View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Ghent University Academic Bibliography

Journal of Thrombosis and Haemostasis, **16**: 2016–2023 DOI: 10.1111/jth.14261

ORIGINAL ARTICLE

Identification of high thrombotic risk triple-positive antiphospholipid syndrome patients is dependent on anti-cardiolipin and anti-β2glycoprotein I antibody detection assays

W. CHAYOUA,*† H. KELCHTERMANS,*† G. W. MOORE,‡ J. MUSIAŁ,§ D. WAHL,¶ B. DE LAAT*† and K. M. J. DEVREESE**

*Cardiovascular Research Institute Maastricht, Maastricht University Medical Centre; †Synapse Research Institute, Maastricht, the Netherlands; ‡Viapath Analytics, Department of Haemostasis and Thrombosis, Guy's and St Thomas' Hospitals, London, UK; §Department of Internal Medicine, Allergy and Immunology, Jagiellonian University Medical College, Kraków, Poland; ¶Inserm, DCAC, Vascular Medicine Division and Regional Competence Center for Rare Vascular and Systemic Autoimmune Diseases, Centre Hospitalier Regional Universitaire de Nancy, Université de Lorraine, Nancy, France; and **Coagulation Laboratory, Department of Diagnostic Sciences, Ghent University Hospital, Gent, Belgium*

To cite this article: Chayoua W, Kelchtermans H, Moore GW, Musiał J, Wahl D, de Laat B, Devreese KMJ. Identification of high thrombotic risk triple-positive antiphospholipid syndrome patients is dependent on anti-cardiolipin and anti-β2glycoprotein I antibody detection assays. *J Thromb Haemost* 2018; **16**: 2016–23.

Essentials

- Triple-positivity is associated with a high risk for a first thrombotic event and recurrence.
- Identification of triple-positives is dependent on the solid phase assay used.
- In triple-positivity, IgM only adds value in thrombotic risk stratification together with IgG.
- Thrombotic risk in triple-positive patients with IgM only, depends on the platform.

Abstract. *Background:* The antiphospholipid syndrome (APS) is characterized by thrombosis and/or pregnancy morbidity with the persistent presence of antiphospholipid antibodies (aPL). Triple-positivity (i.e. positivity for lupus anticoagulant [LAC], anti-cardiolipin [aCL] and anti-β2glycoprotein I [aβ2GPI] antibodies) is associated with a high thrombotic risk. *Objectives:* We investigated the variability in triple-positivity detection by measuring the same samples with four commercially available solid phase assays. In addition, the added clinical value of aPL in LAC-positive patients was investigated, as well as the association of IgM triple-positivity and thrombosis. *Patients/Methods:* We included 851 patients from seven European medical centers. Anti-CL and aβ2GPI IgG/IgM antibodies were determined by four platforms: BioPlex®2200, ImmunoCap®EliA, ACL AcuStar® and QUANTA Lite ELISA®. *Results:* Triple-positivity detection by solid phase assays varied, ranging from 89 up to 118 in thrombotic APS patients (n = 258), of which 86 were detected independent of the platform. Lupus anticoagulant positivity resulted in an odds ratio (OR) for thrombosis of 3.4; triple-positivity (irrespective of the isotype) increased the OR from 4.3 up to 5.2, dependent on the platform. Triple-positivity solely for the IgM isotype did not increase the OR for thrombosis compared with LAC positivity. The highest OR for thrombosis was reached for positivity for IgG and IgM aβ2GPI and aCL (8.6 up to 28.9). *Conclusions:* Triple-positivity proved to be highly associated with thrombosis, but identification is assay dependent. Within triple-positivity, IgM antibodies only have an added clinical value in patients positive for IgG antibodies.

Keywords: antiphospholipid antibodies; immunoassays; immunoglobulin isotypes; risk assessment; thrombosis.

Correspondence: Katrien Devreese, Coagulation Laboratory, Laboratory for Clinical Biology, Ghent University Hospital, Corneel Heymanslaan, 10, B-9000 Gent, Belgium
Tel.: +32 9332 6567
E-mail: katrien.devreese@uzgent.be
Received: 25 July 2018,
Manuscript handled by: P. Toulon
Final decision: F. R. Rosendaal, 19 July 2018

Introduction

The antiphospholipid syndrome (APS) is characterized by thrombosis and/or pregnancy morbidity with the persistent presence of antiphospholipid antibodies (aPL) [1]. Laboratory criteria include aPL detection by phospholipid-dependent coagulation tests referred to as lupus

© 2018 International Society on Thrombosis and Haemostasis

anticoagulant (LAC) or by quantitative solid phase assays measuring anti-$\beta_2$glycoprotein I (aβ2GPI) and anti-cardiolipin (aCL) IgG/IgM antibodies [1]. Given the high frequency of thrombosis irrespective of the syndrome, laboratory tests are of utmost importance for the classification of APS. Detection of aPL by solid phase assays is associated with high inter-laboratory and inter-method variation [2,3]. Reports from external quality control programs illustrate that commercially available aPL assays produce variable results [4–6].

In order to improve the identification of patients at risk, it was suggested that APS patients should be evaluated according to their aPL profile [1,7]. Combined positivity for LAC, aCL and aβ2GPI antibodies (i.e. triple-positivity) has been shown to be associated with a high risk of both a first thrombotic event and recurrence [8–10]. In the NOH-APS study, a large observational study, triple-positivity was a predictor for thrombosis in purely obstetric APS patients [11]. However, computed risks for thrombosis of LAC positivity and triple-positivity were globally concordant, with the exception of pulmonary embolism [11]. Despite the high correlation of triple-positives with thrombosis, the predictive value is argued to originate from LAC positivity [12]. Recently, the detection of triple-positivity (i.e. positivity for LAC, aCL and aβ2GPI antibodies) was suggested to be method and platform independent [13].

Looking at the isotype of aPL, both IgG and IgM antibodies directed against cardiolipin and β2GPI are included in the Sydney criteria [1]. However, the clinical value of IgM antibodies in thrombotic APS is debated [14]. To date, the thrombotic association of IgM antibodies in aPL profiles such as triple-positivity is not known. In this study we included 851 patients from seven European medical centers. Four solid-phase assay platforms were selected based on frequency of use and the willingness of manufacturers to provide their assays. The samples were tested with all assays at one location by a single technician.

In a retrospective multicenter study we aimed to investigate the variability in triple-positivity detection between different aPL detection platforms and the impact of the platform on the association of triple-positivity with thrombosis. In addition, we aimed to assess the added value of aPL detection in LAC-positive patients and the impact of the isotype with respect to the association with thrombosis.

Materials and methods

Study population

We included 851 patients from seven European medical centers. Classification of APS was based on the Sydney criteria [1]. Patients were classified by the local centers, resulting in 258 thrombotic APS patients (APS thrombosis), 204 patients with a history of thrombosis and negative for laboratory criteria of APS (non-APS thrombosis), 196 patients with an autoimmune disease other than APS, such as systemic lupus erythematosus (52%) and systemic sclerosis (27%), without thrombotic complications (AID controls), and 193 controls that were referred for aPL testing for other reasons than the clinical criteria of APS, including subfertility and prolonged activated partial thromboplastin time (controls). Patients were enrolled within a time-span of 1 year, with patient samples stored for less than 5 years. Thrombosis was objectively confirmed according to the Sydney criteria [1]. The majority of thrombotic APS patients received anticoagulant therapy, including vitamin K antagonists (VKAs) (46%), low-molecular-weight heparins (LMWHs) (5%) and direct oral anticoagulants (DOACs) (4%). Twenty-seven patients (10%) received antiplatelet therapy and only 2% of patients with thrombotic APS received both anticoagulant and antiplatelet therapy. Details on anticoagulant and antiplatelet therapy of the remaining 84 patients are not available. Women classified with obstetrical APS were excluded. The study was approved by the local ethical committees. Lupus anticoagulant positivity was determined by the local center, according to the ISTH-SSC (International Society of Thrombosis and Haemostasis-Scientific Standardisation Subcommittee) guideline [15]

Solid phase assays

Commercially available solid phase assays (Table S1) were selected based on frequently used assays in the external quality control program of the ECAT (External quality Control of diagnostic Assays and Tests, Leiden, the Netherlands) and the willingness of manufacturers to collaborate. Anti-CL IgG, aCL IgM, aβ2GPI IgG and aβ2GPI IgM antiphospholipid antibodies were detected by BioPlex®2200 (Bio-Rad, Bio-Rad Laboratories, Hercules, USA), ImmunoCap®EliA (Thermo Fisher Scientific/Phadia, Uppsala, Sweden), ACL AcuStar® (Werfen/Instrumentation Laboratories, Bedford, MA, USA) and QUANTA Lite ELISA® (Inova Diagnostics, San Diego, CA, USA) in the Ghent University Hospital (Ghent, Belgium). Reagents used for the detection of aCL and aβ2GPI IgG/IgM antibodies were measured with their corresponding instruments according to the manufacturer's instructions. All four methods were performed in parallel runs of 40 samples. Antiphospholipid antibody titers were expressed in arbitrary units (GPL, MPL, U mL^{-1}, SGU or SMU). All samples were measured by the same technician and values below the calculated limit of detection (LOD) were replaced by the LOD. Manufacturer's recommended cut-off values were used upon confirmation in 20 healthy volunteers, in accordance with the ISTH-SSC guideline [16].

© 2018 International Society on Thrombosis and Haemostasis

Statistics

Significance of differences between aPL titers was determined with the Mann–Whitney *U*-test. Diagnostic efficacy was assessed within the total population by sensitivity, specificity and odds ratios (ORs) using the Statistical Package for Social Sciences (SPSS 23.0; SPSS, Chicago, IL, USA) and MedCalc Statistical Software version 17.7.2 (MedCalc Software bvba, Ostend, Belgium). Statistical significance was set at P value less than 0.05.

Results

We measured aCL IgG/IgM and aβ2GPI IgG/IgM aPL in 833 individuals with a mean age of 46 years ranging from 16 to 87 years old (Table 1) with four commercially available assays (Table S1). In our study population, venous thrombosis (VT) was more common than arterial thrombosis (AT), both in the APS and control group. In addition, primary APS (PAPS) was more prevalent than APS secondary to an underlying connective tissue disorder (SAPS).

From the 851 samples tested, 274 were LAC positive, and for these triple-positivity ranged from 106 (39%) up to 146 (53%) detected by QUANTA Lite ELISA® and BioPlex®2200, respectively (Table 2). In patients diagnosed with thrombotic APS (n = 258), 202 were positive for LAC. From these 202 LAC-positive samples 118, 101, 111 and 89 were defined as triple-positive (positivity for LAC, aCL IgG or IgM and aβ2GPI IgG or IgM), detected with the solid phase assays: BioPlex® 2200, ImmunoCap® EliA, ACL AcuStar® and QUANTA Lite ELISA®, respectively (Table 2). By a comparison of two proportions, triple-positivity detection was found to be statistically different between BioPlex® 2200 and QUANTA Lite ELISA® (P = 0.0122). Other combinations proved not to be statistically different, although the comparison of ACL AcuStar® and QUANTA Lite ELISA® almost reached statistical significance (P = 0.0586). Of the LAC-positive patients not defined as triple-positive, the majority was isolated LAC positive (Table S2).

Agreement of triple-positivity detection by solid phase assays was assessed by a 2 × 2 contingency table within the total population (Table 3). Discrepancies varied between 14 (BioPlex®2200 vs. ACL AcuStar®) and 48 (BioPlex® 2200 vs. QUANTA Lite ELISA®) individuals. In patients diagnosed with thrombotic APS (n = 258) 118 triple-positives were detected by BioPlex® 2200, of which 86 patients were defined as triple-positive, independent of the solid phase assay used (Table 4). On the other hand, 32 patients were defined as triple-positive by BioPlex® 2200 but negative by all the other tested solid phase assays (Table 4). However, the majority of patients with

Table 1 Demographic and clinical characteristics of the study population

				Antiphospholipid syndrome			Thrombosis				
	Patients (n)	Female	Age, year, mean (range)	Primary APS	Secondary APS	Not specified	Venous	Arterial	Venous and arterial	Small vessel	Not specified
APS thrombosis	259	164 (63%)	50 (17–87)	150	45	64	160	55	26	4	14
Non-APS thrombosis	204	116 (57%)	46 (19–85)	NA	NA	NA	149	47	5	0	3
Autoimmune diseases	196	158 (81%)	46 (16–83)	NA	NA	NA	NA	NA	NA	NA	NA
Controls	194	170 (88%)	39 (18–82)	NA	NA	NA	NA	NA	NA	NA	NA
Total population	853	608 (71%)	46 (16–87)	150	45	64	309	102	31	4	17

APS, antiphospholipid syndrome; NA, not applicable.

Table 2 Triple-positive patients detected with BioPlex® 2200, ImmunoCap® EliA, ACL AcuStar® and QUANTA Lite ELISA® in patients with thrombotic antiphospholipid syndrome (APS), patients with non-APS thrombosis, autoimmune disease (AID) controls and controls

	Lupus anticoagulant positive	Triple-positives			
		BioPlex® 2200	Immuno Cap® EliA	ACL AcuStar®	QUANTA Lite ELISA®
APS thrombosis (n = 258)	202	118	101	111	89
Non-APS thrombosis (n = 204)	0	0	0	0	0
AID controls (n = 196)	56	22	18	19	14
Controls (n = 193)	16	6	6	4	3
Total patient population (n = 851)	274	146	125	134	106

© 2018 International Society on Thrombosis and Haemostasis

Table 3 Discrepancies in triple-positivity detection with pairwise comparison. Number of LAC-positive samples and those positive for aCL and aβ2GPI antibodies (irrespective of the isotype) detected by BioPlex®2200, ImmunoCap®EliA, ACL AcuStar® and QUANTA Lite ELISA®

	BioPlex® 2200		ImmunoCap® EliA		ACL AcuStar®	
	−	+	−	+	−	+
BioPlex® 2200						
−						
+						
ImmunoCap® EliA						
−	696	30				
+	9	116				
ACL AcuStar®						
−	704	13	706	11		
+	1	133	20	114		
Quanta Lite ELISA®						
−	701	44	722	23	713	32
+	4	102	4	102	4	102

discrepant results by the tested solid phase platforms have a history of thrombosis, illustrating clinical relevance (Table 4). Median aPL titers were calculated by aCL and aβ2GPI antibody titers above the cut-off. Triple-positives in agreement across all platforms displayed higher median aPL titers than triple-positive samples not positive for all tested platforms (Fig. 1). Statistical difference was reached for BioPlex®2200 ($P < 0.001$), ACL AcuStar® ($P < 0.001$) and QUANTA Lite ELISA® ($P = 0.0029$), but not for ImmunoCap®EliA ($P = 0.5851$), as shown in Fig. 1.

The sensitivity of triple-positivity for thrombosis was low compared with LAC alone and varied from 19% (95% confidence interval [95% CI], 16–23%) up to 26% (95% CI, 22–30%) between the tested assays (Fig. 2A). However, higher specificity for thrombosis was obtained, ranging from 93% (95% CI, 90–95%) up to 96% (95% CI, 93–97%), as shown in Fig. 2(B). Lupus anticoagulant positivity resulted in an OR of 3.63 (95% CI, 2.76–4.76). Triple-positivity was statistically correlated with thrombosis, independent of the solid phase assay used to detect aCL and aβ2GPI IgG/IgM antibodies (Fig. 2B). Odds ratios for thrombosis ranged from 4.3 (95% CI, 2.7–6.8) up to 5.2 (95% CI, 3.0–8.9) among the platforms (Table 5).

Triple-positivity for aCL IgG and aβ2GPI IgG or aCL IgM and aβ2GPI IgG IgM was significantly correlated with thrombosis, independent of the solid phase assay used. However, positivity for the IgM isotype (aCL IgM and aβ2GPI IgM) was more correlated with thrombosis than triple-positivity for the IgG isotype (aCL IgG and aβ2GPI IgG) upon aCL and aβ2GPI detection by BioPlex® 2200, ImmunoCap® EliA and ACL AcuStar®. On the other hand, triple-positivity for the IgG isotype was more strongly correlated with thrombosis than that for the IgM isotype upon detection of aPL by QUANTA Lite ELISA®. However, the majority of triple-positives for the IgM isotype were also positive for the IgG isotype (data not shown). Isolated triple-positivity for the IgG isotype increased the OR for thrombosis compared with LAC positivity only in two out of the four tested aPL solid phase assays (ACL AcuStar® and QUANTA Lite ELISA®), as shown in Table 5. In triple-positivity, isolated IgM positivity did not increase OR compared with LAC. Moreover, isolated triple-positivity for the IgM isotype did not reach statistical difference when aPL were detected with BioPlex® 2200 and ACL AcuStar® (1.9 [95% CI, 0.6–5.4] and 2.0 [95% CI, 0.7–5.9], respectively). Positivity for all tested aPL (LAC, aCL IgG, aCL IgM, aβ2GPI IgG and aβ2GPI IgM) resulted in the highest OR for thrombosis, ranging from 8.6 (95% CI, 3.1–24.4) up to 28.9 (3.9–212.4), detected by BioPlex® 2200 and ACL AcuStar®, respectively.

Discussion

Detection of aPL antibodies is accompanied by large inter-method and inter-laboratory variation [2,4,5,17,18]. Traditionally, aCL and aβ2GPI antibodies are detected by ELISA. Nowadays, more advanced (automated) systems are available, which are suggested to reduce inter-laboratory variation [4,17,19–21]. We excluded inter-laboratory variation by detection of aCL and aβ2GPI antibodies in the same samples at one laboratory (Ghent, Belgium), carried out by a single technician. Despite many efforts, standardization of antiphospholipid antibody (aPL) assays is far from reached as poor consensus

Table 4 Discrepancies in triple-positivity detection between BioPlex®2200, ImmunoCap®EliA, ACL AcuStar® and QUANTA Lite ELISA®. Number of triple-positives in (dis)agreement among all four solid phase assays in patients with thrombotic APS, patients with non-APS thrombosis, autoimmune disease (AID) controls and controls

	BioPlex®2200	ImmunoCap®EliA	ACL AcuStar®	QUANTA Lite ELISA®	In agreement among all solid phase assays
APS thrombosis	32	15	25	3	86
AID	10	6	7	2	12
Controls	3	3	1	0	3
Total	45	24	33	5	101

© 2018 International Society on Thrombosis and Haemostasis

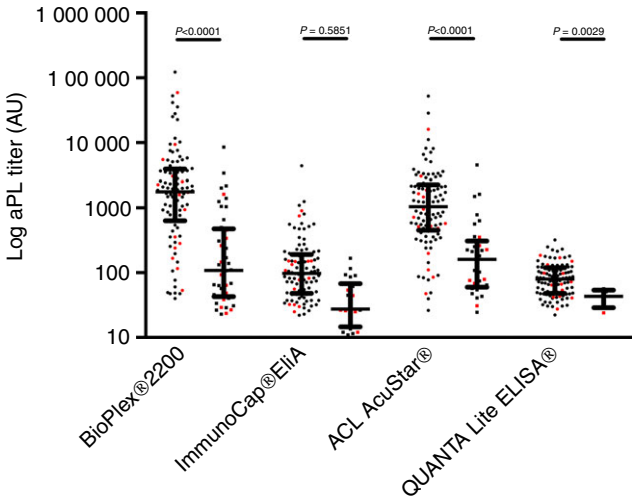

Fig. 1. Antiphospholipid antibody (aPL) titers of triple-positives in (dis)agreement. Log transformed aPL titers in agreement for all platforms tested are indicated by solid dots; disagreements are indicated by solid squares. Patients without the clinical criteria for the antiphospholipid syndrome (APS) are indicated in red and patients with a history of thrombosis are indicated in black. Titers are expressed as the median value of positive aPL titers with interquartile ranges. AU, arbitrary units.

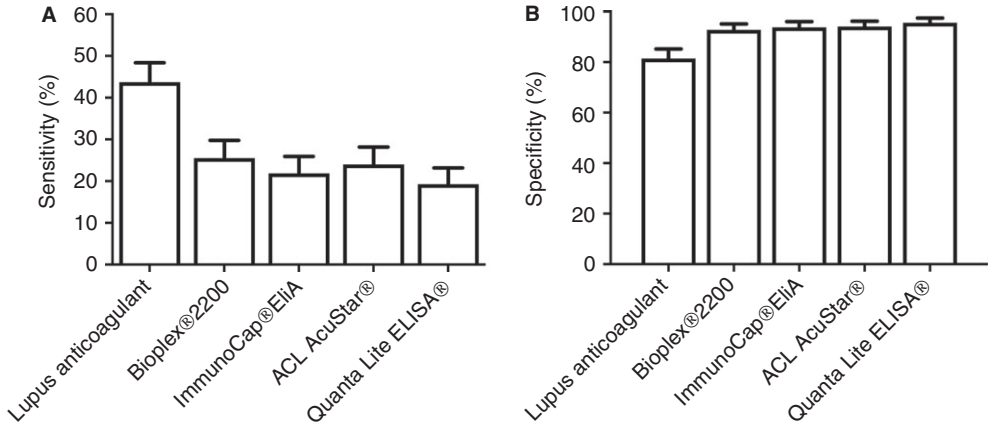

Fig. 2. Diagnostic efficacy of lupus anticoagulant (LAC) and triple-positive patients detected by BioPlex®2200, ImmunoCap®EliA, ACL AcuStar® and QUANTA Lite ELISA®. (A) Sensitivity and (B) specificity for thrombosis (mean ± 95% confidence interval [95% CI]).

Table 5 Correlation of aPL profiles with thrombosis. Odds ratios (ORs) with 95% confidence intervals (CIs) are shown (significant ORs in bold)

Positive	Negative	BioPlex® 2200 (95% CI)	ImmunoCap® EliA (95% CI)	ACL AcuStar® (95% CI)	QUANTA Lite ELISA® (95% CI)
LAC	–	**3.4 (2.5–4.7)**	**3.4 (2.5–4.7)**	**3.4 (2.5–4.7)**	**3.4 (2.5–4.7)**
LAC + aCL + aβ2GPI (irrespective of isotype)	–	**4.4 (2.9–6.9)**	**4.3 (2.7–6.8)**	**5.0 (3.1–8.1)**	**5.2 (3.0–8.9)**
LAC + aCL IgG + aβ2GPI IgG	–	**4.8 (3.0–7.7)**	**4.8 (2.8–8.4)**	**6.0 (3.5–10.2)**	**6.2 (3.1–12.3)**
LAC + aCL IgM + aβ2GPI IgM	–	**5.0 (2.4–10.3)**	**5.0 (2.4–10.3)**	**6.7 (2.8–15.9)**	**4.6 (2.2–9.4)**
LAC + aCL IgG + aβ2GPI IgG	aCL IgM + aβ2GPI IgM	**3.4 (2.0–5.8)**	**3.4 (1.9–6.2)**	**4.0 (2.3–6.9)**	**4.8 (2.2–10.3)**
LAC + aCL IgM + aβ2GPI IgM	aCL IgG + aβ2GPI IgG	1.9 (0.6–5.4)	**2.7 (1.2–6.5)**	2.0 (0.7–5.9)	**2.7 (1.2–6.5)**
LAC + aCL IgG + aβ2GPI IgG + aCL IgM + aβ2GPI IgM	–	**8.6 (3.1–24.4)**	**12.0 (2.8–50.8)**	**28.9 (3.9–212.4)**	**10.1 (2.4–43.3)**

© 2018 International Society on Thrombosis and Haemostasis

is obtained between assays when measuring the same sample [2,20]. The identification of triple-positive patients was recently suggested to be less affected by inter-method variation, thereby better classifying APS patients at risk [13]. In a retrospective cross-sectional study, the authors suggested that identification of patients with triple aPL positivity is platform and method independent, having compared two methods with a different solid phase from the same manufacturer [13]. However, they found a disparity of 6 or 9 triple-positives out of 220 patients (121 with APS and 99 with systemic lupus erythematosus), depending on the cut-off value used, already suggesting the presence of patients with low levels of aβ2GPI and aCL aPL titers and difficulties in reaching consensus in the classification of these patients [13,22]. In our cohort, the highest discrepancy in number of triple-positive samples was found between BioPlex® 2200 and QUANTA Lite ELISA®, with a discrepancy of 29 triple-positives out of 202 LAC-positive samples (14%). Similar to single positivity, identification of triple-positives was found to be assay dependent. In addition, we did not assess the variation of triple-positivity detection introduced by LAC assays. Indeed, an external quality control program concluded that inter-method and inter-laboratory variation is higher in solid phase assays than in LAC detection by dilute Russell's Viper Venom Time (dRVVT) assay [3]. However, difficulties still persist in reaching consensus among weak-positive samples [2–4,23]. The presented variation in triple-positivity detection may therefore be underestimated. A possible limitation of our study is that thrombotic patients under treatment during the time of blood collection could result in an increased risk of false-positive LAC tests.

Samples positive for LAC, aCL and aβ2GPI antibodies by one solid phase platform but not by all four tested platforms have lower median aPL titers, suggesting difficulties in consensus on positivity among low aPL titers (Fig. 1) [24]. We accept that the cut-off calculated by the 99th percentile of a normal population is the best consensus between sensitivity and specificity, and the clinical relevance of aCL and/or aβ2GPI results that are below the 99th percentile needs to be further studied [25]. Few studies showed that low titers of aCL also were predictive for thrombotic recurrence [26]. In this study, we transferred the manufacturer's cut-off after confirmation, as recommend by the ISTH-SSC guidelines [16]. In terms of clinical practice, a recent questionnaire from the SSC showed that the majority of laboratories use the same approach, because only a minority of laboratories have the resources to calculate a cut-off value from at least 120 normals. With this cut-off choice applied for all platforms, the majority of discrepant samples in our study were from patients that experienced a thrombotic event (Table 4).

A minority of non-thrombotic patients (n = 582, autoimmune disease and controls) in our study population showed triple-positivity (n = 17–28/583 or 2.9–4.8%, depending on the platform). These should be regarded as asymptomatic carriers, which is in line with the findings of Mustonen *et al*., who found that 5% of asymptomatic triple-positives were carriers [27].

The association of thrombosis and single aPL positivity is debated because results are contrary. Recent studies showed that the risk of thrombotic events increases with the number of positive tests in APS patients and the creation of antibody profiles and test combinations increases the association with thrombosis [7,8,28–30]. On the other hand, another study showed a strong association between single aPL positivity and thrombosis in pediatric APS patients [31]. Although single positivity is not always significantly correlated with thrombosis, within the current guidelines all aPL have the same diagnostic value [1]. In our cohort, we confirmed the strong correlation between triple-positivity and thrombosis, as triple-positivity was significantly correlated with thrombosis independent of the platform used. A large observational study investigated the incidence of thrombosis in obstetric APS patients. Frequencies of thrombotic events were assessed in 517 APS patients, 279 women carrying a genetic thrombophilia polymorphism and 796 women with negative thrombophilia polymorphism results [11]. Computed risks for thrombosis of LAC positivity and triple-positivity were globally concordant [11]. However, triple-positivity was a predictor for pulmonary embolism, whereas LAC positivity alone was not [11]. In an Italian cohort, 618 patients were referred to aPL testing, of which 55% met the clinical criteria consistent with the Sapporo criteria [7]. A statistically significant correlation between LAC and thrombosis was found (OR, 4.4; 95% CI, 1.5–13.3) [7]. In triple-positive patients the association with thrombosis increased even further (OR, 33.3; 95% CI, 7.0–157.6), suggesting an additional value of triple-positivity detection in thrombotic risk stratification [7]. Patient population and aPL detection method may impact the correlation of thrombosis with triple-positivity and the role of aCL and aβ2GPI antibodies in addition to LAC. It has already been shown that the presence of aCL and aβ2GPI of the same isotype reinforces the clinical probability of APS [32]. We confirmed that ORs for all platforms are higher for triple-positivity with concordance of isotype compared with triple-positivity including combinations of aCL and aβ2GPI irrespective of isotype, except for one platform (QUANTA Lite ELISA®) with lower OR for IgM triple-positivity compared with the OR for triple-positivity irrespective of the isotype.

Our results clearly illustrate the wide variation in thrombotic association introduced by aPL detection methods. In LAC positives, 'isolated' IgM or 'isolated' IgG aPL was less correlated with thrombosis than triple-positivity irrespective of the isotype. Interestingly, positivity for LAC, aCL and aβ2GPI antibodies for both isotypes resulted in the strongest correlation with thrombosis. Therefore, both IgG and IgM antibodies are of

© 2018 International Society on Thrombosis and Haemostasis

added value in stratification of risk of thrombosis in APS. However, IgM did not add any value in thrombotic association to LAC positives in the absence of IgG aPL.

In conclusion, detection of triple-positivity varied among commercially available solid phase assays detecting aCL and aβ2GPI antibodies. However, triple-positivity (irrespective of the isotype) was statistically correlated with thrombosis, independent of the solid phase assay used. Except for one platform, concordance of isotype resulted in the highest OR. Detection of IgM antibodies in triple-positivity was only of added clinical value in combination with LAC, aCL IgG and aβ2GPI IgG positivity. These data confirm the high association of triple-positivity with thrombosis and show that the isotype and solid phase assay used to detect aPL affect the association with thrombosis. As triple-positive APS patients have an increased risk of thrombotic recurrence, standardization in triple-positivity detection is urgently warranted.

Addendum

K. M. J. Devreese, B. de Laat and H. Kelchtermans designed the study. K. M. J. Devreese organized the sample collection at the different centers. K. M. J. Devreese, D. Wahl, G. W. Moore and J. Musiał collected samples and identified sample characteristics. Samples were analyzed under the supervision of K. M. J. Devreese. W. Chayoua, K. M. J. Devreese, B. de Laat and H. Kelchtermans interpreted data, performed statistical analyses and wrote the manuscript. D. Wahl, G. W. Moore and J. Musiał critically reviewed the manuscript.

Acknowledgements

The authors thank M. Luypaert for his technical assistance, Bio-Rad Laboratories, ThermoFisher/Phadia, Werfen/Instrumentation Laboratory and Inova Diagnostics for providing the test kits for the detection of aβ2GPI and aCL IgG and IgM antibodies and S. Zuily, A. Tripodi, P. Fontana and J. Remijn for providing patient samples.

Disclosure of Conflict of Interests

G. W. Moore reports personal fees from DSM Pentapharm, outside the submitted work, and has been a member of the Coagulation Advisory Board for Roche Diagnostics. The other authors state that they have no conflict of interest.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Table S1. Technical specifications of the solid phase assays. NS, Not specified.

Table S2. Distribution of aCL and aβ2GPI antibodies in LAC positive patients with thrombotic APS.

References

1 Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RHWM, de Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; **4**: 295–306.

2 Devreese KM. Antiphospholipid antibody testing and standardization. *Int J Lab Hematol* 2014; **36**: 352–63.

3 Favaloro EJ. Variability and diagnostic utility of antiphospholipid antibodies including lupus anticoagulants. *Int J Lab Hematol* 2013; **35**: 269–74.

4 Favaloro EJ, Franchini M, Lippi G. Biological therapies for von Willebrand disease. *Expert Opin Biol Ther* 2012; **12**: 551–64.

5 Favaloro EJ, McVicker W, Zhang Y, Hamdam S, Huynh M, Peris P, O'Neal M, Hocker N. Improving the inter-laboratory harmonization of the international normalized ratio (INR): utilizing the concept of transference to estimate and/or validate international sensitivity index (ISI) and mean normal prothrombin time (MNPT) values and/or to eliminate measurement bias. *Clin Lab Sci* 2012; **25**: 13–25.

6 Wong R, Wilson R, Pollock W, Steele R, Gillis D. Anti-cardiolipin antibody testing and reporting practices among laboratories participating in a large external Quality Assurance Program. *Pathology* 2004; **36**: 174–81.

7 Pengo V, Biasiolo A, Pegoraro C, Cucchini U, Noventa F, Iliceto S. Antibody profiles for the diagnosis of antiphospholipid syndrome. *Thromb Haemost* 2005; **93**: 1147–52.

8 Pengo V, Ruffatti A, Legnani C, Gresele P, Barcellona D, Erba N, Testa S, Marongiu F, Bison E, Denas G, Banzato A, Padayattil Jose S, Iliceto S. Clinical course of high-risk patients diagnosed with antiphospholipid syndrome. *J Thromb Haemost* 2010; **8**: 237–42.

9 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. *J Thromb Haemost* 2012; **10**: 2512–8.

10 Sciascia S, Cosseddu D, Montaruli B, Kuzenko A, Bertero MT. Risk Scale for the diagnosis of antiphospholipid syndrome. *Ann Rheum Dis* 2011; **70**: 1517–8.

11 Gris JC, Bouvier S, Molinari N, Galanaud JP, Cochery-Nouvellon E, Mercier E, Fabbro-Peray P, Balducchi JP, Mares P, Quere I, Dauzat M. Comparative incidence of a first thrombotic event in purely obstetric antiphospholipid syndrome with pregnancy loss: the NOH-APS observational study. *Blood* 2012; **119**: 2624–32.

12 Krilis SA, Giannakopoulos B. Laboratory methods to detect antiphospholipid antibodies. *Hematology Am Soc Hematol Educ Program* 2014; **2014**: 321–8.

13 Iwaniec T, Kaczor MP, Celinska-Lowenhoff M, Polanski S, Musial J. Identification of patients with triple antiphospholipid antibody positivity is platform and method independent. *Pol Arch Med Wewn* 2016; **126**: 19–24.

14 Kelchtermans H, Pelkmans L, de Laat B, Devreese KM. IgG/IgM antiphospholipid antibodies present in the classification criteria for the antiphospholipid syndrome: a critical review of their association with thrombosis. *J Thromb Haemost* 2016; **14**: 1530–48.

© 2018 International Society on Thrombosis and Haemostasis

15 Pengo V, Tripodi A, Reber G, Rand JH, Ortel TL, Galli M, De Groot PG. Update of the guidelines for lupus anticoagulant detection. *J Thromb Haemost* 2009; **7**: 1737–40.
16 Devreese KM, Pierangeli SS, de Laat B, Tripodi A, Atsumi T, Ortel TL. Testing for antiphospholipid antibodies with solid phase assays: guidance from the SSC of the ISTH. *J Thromb Haemost* 2014; **12**: 792–5.
17 Devreese K, Hoylaerts MF. Laboratory diagnosis of the antiphospholipid syndrome: a plethora of obstacles to overcome. *Eur J Haematol* 2009; **83**: 1–16.
18 Urbanus RT, de Groot PG. Antiphospholipid antibodies–we are not quite there yet. *Blood Rev* 2011; **25**: 97–106.
19 Tincani A, Allegri F, Sanmarco M, Cinquini M, Taglietti M, Balestrieri G, Koike T, Ichikawa K, Meroni P, Boffa MC. Anticardiolipin antibody assay: a methodological analysis for a better consensus in routine determinations–a cooperative project of the European Antiphospholipid Forum. *Thromb Haemost* 2001; **86**: 575–83.
20 Favaloro EJ, Wong RC, Jovanovich S, Roberts-Thomson P. A review of beta2 -glycoprotein-l antibody testing results from a peer-driven multilaboratory quality assurance program. *Am J Clin Pathol* 2007; **127**: 441–8.
21 Devreese KM, Poncet A, Lindhoff-Last E, Musial J, de Moerloose P, Fontana P. A multicenter study to assess the reproducibility of antiphospholipid antibody results produced by an automated system. *J Thromb Haemost* 2017; **15**: 91–5.
22 Iwaniec T, Kaczor MP, Celinska-Lowenhoff M, Polanski S, Musial J. Clinical utility of automated chemiluminescent antiphospholipid antibody assay. *Thromb Res* 2015; **136**: 1033–9.
23 Reber G, Meijer P. In ECAT veritas? *Lupus* 2012; **21**: 722–4.
24 Ruffatti A, Olivieri S, Tonello M, Bortolati M, Bison E, Salvan E, Facchinetti M, Pengo V. Influence of different IgG anticardiolipin antibody cut-off values on antiphospholipid syndrome classification. *J Thromb Haemost* 2008; **6**: 1693–6.
25 Devreese KMJ, Ortel TL, Pengo V, de Laat B; Subcommittee on Lupus Anticoagulant/Antiphospholipid A. Laboratory criteria for antiphospholipid syndrome: communication from the SSC of the ISTH. *J Thromb Haemost* 2018; **16**: 809–13.
26 Schulman S, Svenungsson E, Granqvist S. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. *Am J Med* 1998; **104**: 332–8.
27 Mustonen P, Lehtonen KV, Javela K, Puurunen M. Persistent antiphospholipid antibody (aPL) in asymptomatic carriers as a risk factor for future thrombotic events: a nationwide prospective study. *Lupus* 2014; **23**: 1468–76.
28 Bergrem A, Jacobsen EM, Skjeldestad FE, Jacobsen AF, Skogstad M, Sandset PM. The association of antiphospholipid antibodies with pregnancy-related first time venous thrombosis–a population-based case-control study. *Thromb Res* 2010; **125**: e222–7.
29 Devreese KM. Antiphospholipid antibodies: evaluation of the thrombotic risk. *Thromb Res* 2012; **130**(Suppl. 1): S37–40.
30 Devreese K, Peerlinck K, Hoylaerts MF. Diagnostic test combinations associated with thrombosis in lupus anticoagulant positive patients. *Thromb Haemost* 2011; **105**: 736–8.
31 Ma J, Song H, Wei M, He Y. Clinical characteristics and thrombosis outcomes of paediatric antiphospholipid syndrome: analysis of 58 patients. *Clin Rheumatol* 2017; **37**: 1295–303.
32 Pengo V, Banzato A, Bison E, Bracco A, Denas G, Ruffatti A. What have we learned about antiphospholipid syndrome from patients and antiphospholipid carrier cohorts? *Semin Thromb Hemost* 2012; **38**: 322–7.

© 2018 International Society on Thrombosis and Haemostasis