Lauro Meneghel*, Amelia Ruffatti, Sabrina Gavasso, Marta Tonello, Elena Mattia, Luca Spiezia, Elena Campello, Ariela Hoxha, Marny Fedrigo, Leonardo Punzi and Paolo Simioni

The clinical performance of a chemiluminescent immunoassay in detecting anti-cardiolipin and anti-β_2 glycoprotein I antibodies. A comparison with a homemade ELISA method

DOI 10.1515/cclm-2014-0925
Received September 19, 2014; accepted December 15, 2014; previously published online January 15, 2015

Abstract

Background: Fully automated chemiluminescence immunoassays (CLIAs) are emerging technologies for the detection of anti-cardiolipin (aCL) and anti-β_2 glycoprotein I (anti-β2GPI) antibodies for anti-phospholipid syndrome (APS) classification, which is commonly based on an enzyme-linked immunosorbent assay (ELISA) test result. CLIA and a homemade ELISA were used in this study to detect these antibodies, and their performances were compared.

Methods: Sera were collected from 104 patients with primary APS, 88 seronegative subjects who met the clinical but not the laboratory criteria for APS, and 150 control subjects. IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies were determined in the sera using a CLIA (HemosIL AcuStar®) and a homemade ELISA.

Results: CLIA had a significantly lower comparative sensitivity for IgM aCL and IgG/IgM IgG anti-β2GPI antibodies; its comparative specificity was higher with respect to ELISA for IgM aCL and IgM anti-β2GPI antibodies. The two techniques showed a high, significant agreement ($p<0.001$) and a significant titer correlation ($p<0.001$). CLIA also detected IgG/IgM aCL and IgG anti-β2GPI antibodies in the seronegative patients. There was a significantly higher prevalence of IgG aCL and IgG anti-β2GPI antibodies ($p<0.001$ and $p=0.01$, respectively) in those patients with respect to that in the control population.

Conclusions: Despite a lower comparative sensitivity, CLIA showed a higher comparative specificity for some aPL and a good level of agreement and correlation with a homemade ELISA. CLIA also detected some aCL and anti-β2GPI antibodies in the seronegative patients not usually identified by homemade ELISA.

Keywords: anti-β_2 glycoprotein I (anti-β2GPI) antibodies; anti-cardiolipin (aCL) antibodies; anti-phospholipid antibodies (aPL); anti-phospholipid syndrome (APS); chemiluminescence immunoassay (CLIA); enzyme-linked immunosorbent assay (ELISA).

Introduction

Anti-phospholipid syndrome (APS) classification is based on specific clinical manifestations that include vascular thrombosis and/or pregnancy morbidity associated with the presence in the blood of anti-phospholipid (aPL) antibodies [1]. APL antibodies are a heterogeneous group of autoantibodies directed against plasma protein-phospholipid complexes or single plasma proteins. The aPL antibodies currently considered laboratory criteria for APS classification are lupus anticoagulant (LA), medium-high titers of IgG and/or IgM anti-cardiolipin (aCL) antibodies, and medium-high titers of IgG and/or IgM anti-β_2 glycoprotein I (anti-β2GPI) antibodies. Their presence must be confirmed at least 12 weeks after original detection.

Primary APS (PAPS) is defined as the absence of any other underlying systemic autoimmune disorder; secondary APS is instead associated with another systemic autoimmune disease and in particular systemic lupus

***Corresponding author: Lauro Meneghel,** Rheumatology Unit, Department of Medicine, University of Padua, Via Giustiniani 2, 35128 Padova, Italy, Phone: +39 49 8218397, Fax: +39 49 8212191, E-mail: lauro.meneghel@studenti.unipd.it
Amelia Ruffatti, Marta Tonello, Elena Mattia, Ariela Hoxha and Leonardo Punzi: Rheumatology Unit, Department of Medicine, University of Padova, Padova, Italy
Sabrina Gavasso, Luca Spiezia, Elena Campello and Paolo Simioni: Chair of Internal Medicine, Department of Medicine, University of Padova, Padova, Italy
Marny Fedrigo: Department of Cardiologic, Thoracic, and Vascular Sciences, University of Padova, Padova, Italy

erythematosus (SLE). There are, moreover, subjects who present the typical clinical features consistent with APS classification but are negative for its laboratory criteria [2, 3]. These so-called seronegative patients are not classified as APS patients despite the fact that their negative laboratory results may depend in part or entirely on the poor performance of laboratory tests including enzyme-linked immunosorbent assay (ELISA) [2, 3], hence the importance of reproducible, adequately precise, sensitive, and specific aPL tests to diagnose and consequently to treat these subjects.

As reported in some guidelines [4–6], LA is detected by means of a series of phospholipid-dependent clotting tests; there are nevertheless important differences among these [7]. aCL and anti-β2GPI antibodies are commonly identified by ELISA testing. Although numerous attempts have been made to standardize ELISA testing and several recommendations and updates have been published [8–15], no standardized ELISA methodology is at yet available and intra- and inter-laboratory variability remains at a high level [16–18]. Moreover, validation data concerning homemade ELISA are currently not available in order to define this test as fit for aPL identification.

New, fully automated technologies based on chemiluminescence, a chemical reaction in which light is emitted, have recently been developed for antibody testing [19–22]. In particular, chemiluminescence immunoassay (CLIA) has been available for aCL and anti-β2GPI antibody detection since 2010 [23]. Studies comparing the performance of CLIA with that of ELISA have been performed in heterogeneous groups of patients and have produced divergent results [23–29].

The aim of this work was to compare the performance of a CLIA with that of a homemade ELISA in detecting aCL and anti-β2GPI antibodies in the sera of a large homogeneous cohort of PAPS patients. The diagnostic value of CLIA was also evaluated in a group of seronegative patients with clinical manifestations of APS.

Materials and methods

Study population

One hundred four PAPS patients (89 women and 15 men; mean age 45.0±11.3 years, range 20–71) fulfilling the International Consensus Statement classification criteria for APS [1] were recruited. None of the patients showed any clinical or laboratory features of other systemic autoimmune diseases. Thirty-six of these had pregnancy morbidity alone (one or more fetal deaths occurring at or beyond the 10th week of gestation and/or one or more premature births occurring before the 34th week due to severe pre-eclampsia/eclampsia or clear symptoms of placental insufficiency and/or three or more miscarriages of unknown origin occurring before the 10th week of gestation). Sixty-eight had a history of thrombosis (venous, arterial, or small vessel thrombosis); 19 (27.9%) of these also had pregnancy morbidity. Eighty-eight seronegative patients (81 women and 7 men; mean age 39.3±8.5 years, range 19–70) who met the clinical but not the laboratory criteria for APS classification were also recruited. Fifty-three of these had pregnancy morbidity and 35 had a history of thrombosis. A control group of 150 individuals was also assessed: 100 of these were healthy blood donors matched to the study group for age and sex and 50 (45 women and 5 men; mean age 46.0±14.9 years; range 15–76) were patients affected with various rheumatological diseases (11 SLE, 10 Sjögren syndrome, 7 polymyositis, 10 systemic sclerosis, 6 rheumatoid arthritis, and 6 spondyloarthritis). We included patients with these immune diseases in the control population because aPL may be present as an epiphenomenon. The study was carried out in accordance with the ethical principles outlined in the Declaration of Helsinki and all of the participants gave informed consent.

Chemiluminescence immunoassay

A fully automated chemiluminescent immunoassay (HemosIL AcuStar®; Instrumentation Laboratory, IL, Bedford, MA, USA) was used to detect IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies following the manufacturer's guidelines. During the first step, aPL antibodies were captured from the serum samples by paramagnetic particles coated with cardiolipin or human β2GPI. During the second one, isoluminol-labeled anti-human IgG or IgM antibodies were incubated with the serum samples and bound to the aPL antibodies previously captured by the paramagnetic particles. Finally, reagents triggering the chemiluminescent reaction were added, and the light emitted was measured by the instrument's optical system as relative light units (RLUs). RLUs are directly proportional to aPL antibody concentrations and are converted into U/mL using a standard curve obtained from a pool of positive samples calibrated to Koike's monoclonal antibodies (HCAL for the IgG and EY2C9 for the IgM aPL antibodies) [30]. The intra- and inter-assay coefficient of variation was <10%. The cutoff values for IgG/IgM aCL and IgG/IgM anti-β2GPI tests were calculated as the >99th percentile using sera from the 100 healthy blood donors participating in the study. The cutoff values for IgG/IgM aCL antibodies were 16.2 [95% confidence interval (CI) 15.6–16.9] and 23.6 U/mL (95% CI 22.5–24.6), respectively, and those for IgG/IgM anti-β2GPI antibodies were 35.3 (95% CI 33.8–36.9) and 14.3 U/mL (95% CI 13.7–14.9), respectively.

Homemade ELISA

A homemade ELISA assay was used to detect IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies following the recommendations of the European Forum on aPL [9, 10], as described elsewhere [31]. The cutoff values for medium-high levels of IgG/IgM aCL (calculated as greater than the 99th percentile of the sera from the 100 healthy blood donors participating in the study) were 22.2 GPL (95% CI 21.5–22.9) and 22.9 MPL (95% CI 21.9–24.0), respectively, and of IgG/IgM anti-β2GPI antibodies were 1.9 U/mL (95% CI 1.8–2.0) and 5.7 U/mL (95% CI 5.4–6.0), respectively.

LA assays

LA was assessed by multiple coagulation tests using platelet-poor plasma samples following the updated guidelines [4]. The dilute Russell's viper venom and dilute activated partial thromboplastin times were used as screening tests. Samples with a prolonged screening test not corrected by mixing with a normal pooled plasma underwent a confirmatory study using an excess of phospholipids.

Statistical analysis

The χ^2-test was carried out to compare the sensitivity and specificity of homemade ELISA and CLIA and the antibody prevalence in the seronegative subjects and in the control group. Cohen's κ coefficient was calculated to estimate the agreement between ELISA and CLIA tests [32]. Spearman's correlation coefficient was used to correlate antibody levels determined by the two methods. A p-value of ≤0.05 was considered statistically significant. Statistical analysis was carried out using the SPSS version 19.0 software.

Results

Comparison of ELISA and CLIA data

The study population was classified in accordance with the homemade ELISA results; the same serum samples were then tested for IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies using CLIA. Then, the comparison of the two methods was performed between PAPS patients and control group. The results obtained by ELISA and CLIA are reported in Table 1. As showed in Table 2, ELISA comparative sensitivity was found to be significantly higher than CLIA's for IgM aCL (p<0.001), IgG anti-β2GPI (p=0.01), and IgM anti-β2GPI (p<0.001). There were significant differences between the two methods with regard to specificity, which was comparatively higher for IgM aCL and IgM anti-β2GPI (p=0.03 and p=0.002, respectively) according to CLIA results (Table 2). Concordance between the two techniques was evaluated in the patient and control groups by comparing each ELISA result with the corresponding CLIA one (Table 2). κ Statistics, which ranged between 0.53 and 0.83, revealed a significant concordance between the two assays (p<0.001 for all). The correlation in the PAPS patients between antibody titers detected by ELISA with those detected by CLIA was calculated (Table 2 and Figure 1). The Spearman's ρ coefficients, which ranged between 0.78 and 0.89, showed a significant correlation between the two assay results (p<0.001). PAPS patients with thrombosis and those with pregnancy morbidity were then considered separately. The results obtained using ELISA and CLIA are outlined and compared in Table 3.

Table 1 Positive and negative results obtained from controls, PAPS, and seronegative patients using ELISA and CLIA methods.

	Controls		PAPS		Seronegative patients	
	n	%	n	%	n	%
ELISA						
IgG aCL						
Negative	145	96.7	39	37.5	88	100.0
Positive	5	3.3	65	62.5	0	0
IgM aCL						
Negative	141	94.0	52	50.0	88	100.0
Positive	9	6.0	52	50.0	0	0
IgG anti-β2GPI						
Negative	141	94.0	30	28.8	88	100.0
Positive	9	6.0	74	71.2	0	0
IgM anti-β2GPI						
Negative	136	90.7	52	50.0	88	100.0
Positive	14	9.3	52	50.0	0	0
CLIA						
IgG aCL						
Negative	145	96.7	44	42.3	76	86.4
Positive	5	3.3	60	57.7	12	13.6
IgM aCL						
Negative	147	98.0	74	71.2	87	98.9
Positive	3	2.0	30	28.8	1	1.1
IgG anti-β2GPI						
Negative	145	96.7	44	42.3	83	94.3
Positive	5	3.3	60	57.7	5	5.7
IgM anti-β2GPI						
Negative	148	98.7	74	71.2	88	100.0
Positive	2	1.3	30	28.8	0	0.0

PAPS, primary anti-phospholipid syndrome; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence immunoassay; aCL, anti-cardiolipin antibodies; anti-β2GPI, anti-β_2 glycoprotein I antibodies.

CLIA performance in seronegative patients

As indicated in Table 1, IgG/IgM aCL and IgG anti-β2GPI antibodies were detected by CLIA in the ELISA-seronegative patients. The results in the 88 ELISA-seronegative patients and the 124 ELISA-seronegative controls were compared. When the comparative sensitivity of CLIA in the ELISA-seronegative patients with typical clinical manifestations of APS was evaluated, IgG aCL and IgG anti-β2GPI antibodies showed a significantly higher comparative prevalence with respect to that in the controls (Figure 2). There was no significant difference in the prevalence of IgG/IgM aCL and IgG/IgM anti-β2GPI antibodies in the thrombotic and pregnancy morbidity subsets of the seronegative patients.

Table 2 Parameters of analytical comparison between ELISA and CLIA.

	aCL IgG		aCL IgM		Anti-β2GPI IgG		Anti-β2GPI IgM	
	ELISA	CLIA	ELISA	CLIA	ELISA	CLIA	ELISA	CLIA
Sensitivity, %	62.5	57.7	50.0[a]	28.8[a]	71.2[b]	57.7[b]	50.0[a]	28.8[a]
Specificity, %	96.7	96.7	94.0[c]	98.0[c]	94.0	96.7	90.7[d]	98.7[d]
κ		0.83[a]		0.59[a]		0.79[a]		0.53[a]
Spearman's ρ		0.87[a]		0.80[a]		0.89[a]		0.78[a]

ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence assay; aCL, anti-cardiolipin antibodies; anti-β2GPI, anti-β_2 glycoprotein I antibodies; [a]p<0.001, [b]p=0.01, [c]p=0.03, [d]p=0.002.

Discussion

CLIA, a fully automated technique, takes about 30 min to complete a test, saves time, and reduces operator handling. Automation can also reduce intra- and inter-laboratory variability and improve the reproducibility of the results [22]. Some studies have been performed since CLIA first became available to compare this new technology with ELISA testing, which is more commonly used to detect aCL and anti-β2GPI antibodies [23–29]. These comparison studies were, however, carried out using different CLIA instruments and various ELISA techniques (homemade methods and various commercial kits). As a consequence, different results have been reported. A recently published

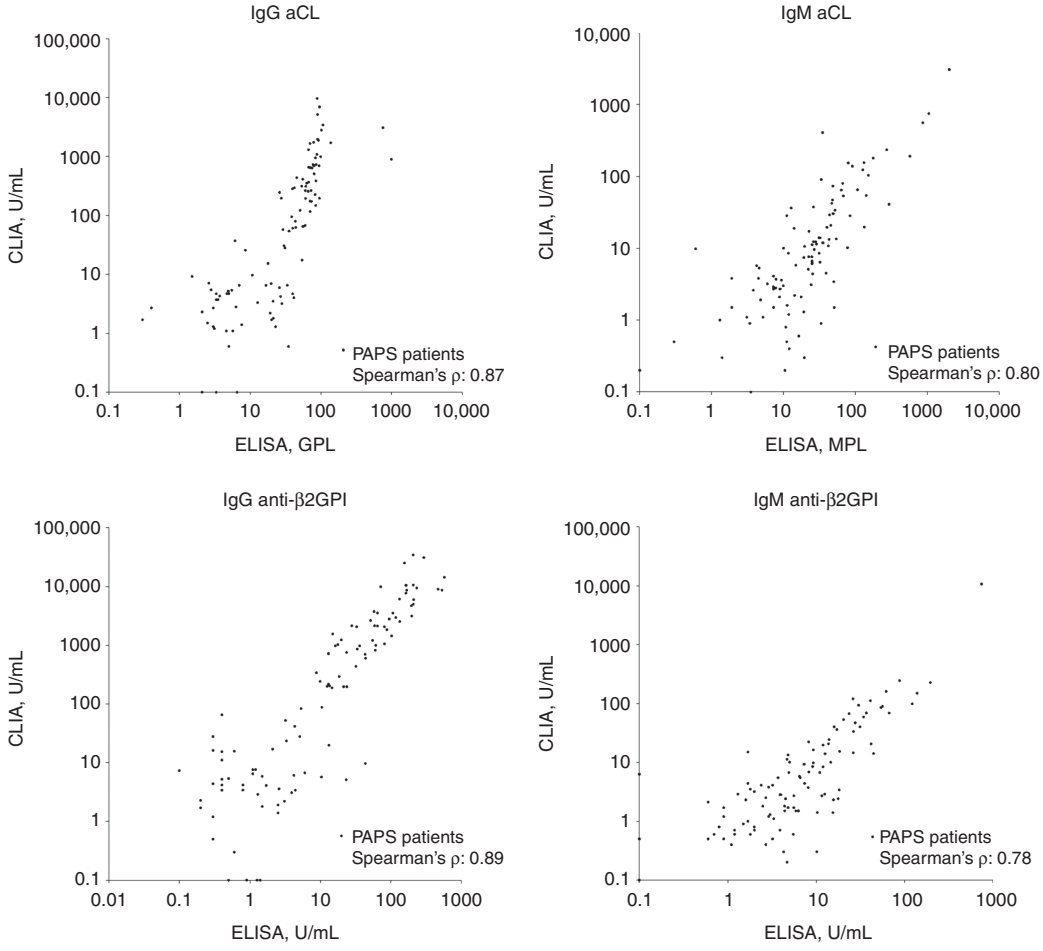

Figure 1 Graphic dispersion of antibody titers detected by ELISA and CLIA.
As demonstrated by Spearman's ρ values, the assays showed a significant correlation. ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence assay; aCL, anti-cardiolipin antibodies; anti-β2GPI, anti-β_2 glycoprotein I antibodies.

Table 3 Results from PAPS patients with thrombosis and pregnancy morbidity using ELISA and CLIA.

	Thrombosis		Pregnancy morbidity		p-Value
	n	%	n	%	
ELISA					
IgG aCL	53	77.9	12	33.3	<0.001
IgM aCL	33	48.5	19	52.8	0.837
IgG anti-β2GPI	59	86.8	15	41.7	<0.001
IgM anti-β2GPI	33	48.5	19	52.8	0.837
CLIA					
IgG aCL	53	77.9	7	19.4	<0.001
IgM aCL	23	33.8	7	19.4	0.172
IgG anti-β2GPI	53	77.9	7	19.4	<0.001
IgM anti-β2GPI	23	33.8	7	19.4	0.172

ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence assay; aCL, anti-cardiolipin antibodies; anti-β2GPI, anti-β_2 glycoprotein I antibodies.

study focused on a refinement of the cutoff values for IgG/IgM aCL and anti-β2GPI antibodies obtained with the same instrument used in the present study. The cutoffs from 626 healthy individuals were, moreover, similar to ours for all the aPL tested except for IgG anti-β2GPI antibodies, which was lower (17.4 vs. 35.3 U/mL) [33].

The present study compared the performance of HemosIL AcuStar CLIA with that of a homemade ELISA performed following international recommendations [9, 10]. The study population included a large cohort of selected PAPS patients to exclude the effects of other underlying autoimmune diseases generally associated to APS.

Study results showed that CLIA had a significantly lower comparative sensitivity for IgM aCL and IgG/IgM anti-β2GPI but a significantly higher comparative specificity for IgM aCL and IgM anti-β2GPI with respect to a homemade ELISA. While in keeping with the results outlined by other studies using the same CLIA instrument [25, 29], they disagree with those described by De Moerloose et al. [23], who found that the HemosIL AcuStar CLIA produced a higher comparative sensitivity than some ELISA kits. That study did not, however, include a statistical comparison. In accordance with other authors [23, 25, 28, 29], our investigation demonstrated a significant agreement and correlation of antibody titers between the two methods. In particular, κ-values and Spearman's ρ coefficients for IgG aCL and IgG anti-β2GPI antibodies were found to be higher than those for the corresponding "M" isotype antibodies (Table 2). The agreement between the two methods was thus superior for the IgG antibody isotype, which is considered, in fact, more clinically relevant for APS [34–36].

The novelty of this study is the significant prevalence of IgG aCL and IgG anti-β2GPI antibodies along with the detection of IgM aCL antibodies in the ELISA-seronegative patients with the typical clinical manifestations of APS. These results would appear to confer a clinical significance to aPL detection by the HemosIL AcuStar CLIA. It would, in fact, be possible to classify 17 out of the 88 seronegative

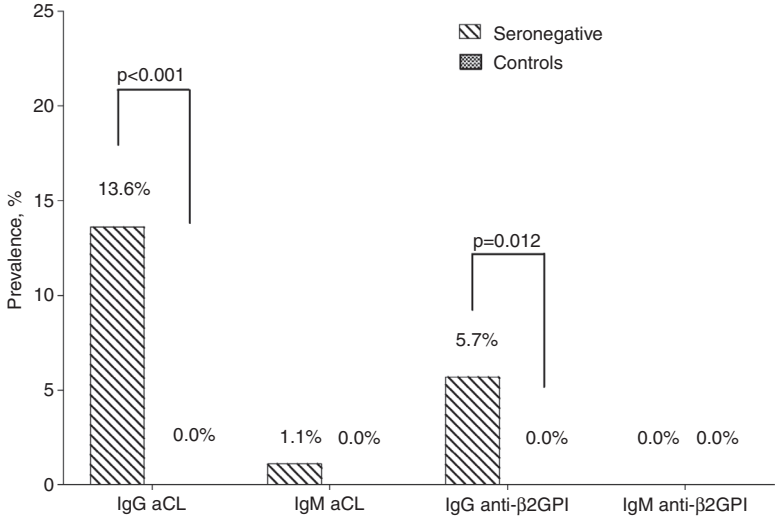

Figure 2 The prevalence of IgG/IgM anti-cardiolipin and IgG/IgM anti-β_2 glycoprotein I antibodies detected by CLIA in ELISA-seronegative patients.

ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence assay; aCL, anti-cardiolipin antibodies; anti-β2GPI, anti-β_2 glycoprotein I antibodies. The comparison of results was made between the 88 ELISA-seronegative patients and the 124 ELISA-seronegative controls.

subjects as APS patients and to treat them accordingly. As the HemosIL AcuStar CLIA showed a high comparative specificity for APS, we could assume that these results have a real clinical value. Technically speaking, the conformation of the antigen-binding site in CLIA is different from that in ELISA; in the former, there are paramagnetic microspheres; in the latter, there is the flat surface of the microwells. It can be hypothesized that the coated antigens expose different epitopes to aPL antibody binding and the HemosIL AcuStar CLIA is able to detect some aCL and anti-β2GPI antibodies not usually uncovered by ELISA.

When the results in the PAPS patients with thrombosis were compared with those in the women with pregnancy morbidity, a significantly higher prevalence of IgG aCL and IgG anti-β2GPI antibodies was found in the former by both ELISA and CLIA. No significant antibody prevalence was instead found in the seronegative thrombotic patients, probably due to the low number of cases examined. Numerous thrombotic subjects with acquired or inherited risk factors for thrombosis were, in fact, excluded during the recruitment process.

It should be noted that the study's primary limitation was that the study population was selected on the basis of homemade ELISA results; CLIA's relative sensitivity and specificity were consequently influenced by ELISA's ones.

In conclusion, despite a lower comparative sensitivity, the HemosIL AcuStar CLIA showed a higher comparative specificity and a good level of agreement and titer correlation with homemade ELISA. Surprisingly, the IgG aCL and IgG anti-β2GPI antibodies detected using this fully automated method were found to be significant in the seronegative patients with typical clinical manifestations of APS. If confirmed by further studies, CLIA could be considered a valuable method to assess patients with clinical manifestations of APS but testing negative for aPL using a homemade ELISA.

Acknowledgments: The authors are grateful to Dr Elisa Salvan for the statistical calculations and to Mrs Linda Inverso Moretti for editing the English version of the manuscript.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Financial support: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. 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). J Thromb Haemost 2006;4:295–306.
2. Conti F, Capozzi A, Truglia S, Lococo E, Longo A, Misasi R, et al. The mosaic of "seronegative" antiphospholipid syndrome. J Immunol Res 2014;2014:1–7.
3. Hughes GR, Khamashta MA. Seronegative antiphospholipid syndrome. Ann Rheum Dis 2003;62:1127.
4. Pengo V, Tripodi A, Reber G, Rand JH, Ortel TL, Galli M, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost 2009;7:1737–40.
5. Keeling D, Mackie I, Moore GW, Greer IA, Greaves M. British Committee for Standards in Haematology Guidelines on the investigation and management of antiphospholipid syndrome. Br J Haematol 2012;157:47–58.
6. Clinical and Laboratory Standards Institute (CLSI). Laboratory testing for the lupus anticoagulant; approved Guideline. CLSI document H60-A. Wayne, PA: CLSI, 2014.
7. Favaloro EJ, Wong RC. Antiphospholipid antibody testing for the antiphospholipid syndrome: a synopsis of challenges and recent guidelines. Pathology 2014;46:481–95.
8. Favaloro EJ, Plebani M, Lippi G. Standardization and harmonization of antiphospholipid antibody assays. Semin Thromb Hemost 2014;40:161–2.
9. Reber G, Tincani A, Sanmarco M, de Moerloose P, Boffa MC. Proposals for the measurement of anti-b2-glycoprotein I antibodies. Standardization Group of the European Forum on Antiphospholipid Antibodies. J Thromb Haemost 2004;2:1860–2.
10. Tincani A, Allegri F, Balestrieri G, Reber G, Sanmarco M, Meroni P, et al. Minimal requirements for antiphospholipid antibodies ELISAs proposed by the European Forum on antiphospholipid antibodies. Thromb Res 2004;114:553–8.
11. Wong RC, Gillis D, Adelstein S, Baumgart K, Favaloro EJ, Hendle MJ, et al. Consensus guidelines on anti-cardiolipin antibody testing and reporting. Pathology 2004;36:63–8.
12. Wong RC, Favaloro EJ, Adelstein S, Baumgart K, Bird R, Brighton TA, et al. Consensus guidelines on anti-beta 2 glycoprotein I testing and reporting. Pathology 2008;40:58–63.
13. Harris EN, Pierangeli SS. Revisiting the anticardiolipin test and its standardization. Lupus 2002;11:269–75.
14. Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC, et al. International consensus guidelines on anticardiolipin and anti-β2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. Arthritis Rheum 2012;64:1–10.
15. Pierangeli SS, Favaloro EJ, Lakos G, Meroni PL, Tincani A, Wong RC. Standards and reference materials for the anticardiolipin and anti-β2glycoprotein I assays: a report of recommendations from the APL Task Force at the 13th International Congress on Antiphospholipid Antibodies. Clin Chim Act 2012;413:358–60.
16. Pierangeli SS, Harris EN. A quarter of a century in anticardiolipin antibody testing and attempted standardization has led us to here, which is? Semin Thromb Hemost 2008;34:313–28.

17. Reber G, Boehlen F, de Moerloose P. Technical aspects in laboratory testing for antiphospholipid antibodies: is standardization an impossible dream? Semin Thromb Hemost 2008;34:340–6.
18. Wong R, Favaloro E, Pollock W, Wilson R, Hendle M, Adelstein S. A multi-centre evaluation of the intra-assay and inter-assay variation of commercial and in-house anti-cardiolipin antibody assays. Pathology 2004;36:182–92.
19. Zhao L, Sun L, Chu X. Chemiluminescence immunoassay. Trends Analyt Chem 2009;28:404–14.
20. Roda A, Guardigli M. Analytical chemiluminescence and bioluminescence: latest achievements and new horizons. Anal Bioanal Chem 2012;402:69–76.
21. Baeyens WR, Schulman SG, Calokerinos AC, Zhao Y, García Campaña AM, Nakashima K, et al. Chemiluminescence-based detection: principles and analytical applications in flowing streams and in immunoassays. J Pharm Biomed Anal 1998;17:941–53.
22. Andreoli L, Rizzini S, Allegri F, Meroni P, Tincani A. Are the current attempts at standardization of antiphospholipid antibodies still useful? Emerging technologies signal a shift in direction. Semin Thromb Hemost 2008;34:356–60.
23. De Moerloose P, Reber G, Musial J, Arnout J. Analytical and clinical performance of a new, automated assay panel for the diagnosis of antiphospholipid syndrome. J Thromb Haemost 2010;8:1540–6.
24. Persijn L, Decavele AS, Schouwers S, Devreese K. Evaluation of a new set of automated chemiluminescense assays for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome. Thromb Res 2011;128:565–9.
25. Van Hoecke F, Persijn L, Decavele AS, Devreese K. Performance of two new, automated chemiluminescence assay panels for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome. Int J Lab Hematol 2012;34:630–40.
26. Capozzi A, Lococo E, Grasso M, Longo A, Garofalo T, Misasi R, et al. Detection of antiphospholipid antibodies by automated chemiluminescence assay. J Immunol Methods 2012;379:48–52.
27. Forastiero R, Papalardo E, Watkins M, Nguyen H, Quirbach C, Jaskal K, et al. Evaluation of different immunoassays for the detection of antiphospholipid antibodies: report of a wet workshop during the 13th International Congress on Antiphospholipid Antibodies. Clin Chim Acta 2014;428:99–105.
28. Mondejar R, González-Rodríguez C, Toyos-Sáenz de Miera FJ, Melguizo-Madrid E, Zohoury N, Mahler M. Role of antiphospholipid score and anti-β2-glycoprotein I Domain I autoantibodies in the diagnosis of antiphospholipid syndrome. Clin Chim Acta 2014;431:174–8.
29. Chung Y, Kim JE, Lim HS, Kim HK. Clinical performance of anticardiolipin and antiβ2 glycoprotein I antibodies using a new automated chemiluminescent assay: superior thrombotic prediction of combined results measured by two different methods. Blood Coagul Fibrinolysis 2014;25:10–5.
30. Ichikawa K, Tsutsumi A, Atsumi T, Matsuura E, Kobayashi S, Hughes GR, et al. A chimeric antibody with the human gamma1 constant region as a putative standard for assays to detect IgG beta2-glycoprotein I-dependent anticardiolipin and anti-beta2-glycoprotein I antibodies. Arthritis Rheum 1999;42:2461–70.
31. Ruffatti A, Olivieri S, Tonello M, Bortolati M, Bison E, Salvan E, et al. Influence of different IgG anticardiolipin antibody cut-off values on antiphospholipid syndrome classification. J Thromb Haemost 2008;6:1693–6.
32. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med 2005;37:360–3.
33. Fontana P, Poncet A, Lindhoff-Last E, de Moerloose P, Devreese KM. Refinement of the cutoff values of the HemosIL AcuStar assay for the detection of anticardiolipin and anti-beta2-glycoprotein-1 antibodies. J Thromb Haemost 2014;12:2034–7.
34. Galli M, Luciani D, Bertolini G, Barbui T. Lupus anticoagulants are stronger risk factors for thrombosis than anticardiolipin antibodies in the antiphospholipid syndrome: a systematic review of the literature. Blood 2003;101:1827–32.
35. Galli M, Reber G, de Moerloose P, de Groot PG. Invitation to a debate on the serological criteria that define the antiphospholipid syndrome. J Thromb Haemost 2008;6:399–401.
36. Galli M, Luciani D, Bertolini G, Barbui T. Anti-beta 2-glycoprotein I, antiprothrombin antibodies, and the risk of thrombosis in the antiphospholipid syndrome. Blood 2003;102:2717–23.