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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- β_2 glycoprotein I (a β_2 GPI) 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 antiprothrombin 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].

Antiprothrombin 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- β 2GPI 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 a β 2GPI, 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- β 2GPI and LA [36], quadruple positivity for LA, aCL, anti- β 2GPI 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 aCL or anti- β 2GPI. 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- β 2GPI 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- β 2GPI + 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.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. Antiphospholipid syndrome. *Lancet*. 2010;376(9751):1498–509.
2. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. *N Engl J Med*. 2013;368(11):1033–44.
3. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *JTH*. 2006;4(2):295–306.
4. Bertolaccini ML. Antibodies to prothrombin. *Lupus*. 2012;21(7): 729–31.
5. Bertolaccini ML, Gomez S, Pareja JF, Theodoridou A, Sanna G, Hughes GR, et al. Antiphospholipid antibody tests: spreading the net. *Ann Rheum Dis*. 2005;64(11):1639–43.
6. Bertolaccini ML, Amengual O, Atsumi T, Binder WL, de Laat B, Forastiero R, et al. 'Non-criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX, USA, April 2010. *Lupus*. 2011;20(2):191–205. This report summarizes the findings, conclusions, and recommendations of the 13th International Congress on Antiphospholipid Antibodies Diagnostic task force.
7. Mann KG, Nesheim ME, Church WR, Haley P, Krishnaswamy S. Surface-dependent reactions of the vitamin K-dependent enzyme complexes. *Blood*. 1990;76(1):1–16.

8. Loeliger A. Prothrombin as a co-factor of the circulating anticoagulant in systemic lupus erythematosus? *Thromb Diath Haemorrh.* 1959;3:237–56.
9. Bertolaccini ML, Atsumi T, Koike T, Hughes GR, Khamashta MA. Antiprothrombin antibodies detected in two different assay systems. Prevalence and clinical significance in systemic lupus erythematosus. *Thromb Haemost.* 2005;93(2):289–97.
10. Atsumi T, Ieko M, Bertolaccini ML, Ichikawa K, Tsutsumi A, Matsuura E, et al. Association of autoantibodies against the phosphatidylserine-prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. *Arthritis Rheum.* 2000;43(9):1982–93.
11. Galli M, Beretta G, Daldossi M, Bevers EM, Barbui T. Different anticoagulant and immunological properties of anti-prothrombin antibodies in patients with antiphospholipid antibodies. *Thromb Haemost.* 1997;77(3):486–91.
12. Bevers EM, Galli M, Barbui T, Comfurius P, Zwaal RF. Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb Haemost.* 1991;66(6):629–32.
13. Rao LV, Hoang AD, Rapaport SI. Differences in the interactions of lupus anticoagulant IgG with human prothrombin and bovine prothrombin. *Thromb Haemost.* 1995;73(4):668–74.
14. Wu JR, Lentz BR. Phospholipid-specific conformational changes in human prothrombin upon binding to procoagulant acidic lipid membranes. *Thromb Haemost.* 1994;71:596–604.
15. Rao LV, Hoang AD, Rapaport SI. Mechanism and effects of the binding of lupus anticoagulant IgG and prothrombin to surface phospholipid. *Blood.* 1996;88(11):4173–82.
16. Akimoto T, Akama T, Kono I, Sumida T. Relationship between clinical features and binding domains of anti-prothrombin autoantibodies in patients with systemic lupus erythematosus and antiphospholipid syndrome. *Lupus.* 1999;8(9):761–6.
17. Malia RG, Brookfield C, Bulman L, Greaves M. Prothrombin fragment 1–2: the epitope for antiphospholipid antibody expression. *Thromb Haemost.* 1997;171:689.
18. Bajaj SP, Rapaport SI, Fierer DS, Herbst KD, Schwartz DB. A mechanism for the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome. *Blood.* 1983;61(4):684–92.
19. Rapaport SI, Ames SB, Duvall BJ. A plasma coagulation defect in systemic lupus erythematosus arising from hypoprothrombinemia combined with antiprothrombinase activity. *Blood.* 1960;15:212–27.
20. Erkan D, Bateman H, Lockshin MD. Lupus anticoagulant-hypoprothrombinemia syndrome associated with systemic lupus erythematosus: report of 2 cases and review of literature. *Lupus.* 1999;8:560–4.
21. Edson JR, Vogt JM, Hasegawa DK. Abnormal prothrombin crossed-immunoelectrophoresis in patients with lupus inhibitors. *Blood.* 1984;64(4):807–16.

22. Fleck RA, Rapaport SI, Rao LV. Anti-prothrombin antibodies and the lupus anticoagulant. *Blood*. 1988;72(2):512–9.
23. Pengo V, Denas G, Bison E, Banzato A, Jose SP, Gresele P, et al. Prevalence and significance of anti-prothrombin (aPT) antibodies in patients with Lupus Anticoagulant (LA). *Thromb Res*. 2010;126(2):150–3.
24. Horbach DA, van Oort E, Derksen RH, de Groot PG. The contribution of anti-prothrombin-antibodies to lupus anticoagulant activity—discrimination between functional and non-functional antiprothrombin-antibodies. *Thromb Haemost*. 1998;79(4):790–5.
25. Atsumi T, Amengual O, Yasuda S, Koike T. Antiprothrombin antibodies—are they worth assaying? *Thromb Res*. 2004;114(5–6):533–8.
26. Pengo V, Biasiolo A, Brocco T, Tonetto S, Ruffatti A. Autoantibodies to phospholipid-binding plasma proteins in patients with thrombosis and phospholipid-reactive antibodies. *Thromb Haemost*. 1996;75(5):721–4.
27. Roubey RA. Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulants and other "antiphospholipid" autoantibodies. *Blood*. 1994;84(9):2854–67.
28. Bertolaccini ML, Atsumi T, Khamashta MA, Amengual O, Hughes GR. Autoantibodies to human prothrombin and clinical manifestations in 207 patients with systemic lupus erythematosus. *J Rheumatol*. 1998;25(6):1104–8.
29. Vaarala O, Puurunen M, Manttari M, Manninen V, Aho K, Palosuo T. Antibodies to prothrombin imply a risk of myocardial infarction in middle-aged men. *Thromb Haemost*. 1996;75(3):456–9.
30. Hoxha A, Ruffatti A, Pittoni M, Bontadi A, Tonello M, Salvan E, et al. The clinical significance of autoantibodies directed against prothrombin in primary antiphospholipid syndrome. *Clin Chim Acta*. 2012;413(9–10):911–3.
31. • Sciascia S, Murru V, Sanna G, Roccatello D, Khamashta MA, Bertolaccini ML. Clinical accuracy for diagnosis of antiphospholipid syndrome in systemic lupus erythematosus: evaluation of 23 possible combinations of antiphospholipid antibody specificities. *JTH*. 2012;10(12):2512–8. This study aimed to evaluate the clinical accuracy of aPL specificities both individually and/ or in combination, in an attempt to identify a panel of tests that may provide the best accuracy for diagnosing APS.
32. Galli M, Luciani D, Bertolini G, Barbui T. Anti-beta2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. *Blood*. 2003;102(8):2717–23.
33. •• Sciascia S, Sanna G, Murru V, Roccatello D, Khamashta MA, Bertolaccini ML. Anti-prothrombin (aPT) and antiphosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome. A systematic review. *Thromb Haemost*. 2013;111(2). This systematic review supports the concept that antibodies to prothrombin (both aPT and aPS/PT) increase the risk of thrombosis.

34. Forastiero R, Martinuzzo M, Pombo G, Puente D, Rossi A, Celebrin L, et al. A prospective study of antibodies to beta2- glycoprotein I and prothrombin, and risk of thrombosis. *JTH*. 2005;3(6):1231–8.
35. Bizzaro N, Ghirardello A, Zampieri S, Iaccarino L, Tozzoli R, Ruffatti A, et al. Anti-prothrombin antibodies predict thrombosis in patients with systemic lupus erythematosus: a 15-year longitudinal study. *JTH*. 2007;5(6):1158–64.
36. Pengo V, Biasiolo A, Pegoraro C, Cucchini U, Noventa F, Iliceto S. Antibody profiles for the diagnosis of antiphospholipid syndrome. *Thromb Haemost*. 2005;93(6):1147–52.
37. Zigon P, Cucnik S, Ambrozic A, Kveder T, Semrl SS, Rozman B, et al. Detection of antiphosphatidylserine/prothrombin antibodies and their potential diagnostic value. *Clin Dev Immunol*. 2013;2013: 724592.
38. Tselios K, Sarantopoulos A, Gkougkourellas I, Boura P. The role of anti-phosphatidylserine/prothrombin antibodies in thrombotic manifestations of systemic lupus erythematosus patients. *Front Neurol*. 2013;4:66.
39. Mullen MT, Messe SR, Kasner SE, Sansing L, Husain MR, Norman GL, et al. Anti-phosphatidylserine-prothrombin antibodies are associated with outcome in a TIA cohort. *Front Neurol*. 2012;3:137.
40. Sanfelippo M, Joshi A, Schwartz S, Meister J, Goldberg J. Antibodies to phosphatidylserine/prothrombin complex in suspected antiphospholipid syndrome in the absence of antibodies to cardiolipin or Beta-2-glycoprotein I. *Lupus*. 2013;22(13):1349–52.
41. Tsutsumi A, Hayashi T, Chino Y, et al. Significance of antiprothrombin antibodies in patients with systemic lupus erythematosus: clinical evaluation of the antiprothrombin assay and the antiphosphatidylserine/prothrombin assay, and comparison with other antiphospholipid antibody assays. *Mod Rheumatol Jpn Rheum Assoc*. 2006;16(3):158–64.
42. Bardin N, Alessi MC, Dignat-George F, et al. Does the antiprothrombin antibodies measurement provide additional information in patients with thrombosis? *Immunobiology*. 2007;212(7): 557–65.
43. Sailer T, Vormittag R, Zoghalmi C, et al. The clinical significance of anti-prothrombin antibodies for risk assessment of thromboembolism in patients with lupus anticoagulant. *Thromb Res*. 2007;120(2):295–302.
44. Hasegawa M, Fujimoto M, Hayakawa I, et al. Antiphosphatidylserine-prothrombin complex antibodies in patients with localized scleroderma. *Clin Exp Rheumatol*. 2006;24(1):19–24.
45. Tzoulaki I, Liberopoulos G, Ioannidis JP. Assessment of claims of improved prediction beyond the Framingham risk score. *JAMA*. 2009;302(21):2345–52.
46. Sciascia S, Cosseddu D, Montaruli B, Kuzenko A, Bertero MT. Risk Scale for the diagnosis of antiphospholipid syndrome. *Ann Rheum Dis*. 2011;70(8):1517–8.
47. Otomo K, Atsumi T, Amengual O, Fujieda Y, Kato M, Oku K, et al. Efficacy of the antiphospholipid score for the diagnosis of antiphospholipid syndrome and its predictive value for thrombotic events. *Arthritis Rheum*. 2012;64(2):504–12.

48. Sciascia S, Sanna G, Murru V, Roccatello D, Khamashta MA, Bertolaccini ML. GAPSS: the Global Anti-Phospholipid Syndrome Score. *Rheumatology (Oxford)*. 2013;52(8):1397–403.
49. Sciascia S, Bertolaccini ML, Roccatello D, Khamashta MA. Independent validation of the antiphospholipid score for the diagnosis of antiphospholipid syndrome. *Ann Rheum Dis*. 2013;72: 142–3.

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- β 2-glycoprotein IgG/IgM	4
Lupus anticoagulant	4
Anti-prothrombin/phosphatidylserine complex (aPS/PT) IgG/IgM	3
Hyperlipidemia	3
Arterial hypertension	1