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Anti-prothrombin (aPT) and anti phosphatidylserine/prothrombin (aPS/PT) antibodies and the risk of thrombosis in the antiphospholipid syndrome

A systematic review

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Abstract:

Background: Antibodies to prothrombin are detected by directly coating prothrombin on irradiated ELISA plates (aPT) or by using the phosphatidylserine/prothrombin complex as antigen (aPS/PT). Although these antibodies have both been associated with antiphospholipid syndrome (APS) and a correlation between the two assays have been reported, it seems that aPT and aPS/PT belong to different populations of autoantibodies. **Objectives:** To systematically review the available evidence on aPT and aPS/PT antibodies and the risk of thrombosis in APS. **Methods:** Medline-reports published between 1988 and 2013 investigating aPT and aPS/PT as a risk factor for thrombosis were included. Whenever possible, antibody isotype(s) and site of thrombosis were analysed. **Results:** This systematic review is based on available data from more than 7000 patients and controls from 38 studies analyzing aPT and 10 aPS/PT. Antibodies to prothrombin (both aPT and aPS/PT) increased the risk thrombosis (OR 2.3 [95%CI 1.72-3.5]). aPS/PT seemed to represent a stronger risk factor for thrombosis, both arterial and/or venous than aPT (OR 5.11 [95%CI 4.2-6.3] and 1.82 [95%CI 1.44-2.75], respectively). **Conclusion:** Routine measurement of aPS/PT (but not aPT) might be useful in establishing the thrombotic risk of patients with previous thrombosis and/or systemic lupus erythematosus. Their inclusion as laboratory criteria for the APS should be indisputably further explored.

INTRODUCTION: Antiphospholipid antibodies (aPL) are a wide and heterogeneous family of immunoglobulin G, M and, less frequently A, initially thought to target negatively charged phospholipids (1). 2GPI) and the lupus β 2 glycoprotein-I (anti- β 2GPI). Although anticardiolipin (aCL), anti- β 2GPI and anticoagulant (LA) are the routinely tested antibodies for the diagnosis of antiphospholipid syndrome (APS) (2), research on aPL is continuously expanding in the search for new clinically useful markers. Prothrombin, a plasma protein, was described as a cofactor for a 'circulating anticoagulant' by Loeliger in 1959 (3). Prothrombin is a common antigenic target for aPL, reported in around 50–90% of patients with other aPL (4). Because antiprothrombin antibodies have heterogeneous immunologic and functional properties, their clinical significance is still under debate. Evidence of pathogenicity comes from animal model. Active immunization with prothrombin was associated with prothrombotic activity of blood in an ex-vivo mice model, producing direct evidence for thrombus induction by antibodies to prothrombin (5). Antibodies to prothrombin are usually detected by two different system assays, by directly coating prothrombin on irradiated ELISA plates (aPT) or by using the phosphatidylserine/prothrombin complex as antigen (aPS/PT). Although aPT and/or aPS/PT have both been associated with APS and a correlation between the two assays have been reported (6), it seems that aPT and aPS/PT belong to different populations of autoantibodies even though they can both be present in the same patient (7).

Studies on the significance of antiprothrombin antibodies in thrombosis, including a systematic review (8) have shown controversial results. Therefore the value of antiprothrombin antibodies as markers of APS remains to be determined whilst the strength of the association of anti-prothrombin antibodies, detected either as aPT or aPS/PT, with thrombosis remains to be established. To contribute to this issue, we carried out a systematic review

of the literature. In an attempt to summarise all available data on this subject, we selected papers whose design allowed us to objectively verify the clinical events and to establish the odds ratio with their 95% confidence interval (OR [95%CI]) for aPT and/or aPS/PT for thrombosis. Whenever possible, the antibody isotype(s) and the site of thrombosis were taken into account.

Patients, materials, and methods

Literature search and selection of studies

Articles were identified by a computer-assisted search of the literature. The search strategy was applied to Ovid MEDLINE (R) In Process & other non-indexed citations and Ovid MEDLINE from 1988 through 2013. The grey literature was searched by applying a similar strategy to Google ScholarR, PubMedR and the Proquest Dissertation & Theses databases. Additional references were identified from manual review of the reference lists of included articles. A schematic representation of the search strategy, key words and subject terms used in the search is given in Figure 1. This approach has been previously reported and validated (8, 9). The search was further refined using the following limitations: "English language" and "human." Review articles were excluded from the search. For aPT and aPS/PT we mainly focused on studies that met specific requirements such as specification of the temporal sequence between measurement of the antibodies and the events, or the presence of a control group besides the objective documentation of thrombosis. Prospective, cross-sectional, case-control, and ambispective studies met these criteria, although retrospective studies were also included. All series of 7 or more patients were classified according to the aPL type and the underlying disease, and information about the study design and the assay methods was recorded.

Assessment of validity

Study Selection

Potential studies identified with the above search strategy were exported to an electronic reference management software program (RefWorks v.2.0). Duplicate studies were identified and removed using the filter functions "exact duplicates" and "close duplicates." Two independent reviewers (SS, MLB) reviewed all potential studies. Eligibility was first determined by review of the title and abstract and then by full article review. Disagreements were resolved by consensus; if consensus could not be achieved, a third party (GS) would provide an assessment of eligibility. As the data on eligibility were dichotomous (eligible: yes / no), inter-rater agreement at both the title and abstract review and the full article review stages was determined by calculation of Cohen's kappa coefficient (10). The patient population and laboratory methods were systematically examined.

Patient population: We noted whether eligibility criteria and confounding factors were specified for patients and, when included, controls. Patients were classified according to their underlying disease or syndrome. The major categories included systemic lupus erythematosus (SLE), other autoimmune diseases, non autoimmune diseases, arterial thrombosis, venous thrombosis and aPL positivity.

Assay methods: Only studies that objectively verified the thromboembolic events were included. The following methods were considered: computerized ultrasonography or venography for deep vein thrombosis; radionuclide lung scanning or angiography for pulmonary embolism; arteriography for peripheral arterial occlusions; computed tomography, resonance imaging, or angiography for ischemic stroke; and electrocardiogram and cardiac enzymes for myocardial infarction. Among the details of thrombosis, we noted the site (arterial or venous) and whether it was the first episode or a recurrence. Only studies that objectively described the methodological approach for aPT and/or aPS/PT were included. We registered the aPT and aPS/PT antibody isotype (s) and how normal cut off was expressed (i.e. the number of standard deviations above the mean of controls, the multiple of the mean, the

percentile, or quartile) (data not shown). In view of the current lack of standardization for these assays, the decision to include or exclude a study did not take into account the method used to detect aPL, as long as the determination of the cut-off values was fully detailed. Articles were then analyzed both pooled together and separately, according to the methods used for anti-prothrombin antibodies testing (i.e. aPT or aPS/PT).

Statistical analysis

Odds ratios with 95% CI (OR [95%CI]) for arterial and/or venous thrombosis were recorded. If not available, they were calculated, whenever possible, by means of contingency tables. In case-control and cross-sectional studies, contingency tables were used to compare the proportion of aPT and/or aPS/PT in patients with and without thrombosis. In prospective studies, contingency tables were established as previously reported (8, 9). Briefly, if SLE was the enrolment criterion, the OR [95%CI] was calculated by comparing the proportion of aPT and/or aPS/PT antibodies in patients who did or did not develop thrombosis during followup. If thrombosis was the enrolment criterion, the OR [95%CI] was calculated by comparing the proportion of aPT and/or aPS/PT in patients with or without recurrent thrombosis during follow up. If positivity for aPL was the enrolment criterion, the OR [95%CI] was calculated by comparing the rates of thrombosis during follow-up of patients grouped according to different antibody types and titers.

Risk of Bias Assessment

One reviewer (SS) assessed the risk of bias of individual studies using the Newcastle-Ottawa Scale (NOS) for cohort studies, and the NOS for case control studies. The NOS is a scoring tool used to assess quality of evidence and risk of bias for non-randomized studies included in meta-analyses(11) The overall quality of evidence was determined using GRADE criterion and summarized using GRADE profiler (12).

Results Literature search strategy and articles retrieved

Sixty-six articles were retrieved: 48 investigated aPT (6, 7, 13-59) and 18 dealt with aPS/PT (6, 7, 24, 39, 43, 44, 52, 57, 58, 60-68) (Figure 1). Of them, 7 studies (6, 7, 24, 39, 42, 43, 64) analyzed both aPT and aPS/PT. After full article review, 48 publications met the eligibility criteria for inclusion in the review (kappa 0.94). Two further publications (69, 70) were obtained from review of the reference lists of included articles; however they were excluded because it was not possible to differentiate APS patients with thrombosis from those with pregnancy morbidity. No further publications were obtained from review of the grey literature. The majority of the excluded articles for the following reasons: in 8 papers (49, 51, 61-65, 68), the OR [95%CI] for thrombosis was not provided and could not be calculated; 10 other studies (47, 48, 50, 53-59) were found not pertinent for the purpose of this review. The main characteristics of the remaining 48 articles are depicted in Tables 1 and 2. Overall, these studies provided information on 6006 patients and 1422 controls. Four prospective studies contributed with 417 patients. As most of the available studies were retrospective, we failed to separately analyze them on the basis of their design. In some studies healthy controls were involved only in the setting up of cut off values for aPT and/or aPS/PT testing. Indeed, they were reported but not 2GPI were theβincluded in the analysis. SLE, APS, and the presence of LA, aCL and/or anti– enrolment criteria in 37 studies. Only 16 studies performed multivariate analysis using logistic regression, allowing a risk assessment analysing the joint contribution of each risk factor.

Studies on aPT

The OR with 95% CI of aPT for thrombosis were calculated in 38 studies on 5705 patients and 1262 controls (Table 1, Figure 3). One study (42) used 2 methods to detect the antibodies and, therefore, results are reported for both of the assays. Overall, 22 (49%) of 45 associations reached significance: 2 of 7 associations with arterial thrombosis, 7 of 11 with venous thrombosis, and 13 of 27 with any thrombosis (as no distinction was possible

between venous and arterial thrombosis). In one case, the association was confirmed after multivariate analysis only if associated to positive LA (26). Isotype analysis was performed in a few studies. These showed that IgG aPT were significantly associated with thrombosis as a whole in 6 out of 7 studies (86%) (6, 7, 31, 34, 38, 44, 46) and with venous thrombosis in 3 of 5 studies (60%) (6, 27, 43, 44, 71). No associations between IgG aPT with arterial thrombosis were reported (6, 42, 44, 71). IgM aPT was associated with thrombosis as a whole in 3 (42%) of 7 studies (6, 7, 31, 34, 38, 44, 46). However, no associations were sustained after subgrouping between h arterial (6, 42, 44, 71) or venous thrombosis (6, 27, 43, 44, 71). Studies that included multivariate analysis are shown in Table 1. Overall, IgG aPT seemed more consistently associated with thrombosis than IgM antibodies. The lack of reference materials to quantify aPT meant we could not assess whether the risk correlated with their titers.

Studies on aPS/PT

The OR [95%CI] of aPS/PT for thrombosis were available in 10 studies on 1775 patients and 628 controls (Table 2; Figure 4). Overall, 15 out of 18 analyses (83 %) reported significant associations: 3 out of 6 with arterial thrombosis, 4 out of 4 with venous thrombosis, and 8 out of 8 with thrombosis as a whole. Isotype analysis showed that IgG aPS/PT was associated with thrombosis as a whole, particularly with venous thrombosis in all the studies. Three studies failed to confirm the association between IgG aPS/PT and arterial thrombosis (39,55,62). The IgM isotype reached significance with thrombosis as a whole in all the studies but one (44). When analysed separately, the association with venous thrombosis was confirmed in all the studies, while the association with arterial events was confirmed in 2 studies (66, 67) out of 4 (Figure 4). Five studies (24, 52, 60, 66, 67) performed multivariate analysis. The association between aPS/PT and thrombosis as a whole and venous thrombosis was confirmed in all. In two studies, a significant association with arterial thrombosis was lost after multivariate analysis (37, 67).

Comparison of aPT and aPS/PT

There were 7 studies (6, 7, 24, 39, 42, 43, 64) that directly compared aPT and aPS/PT and their OR for thrombosis in 1196 Patients. When analysed within the same studies, aPS/PT is shown to be more strongly associated with thrombosis, both arterial and venous, than aPT. Overall, 10 out of 11 (90%) possible analyses derived from the 7 studies reported a significant association between any thrombosis and aPS/PT compared to 5/11 for aPT. Analysis in relation to the type of thrombosis showed that aPS/PT only seemed to be associated with arterial events. The association with venous thrombosis was confirmed in all the studies for between aPS/PT and in 4 out 6 for aPT.

DISCUSSION

This systematic review of the literature aimed at establishing the strength of the association between anti-prothrombin antibodies, tested either as aPT and/or aPS/PT, with thrombosis. Data on more than 7000 patients and controls from 38 studies analyzing aPT and 10 aPS/PT were available for our systematic review. Although 10 studies measured aPS/PT, only 7 directly compared the odds ratio for thrombosis in about a thousand patients and controls. The aPS/PT antibodies were more significantly associated with thrombosis. Although indirect and potentially risky, comparison of the studies that analyzed only one antibody confirmed this increasing awareness. Overall, this review formally establishes aPS/PT are strong risk factors for thrombosis, irrespective of the site and type of thrombosis. aPS/PT have an odds ratio for thrombosis three to eighteen times higher than controls (6, 7, 24, 39, 43, 44, 52, 57, 58, 60-68). As already demonstrated for Lupus Anticoagulant (28), the aPS/PT estimated risk is very close to that reported for deep vein thrombosis in patients with (72) and without (73) SLE. This risk has also been reported to be similar to and, in some instances, even higher than that in patients with genetically determined risk factors for venous thrombosis(74).

Although the general population is unlikely to benefit from indiscriminate screening for these antibodies, their detection in patients with SLE, connective tissue diseases and/or previous thrombosis is justified by the high thrombotic risk associated with these clinical conditions. The aPT does not seem to be such a strong risk factor for APS, as less than 50% of their associations with thrombosis reached statistical significance. A sub-analysis of the different types of thrombosis showed that aPT are mainly associated with venous thrombosis although data available is vastly skewed. Whilst some data suggest that aPT antibodies are associated with thrombosis, a number of issues raise concern. Firstly, significant associations mainly come from retrospective studies, which contain a low level of evidence. Secondly, only a minority of studies confirm their findings by multivariate analysis. And finally, when the antibodies are analyzed in relation to the type of thrombosis, they are not associated with arterial events, and only marginally with thrombotic events as a whole. Overall, the potential role of aPT antibodies as laboratory tool for the diagnosis of APS still remains to be established. In this review we were unable to fully address the importance of antibody isotypes, as most studies investigated only IgG antibodies or did not distinguish between IgG and/or IgM. With these limitations, the association between IgM aPT and thrombosis is still elusive limiting their value in clinical practice. In contrast, our analysis strongly suggests that aPS/PT are associated with thrombosis. In this case, as well as per aPT, the fact that significant associations mainly came from retrospective studies, with a low level of evidence needs to be acknowledged. Intriguingly, despite a two-fold-increase in the risk for arterial thrombosis in aPS/PT positive, two recent studies failed in showing this association (60, 67). All in all, these findings need to be clearly substantiated by well-designed prospective clinical studies. The strengths of this analysis lie on a comprehensive search strategy, the inclusion of grey literature searches and manual review of reference lists minimized the risk of missing eligible studies. We performed independent and duplicate review for study selection, and data extraction. The use of the GRADE system and the NOS scale increases our confidence in our conclusions, and allows for reliable assessment of the quality of individual studies and the overall body of evidence. There are also limitations. All of the included studies were observational studies, subject to the biases inherent in this study design. The majority of the studies enrolled small numbers of patients, resulting in a loss of precision. Additionally, there was heterogeneity in the data. This resulted in the overall quality of the evidence being rated as “low,” as per GRADE criteria. Overall, based on our data, routine measurement of aPS/PT might be useful in establishing the thrombotic risk of patients with previous thrombosis and/or SLE. In conclusion, aPS/PT and not aPT seem to be a potential candidate as a laboratory tool for the diagnosis of APS whereas their inclusion as laboratory criteria for the APS should be indisputably explored. Acknowledgements: MLB is funded by the Louise Gergel Fellowship. This work was supported by grants from the St Thomas’ Lupus Trust.

Author Contributions

Study design. SS, MLB, GS, MK

Acquisition of data. SS, MLB, VM, DR

Analysis and interpretation of data. SS, MLB, GS, DR

Manuscript preparation. SS, MLB, GS, MK

Statistical analysis. SS, MLB

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Table 1: aPT and thrombosis: main characteristics of 38 articles on 5705 patients and 1262 controls

Article and Ref Number	Year	Study design	N.patients and Enrolment criteria	N. Controls	Isotypes
Horbach et al (9)	1996	R (M)	175 SLE	23 thrombosis, 40 aPL carriers, 42 HC	IgG/IGM
Pengo et al (10)	1996	R	22 APS		IgG
Puurunen et al (11)	1996	R	139 APS		IgG/IGM
Forastiero et al (12)	1997	R (M)	233 aPL positive		IgG/IGM
Palosuo et al (13)	1997	CS	265 DVT	265 HC	IgG
Bertolaccini et al (14)	1998	R	207 SLE		IgG/IGM
Vaarala et al (15)	1998	CS	106 IMA	106 coronary episodes	Ig G
Martinuzzo et al (16)	1998	R	54 pulmonary hypertension		IgG/M
Akimoto et al (17)	1999	R(M)	13 aPL positive		IgG/IGM
Galli et al (18)	2000	R	75 aPL positive		IgG/IGM
Lakos et al (19)	2000	R	70 SLE		IgG/IGM
Atsumi et al (20)	2000	R(M)	265 SLE	36 HC	IgG/IGM
Donohoe et al (21)	2001	R	66 APS	30 HC, 42 SLE	IgG/IGM
Nojima et al (22)	2001	R(M)	124 SLE		IgG/IGM
Nojima et al (23)	2001	R(M)	168 SLE	80 HC	IgG/IGM
Pasquier et al (24)	2001	CS	398 suspected VTE		IgG/IGM
Sands et al (25)	2001	R	88 haemodialysis		IgG/IGM
Salcido-Ochoa et al (27)	2002	R	38 PAPS, 466 SLE		IgG/IGM
Simmelink et al (28)	2003	R	46 SLE	40HC	IgG/IGM
Previtali et al (29)	2002	CC	79 arterial/venous thrombosis	85 HC	IgG/M
Musial et al (30)	2003	R	160 SLE, 22 Lupus-Like, 22 APS		IgG/IGM
Ishikura et al (31)	2004	R	78 DVT/cerebral thrombosis	22 SLE, 30 ITP, 40HC	IgG/IGM
Koskenmies et al (32)	2004	R	292 SLE		IgG/IGM
Zanon et al (33)	2004(M)	R	236 DVT		IgG/M
Forastiero et al (34)	2005(M)	P	194 aPL positive		IgG/IGM
Bertolaccini et al (5)	2005	R	212 SLE	100 HC	IgG/IGM
Bertolaccini et al (6)	2005	R	168 SLE		IgG/IGM
Tsutsumi et al (35)	2006	R	139 SLE		IgG/IGM
Gould et al (36)	2006	CS	100 SLE		IgG/IGM/IgA
Bizzarro et al (37)	2006	P(M)	101 SLE		IgG
Sailer et al (38)	2007	R(M)	79 LA positive		IgG/M
Bardin et al (39)	2007	R	152 thrombosis	120 HC	IgG
Szodoray et al (40)	2009	R	85 SLE		IgG/IgM
Rask et al (41)	2010	R	57 acute thrombosis	47 HC	IgG/IGM
Pengo et al (65)	2010	R	231 LA positive		IgG/IGM
Hoxha et al (42)	2012	R	158 PAPS	100 HC, 114 autoimmune conditions	IgG/IGM

SLE: systemic lupus erythematosus; APS: antiphospholipid syndrome; HC: healthy controls; aPL: antiphospholipid antibodies; DVT: deep vein thrombosis;

IMA: ischemic myocardial attack; PAPS: Primary antiphospholipid syndrome; LA: lupus anticoagulant; VTE: venous thrombo- embolism; HCV: hepatitis C

virus

R: retrospective study; CS: cross-sectional study; CC: case-control study; L: longitudinal study; M: multivariate analysis

Table 2: aPS/PT and thrombosis: main characteristics of 10 articles on 1775 patients and 628 controls

Article and Ref Number	Year	Study design	N patients and Enrolment criteria	N. Controls	Isotypes
Atsumi et al (20)	2000	R(M)	265 SLE		IgG/IGM
Nojima et al (62)	2004	R(M)	126 SLE	80 HC	IgG
Bertolaccini et al (5)	2005	R	212 SLE	100 HC	IgG/IGM
Bertolaccini et al (6)	2005	R	168 SLE		IgG/IGM
Tsutsumi et al (35)	2006	R	139 SLE	148 HC	IgG/IGM
Nojima et al (48)	2006	R(M)	175 SLE	80 HC	IgG
Bardin et al (39)	2007	R	152 pts with thrombosis	120 HC	IgG
Szodoray et al (40)	2009	R	85 SLE		IgG/IgM
Hoxha et al (42)	2012	R(M)	158 PAPS	100 HC	IgG/IGM
Vlagea et al (63)	2012	R(M)	295 suspected APS or Autoimmune disease		IgG/IGM

SLE: systemic lupus erythematosus, APS: antiphospholipid syndrome, HC: healthy controls, PAPS: Primary antiphospholipid syndrome; R: retrospective study; M: multivariate analysis