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New tests to detect antiphospholipid antibodies: antiprothrombin (aPT) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies.

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Abstract

Antiprothrombin antibodies have been proposed as potential new biomarkers for thrombosis and/or pregnancy morbidity in the setting of the antiphospholipid syndrome (APS). Antiprothrombin antibodies are commonly detected by ELISA, using prothrombin coated onto irradiated plates (aPT), or prothrombin in complex with phosphatidylserine (aPS/PT), as antigen. Although these antibodies can co-exist in the same patient, aPT and aPS/PT seem to belong to different populations of autoantibodies. Early research explored the role of antibodies to prothrombin as potential antigenic targets for the lupus anticoagulant (LA). To date their clinical significance is being investigated and their potential role in identifying patients at higher risk of developing thrombotic events or pregnancy morbidity is being probed.

Keywords

Antiphospholipid antibodies, Antiphospholipid syndrome, Thrombosis, Venous thrombosis, Antiprothrombin, Anti-phosphatidylserine, Stroke

Introduction

Antiphospholipid antibodies (aPL) are immunoglobulins shown to be related to diverse clinical events, such as arterial and venous thrombosis, complications of pregnancy, livedo reticularis, valvular disease, neurologic disorders, and thrombocytopenia [1]. Despite their name, aPL are not directed against anionic phospholipids but are part of a large family of autoantibodies targeting phospholipid-binding plasma proteins or their complex with phospholipids [2].

The term antiphospholipid syndrome (APS) links thrombosis and/or pregnancy morbidity to persistently positive aPL. The APS is now recognised as one of the most common causes of acquired thrombophilia [3].

In clinical practice, anticardiolipin antibodies (aCL), anti-β2 glycoprotein I (aβ2GPI) antibodies and the lupus anticoagulant (LA) have been the most established tests for the diagnosis of APS [3]. The clinical utility of aPL assays for autoantibodies other than the routinely used is now under debate [4]. Indeed, current lines of research are examining the usefulness of testing for new aPL specificities in helping to identify APS in patients with thrombosis and/or pregnancy morbidity, particularly in those who are repeatedly negative for the currently used tests [5]. Moreover, non-criteria aPL tests, among those antiphrothrombin antibodies, are proposed to help in assessing the risk for both thrombosis and pregnancy morbidities in patients suspected of APS [6••].

Prothrombin, also known as clotting factor II, is a vitamin K–dependent proenzyme of 72 kD. It is synthesized in the liver as an inactive zymogen and exerts a procoagulant activity via a prothrombinase complex, triggering

fibrinogen conversion to fibrin [7]. Prothrombin binds to negatively charged phospholipids through the vitamin K-dependent carboxylation/gamma-carboxyglutamic domains, located in fragment 1 of the prothrombin molecule (Fig. 1). In 1959, Loeliger first reported prothrombin as a cofactor for a 'circulating anticoagulant' in one patient with hypoprothrombinemia [8]. After this first observation, several groups have explored the clinical association of antibodies directed to this protein with the clinical manifestation of APS.

Antiprothrombin Antibodies: Immunological Features and Detection Methods

Antibodies to prothrombin can be detected by ELISA using prothrombin coated onto irradiated plates (aPT) or the phosphatidylserine/prothrombin complex as antigen (aPS/PT) [9, 10]. Both aPT and aPS/PT have been showed to be associated with the clinical manifestation of APS. Although they seem to belong to different populations of autoantibodies, they can both be detected simultaneously in one patient [11].

Antiprothombin antibodies are human species-specific [12], but binding with bovine prothrombin has also been reported in some cases [13].

The recognition of prothrombin by the antibodies largely depends on the mode of presentation of the antigen in solid phase. Antibodies are unable to bind prothrombin when prothrombin is immobilized onto non-irradiated plates [9]. On the contrary, they bind when prothrombin is immobilized on a suitable anionic surface, adsorbed on gamma-irradiated plates or exposed to immobilized anionic phospholipids (Fig. 2). The behaviour of these antibodies in binding the antigen resembles that of anti-β2GPI antibodies. Human prothrombin undergoes a conformational change upon binding to phosphatidylserine-containing surfaces in the presence of calcium [14]. Prethrombin 1 and fragment 1 [15, 16] and fragment 1 + 2 [17] have been reported as potential antigens recognized by antiprothrombin antibodies, suggesting that the dominant epitopes are likely to be located near the phospholipid-binding site of the prothrombin molecule.

Antiprothrombin Antibodies and the Lupus Anticoagulant

Prothrombin was first reported by Loeliger in 1959 [8] as a cofactor for LA. Bajaj et al. [18] were the first to identify antibodies that bound prothrombin without neutralising its coagulant activity in two cases of hypoprothrombinemia associated to LA positivity. The authors suggested that acquired hypoprothrombinemia-lupus anticoagulant syndrome resulted from rapid clearance from the circulation of prothrombin antigen–antibody complexes. Lupus anticoagulant-hypoprothrombinemia syndrome is a rare clinical entity that can occur in association with SLE, transient viral infections, drug reactions or even in healthy individuals [19]. It has been speculated that antibodies found in the presences of LA-associated hypoprothrombinemia may be high affinity, in contrast to those detected in APS in which patients have low affinity antibodies. This difference may determine the clinical presentation of thrombosis versus bleeding [20]. Edson et al. reported the presence of circulating prothrombin–aPT complexes in patients who tested positive for LA and had normal prothrombin levels [21]. Fleck et al. [22] subsequently confirmed that some aPT showed LA activity, and, in 1991, Bevers et al. [12] showed that prothrombin was required for expressing LA activity in 69 % of aCL depleted LA-positive plasma samples.

Available data support the existence of two types of aPT, those with and those without LA activity [23]. Recently, Pengo and colleagues reported a significantly lower prevalence of aPT, when compared to aβ2GPI, in a cohort of patients with LA. No association with the clinical features of APS was reported for aPT [24]. In contrast, some studies have shown a strong correlation between aPS/PT and the LA [25] and the nature of this correlation is currently being investigated. Our group has recently shown that aPS/PT, either IgG or IgM are associated with thrombosis in patients with and without LA. In addition, LA and aPS/PT, individually, were found to be independent risk factors for thrombosis and pregnancy loss.

Clinical Significance

The association between APS and antibodies to prothrombin, detected either as aPT or aPS/PT, has been evaluated with contradictory results [23, 26–30, 31•].

Although no association between aPT and the risk of thrombosis was found in a previous meta-analysis [32], a more recent systematic review suggests that aPT and aPS/PT are risk factors for thrombotic events [33••]. These data are based on more than 7,000 patients and controls from 38 studies analysing aPT and 10 studies analysing aPS/PT. While both antibodies to prothrombin (aPT and aPS/PT) have been shown to increase the risk thrombosis [OR 2.3 (95 % CI 1.72–3.5)], aPS/PT represent a stronger risk factor for both arterial and/or venous thrombosis when compared to aPT [OR 5.11 (95 % CI 4.2–6.3) vs. 1.82 (95 % CI 1.44–2.75), respectively].

Two prospective studies have shown that the presence of aPT is a predictor of first or recurrent thrombosis in aPL patients [34, 35]. Forastiero et al. studied a cohort of patients persistently positive for aCL and/or LA. The authors showed that aPT predicted a higher risk of thromboembolic events, mainly in those patients also positive for the LA [34]. In an inception cohort of 101 SLE patients longitudinally followed for 15 years, Bizzarro et al. showed IgG aPT as the most useful predictor for thrombosis in SLE patients [35].

The notion of aPL has evolved from aPL being considered as pure diagnostic markers to becoming recognised as risk factor for thrombosis. Most importantly, it appears that the risk of thrombosis progressively increases with the number of positive aPL tests. While this concept has already been described for the so-called “triple positivity” for aCL, anti-β2GPI and LA [36], quadruple positivity for LA, aCL, anti-β2GPI and aPT has been shown to confer a 30-fold higher risk of thrombosis, much higher risk than with the former combination [34, 35].

Many reports have also shown the clinical utility of aPS/PT in the diagnosis of APS [9]. Early data from Atsumi et al. showed that the presence of aPS/PT conferred an odds ratio for APS of 3.6 in his large cohort of 265 Japanese patients with systemic autoimmune diseases [10]. Recently, Zigon et al. showed aPS/PT as the strongest independent risk factor for the presence of obstetric complications in a cohort of 156 patients with systemic autoimmune diseases [37]. Interestingly, while some studies support the association of aPS/PT with arterial thrombosis, mainly in the setting of ischemic/thrombotic cerebrovascular events [38, 39], Zigon et al. failed to confirm this association. Both IgG and IgM aPS/PT were only associated with venous thrombosis [37].

Sanfelippo et al. [40] measured aPS/PT in 728 patients suspected of having APS, in the absence of of aCL or anti-β2GPI. Of the 728 tested samples, 41 had elevated levels of aPS/PT with thrombotic events occurring in 11 out of the 22 patients with accessible medical histories. Overall, these data support the notion that testing for aPS/PT in

those negative for aCL, anti-β2GPI and the LA can contribute to the identification of APS in patients that may otherwise go undetected with current testing methods.

The importance of aPS/PT as a diagnostic marker of APS was also recently reported by our group [31•]. In this study, we evaluated several possible aPL specificities combination in an attempt to establish the profile that would provide us with the best diagnostic accuracy. Testing for six aPL derived in 23 possible combinations of results. The profile including LA + anti-β2GPI + aPS/PT held the best diagnostic accuracy for APS as a whole and, individually, for each thrombosis and pregnancy loss [OR 3.73 (95 % CI 1.82–5.38); OR 3.75 (95 % CI 2.13–6.62) and OR 4.82 (95 % CI 2.17–10.72), respectively] and the best specificity when compared with all the other attainable combination of tests, including the current classification criteria profile.

When systematically reviewing the topic [33••], we found 7 studies that directly compared aPT and aPS/PT and their OR for thrombosis in 1196 patients [5, 9, 41–44]. When analysed, aPS/PT was shown to be more strongly associated with thrombosis, both arterial and venous, than aPT.

While evidence tends to support the importance of testing for antiprothrombin antibodies, particularly aPS/PT, in routine practice, methodological issues appertaining to all aPL are the subject of debate and concern. Future efforts of harmonisation of tests used to detect antibodies binding prothrombin are urgently needed. Potentially, the use of reference material for these, as well as all aPL detection, will aid in solving many of the problems caused by a lack of standardisation of aPL assays.

aPT and aPS/PT and Scoring Systems

Risk prediction models are becoming increasingly common in both medical research and clinical practice, due in part to the enhanced focus on individualised medicine [45]. Recently, three score systems have been formulated to quantify the risk of thrombosis/obstetric events in APS, aiming to help physicians to stratify patients according to risk [46–48]. Two of those scores include antibodies to prothrombin among the variables computed when assessing the risk for thrombosis or pregnancy morbidity [47, 48]. Otomo et al. [47] designed the “antiphospholipid score” (aPL-S) with the purpose of quantifying the risk based on the aPL profile. An algorithm was created based on multiple aPL assays, with each assay being assigned a different score weighted on the relative risk of having clinical manifestations of APS. In order to independently validate the aPL-S, we applied the proposed score system to a cohort of 211 consecutive SLE patients, proving that the aPL-S correlated with a history of thrombosis or pregnancy loss in our cohort, suggesting that the aPL-S is a suitable quantitative marker of APS [49].

Recently, we formulated an alternative score derived from the combination of independent risk factors for thrombosis and pregnancy loss in a large cohort of well-characterised SLE patients: the Global APS Score or GAPSS [48]. This score takes into account not only the aPL profile (criteria [3] and non-criteria aPL [31•]) but also includes the conventional cardiovascular risk factors and the autoimmune antibodies profile [48] into the equation (Table 1). Positivity for aPS/PT was found to be an important variable when assessing the risk by using the GAPSS, suggesting that the addition of these antibodies can help in predicting APS-related clinical manifestations risk. The GAPSS scoring system is derived from the combination of independent risk for both thrombosis and pregnancy loss, and accounted for multiple factors, including the patient’s aPL profile, conventional cardiovascular risk

factors, autoimmune antibody profile, and thromboprophylactic drug use [42]. The GAPSS can be calculated for each patient by adding the points corresponding to the different risk factors, weighted as shown. GAPSS values ≥10 have demonstrated the best diagnostic accuracy compared with the different thresholds for APS diagnosis.

Conclusions

Although some controversial data exist, most of the studies in the literature support the association between antibodies directed to prothrombin, particularly aPS/PT, and the clinical manifestations of the APS. In addition, the correlation with the LA make these antibodies an interesting subpopulation in APS. Many groups are currently working towards further characterisation of aPS/PT and their mechanisms of action, although additional laboratory and clinical studies are needed to conclusively define the relevance and prognosis impact of testing for these antibodies in the daily routine clinical practise. The possibility of antiprothrombin antibodies, particularly aPS/PT, becoming an additional serological classification criterion for APS is being eagerly discussed, especially when considering how to identify APS patients negative for classical aPL.

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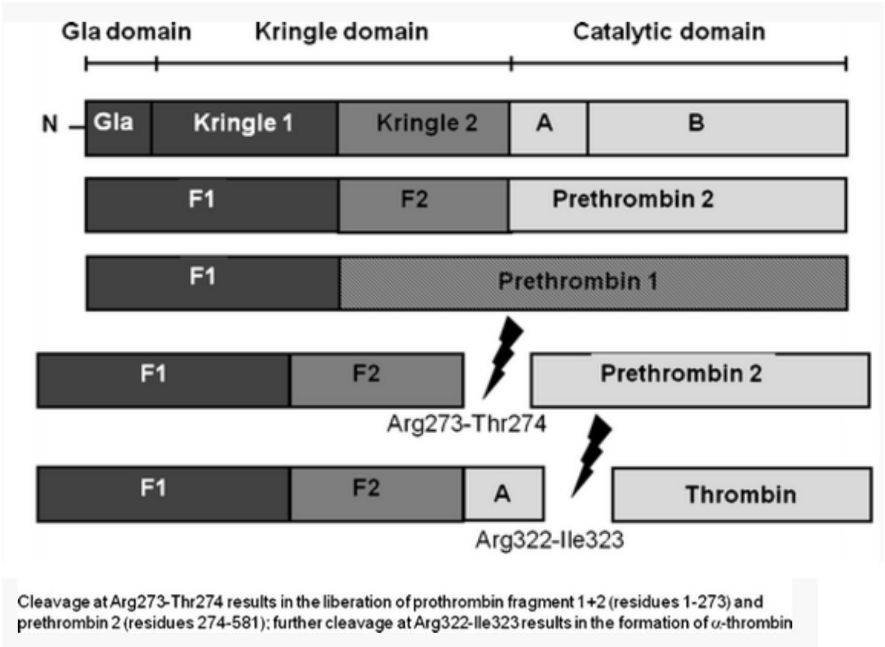

Fig. 1

Schematic structure of the prothrombin molecule. Cleavage at Arg273-Thr274 results in the liberation of prothrombin fragment 1 + 2 (residues 1–273) and prethrombin 2 (residues 274–581); further cleavage at Arg322-Ile323 results in the formation of α-thrombin

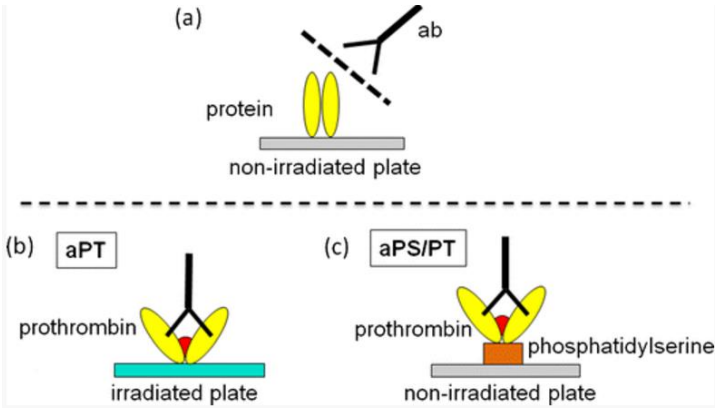

Fig. 2

ELISA systems for the detection of antiprothrombin antibodies. Antibodies are unable to bind prothrombin when prothrombin is immobilised onto non-irradiated plates (a), but they bind when prothrombin is immobilised on a suitable anionic surface, adsorbed on gamma-irradiated plates (b) or exposed to immobilised anionic phospholipid (c)

Table 1
The Global AntiPhospholipid Syndrome Score (GAPSS)

Factor	Point value
Anticardiolipin IgG/IgM	5
Anti-β2-glycoprotein IgG/IgM	4
Lupus anticoagulant	4
Anti-prothrombin/phosphatidylserine complex (aPS/PT) IgG/IgM	3
Hyperlipidemia	3
Arterial hypertension	1