autoimmune diseases (clinical false positive). Further, there are many patients, strongly suspected to have APS by their clinical phenotype, but negative for any current aPL (laboratorical and/or clinical false negative). For the better recognition of APS patients, we investigators have to maintain our efforts to polish the modality in order to identify 'true aPL'.

Prothrombin

Prothrombin (factor II) is a vitamin K-dependent single-chain glycoprotein of 579 amino acid residues with a molecular weight of 72 kDa, and present at a concentration of approximately 100 μg/ml in normal plasma. Prothrombin undergoes γ-carboxylation during its liver biosynthesis. These γ-carboxyglutamic residues, known as the Gla domain, are located on fragment 1 of the prothrombin molecule. The Gla domain is essential for the calcium-dependent binding of phospholipids to prothrombin, which is necessary for the conversion of prothrombin to biologically active α-thrombin.

Prothrombin is physiologically activated by the prothrombinase complex (factor Xa, factor Va, phospholipids and calcium). Once negatively charged phospholipids bind prothrombin, prothrombinase complex converts prothrombin to thrombin, which triggers fibrinogen polymerization into fibrin. Moreover, thrombin binds thrombomodulin on the surface of endothelial cells and activates protein C, which then exerts its anticoagulant

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activity by digesting factor Va and depriving the prothrombinase complex of its most important cofactor. Owing to this negative feedback pathway, prothrombin/thrombin behaves as an 'indirect' anticoagulant.

Antiprothrombin antibodies and their properties

Antiprothrombin antibodies bind to prothrombin coated on gamma irradiated or activated polyvinyl chloride enzyme-linked immunosorbent assay (ELISA) plates (aPT-A)[1] or exposed to immobilized phosphatidylserine (phospatidylserine-dependent antiprothrombin antibodies, aPS/PT).[2] Moreover, antiprothrombin antibodies have been detected against prothrombin bound hexagonal (II) phase phosphatidylethanolamine.[3]

Unlike β_2GPI, prothrombin requires calcium ions for its binding to anionic phospholipids. Antiprothrombin antibodies may be directed against cryptic or neoepitopes exposed when prothrombin binds to anionic phospholipids. Upon binding to a phosphatidylserine-containing surface in the presence of calcium ions, human prothrombin undergoes a conformational change that results in the exposure of a hydrophobic patch thought to be crucial for functional phospholipid binding. It was proposed that a second conformational change creates a surface-exposed hydrophilic cleft that may be complementary in shape and charge to that of the polar group.[4]

On the other hand, antiprothrombin antibodies may be low affinity antibodies recognized more efficiently when the prothrombin is bound to phosphatidylserine coated on ELISA plates, or may bind bivalently to immobilized prothrombin.[5] Thus, prothrombin complexed with phosphatidylserine could allow clustering and better orientation of the antigen, offering optimal conditions for antibody recognition.

The mechanisms by which antiprothrombin antibodies cause LA activity are not completely elucidated. It was suggested that antiprothrombin antibodies cause prolongation of *in vitro* clotting time by inhibiting the conversion of prothrombin into thrombin.[6] However, it seems unlikely that antiprothrombin antibodies prolong the coagulation times by hampering the activation of prothrombin through binding near the activation sites in the molecule, since such a mechanism would not explain the neutralizing effects of high phospholipid concentrations. Affinity-purified antiprothrombin antibodies from LA positive plasma inhibited both prothrombinase and tenase complex.[7] Thus, in the procoagulant pathway, antiprothrombin antibodies might increase the affinity of prothrombin for negatively charged phospholipids, thereby competing with clotting factors for the available catalytic phospholipid surface, a mechanism similar to that of anti-β_2GPI antibodies. The model, based on an increased affinity of protein-antibody complexes (β_2GPI or prothrombin) for negatively charged phospholipids can explain why LA activity caused by both anti-β_2GPI antibodies and antiprothrombin antibodies can be neutralized by the addition of extra phospholipids.

Recently, we established a phosphatidylserine-dependent monoclonal antiprothrombin antibody, 231D.[8] The 231D spiked plasma showed strong LA activity, and *in vitro* thrombin generation was significantly reduced in the presence of a high factor Va/factor Xa ratio. The anticoagulant activity of 231D may depend on its interaction onto the factor Va binding site of prothrombin molecule.

Clinical significance of aPS/PT

No association between aPT-A and the risk of thrombosis was found in a systematic review.[9] In contrast, we first reported the clinical utility of aPS/PT assay for the diagnosis of APS.[10] The aPS/PT strongly correlate with the presence of LA and both sensitivity and specificity of aPS/PT for the diagnosis of APS were demonstrated to be higher than those for aPT-A and comparable to that for aCL (Table 1); moreover, most of the APS patients with aPS/PT had positive LA (44/47 in APS group but 6/11 in the non-APS group). These data suggest that aPS/PT may also be one of the 'confirming' assays for APS-associated LA.

Table 1 Sensitivity, specificity and odds ratio of aPL tests for the diagnosis of APS

Assay	*Sensitivity (95% CI)*	*Specificity (95% CI)*	*Odds ratio for diagnosis of APS (95% CI)*
LA	84% (74–91%)	79% (71–85%)	19.8 (9.6–40.6)
aCL/β_2GPI	56% (45–67%)	86% (79–91%)	15.4 (7.2–32.7)
aPS/PT	57% (46–68%)	92% (86–96%)	7.9 (4.1–15.2)

APS, antiphospholipid syndrome; LA, lupus anticoagulant; aCL/β_2GPI, β_2 glycoprotein I-dependent anticardiolipin antibodies; aPS/PT, phosphatidylserine-dependent antiprothrombin antibodies. Data taken from Atsumi *et al.*[10] 95% Confidence intervals (CI) for sensitivity and specificity were calculated using the binomial method.

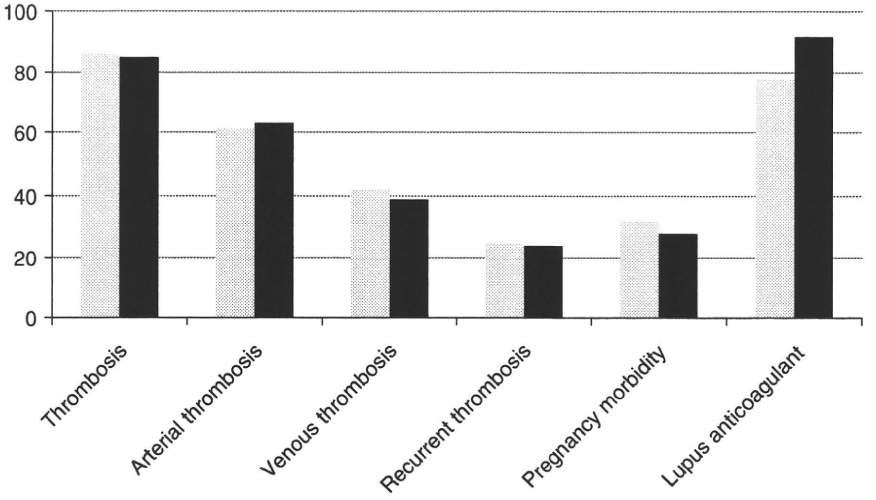

Figure 1 Prevalence of clinical and laboratory manifestations in patients with APS. In our cohort of 126 patients of well-characterized APS, 72 patients had IgG/M aCL and 88 had IgG/M aPS/PT (some patients had both). The gray and black bars represent the prevalence of each manifestation in patients with aCL and aPS/PT, respectively.

Table 2 Prevalence of IgG phosphatidylserine-dependent antiprothrombin antibodies (aPS/PT) in 441 patients with autoimmune diseases

Disease	*Positive/total (n)*	*Prevalence (%)*
Primary APS	33/84	39
APS with SLE	32/68	47
SLE without APS	13/136	10
Rheumatoid arthritis	0/46	0
Primary Sjögren's syndrome	0/36	0
Other systemic autoimmune diseases	3/71	4

Previously our group has used the aPS/PT assay in a large population of patients with autoimmune diseases (British and Japanese population).[11] The investigated population comprised 441 patients including 152 patients with APS (84 primary APS and 68 APS with systemic lupus erythematosus) and 289 patients without APS (136 systemic lupus erythematosus, 46 rheumatoid arthritis, 36 primary Sjögren's syndrome and 71 other rheumatic diseases); see Table 2. We found that IgG aPS/PT were highly prevalent in patients with APS compared with patients with other diseases (odds ratio, OR [95% confidence interval, CI]; 12.8 [7.0–23.2]). In our current cohort of 126 patients with well-characterized APS who visited our autoimmune and rheumatology clinic, clinical manifestations in patients with aPS/PT are very similar to those in patients with aCL. Therefore, aPS/PT may play a role as a marker of the disease as well as aCL.

Apart from its potential role as 'ELISA-based LA confirming', aPS/PT assay showed a very high specificity for the diagnosis of APS. Considering that aPS/PT and aCL have a similar diagnostic value for APS, we propose that aPS/PT should be further explored, not only for research purposes, but also as a candidate for one of the laboratory criteria for the classification of the APS.

Conclusions

aPS/PT, as well as other standard aPL, are useful tools for the diagnosis of APS. We propose that these assays should be performed in conjunction with the LA test. Additional and prospective studies on aPS/PT, however, are needed to establish the clinical relevance of these antibodies.

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