Original Research Article

Diagnosis of Antiphospholipid Syndrome by Chemiluminescent or Enzyme-Linked Immunosorbent Assay – A Comparison Study and Comprehensive Literature Review

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Abstract

Objective: Enzyme-linked immunosorbent assay (ELISA) is the established method for detecting antiphospholipid antibodies (aPL) in the diagnosis of antiphospholipid syndrome (APS) but is labor-intensive compared with the newer automated chemiluminescent assay (CLIA). This study aims to evaluate CLIA versus ELISA for aPL, correlate each method with clinical manifestations and perform a comprehensive literature review.

Methods: Patient samples were concurrently tested by ELISA (QUANTA Lite®) and CLIA (ACL AcuStar®) for anti-cardiolipin antibody (aCL) and anti-β2-glycoprotein-I (aβ2GPI) IgG and IgM. Assay results were correlated with any of the revised Sapporo APS clinical criteria.

Results: Of the 107 patients, 67% fulfilled at least one clinical criterion. 38 patients (35.5%) had APS. For aCL IgG, aCL IgM and aβ2GPI IgM, CLIA showed above 77% concordance and fair to excellent agreement (Cohen's kappa 0.39–0.86) with moderate/high positive ELISA of ≥40 units. Both methods showed good correlation (Spearman's r 0.60–0.80, $p < 0.0001$) that was non-linear over the range of titers. CLIA sensitivity and specificity was 46%–100% and 68%–95%, with AUROC ranging from 0.80–0.93. For aβ2GPI IgG, concordance was 36.7% and agreement was low (kappa −0.23). Correlation with clinical criteria revealed no statistically significant difference in the occurrence of clinical manifestations in ELISA-positive versus CLIA-positive groups.

Conclusions: aPL detection by CLIA showed close but incomplete concordance with ELISA. CLIA positivity correlated well with moderate/high ELISA positivity, but antibody titers should not be directly compared across systems. CLIA is an acceptable alternative to ELISA in the routine non-research setting. Our findings are congruent with the reviewed literature.

Keywords

antiphospholipid antibodies, antiphospholipid syndrome, diagnosis, solid phase assay, chemiluminescent, ELISA

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Introduction

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by vascular thrombosis in venous, arterial or microvascular beds and/or obstetric morbidity such as fetal losses, intrauterine growth restriction and severe pre-eclampsia, in the presence and persistence of antiphospholipid antibodies (aPL). A severe syndrome of thrombosis in multiple organs, termed catastrophic APS, as well as non-thrombotic manifestations including thrombocytopenia, hemolytic anemia, valvular heart disease and nephropathy are other well-known manifestations of APS.[1]

The diagnosis of APS relies on the laboratory detection of aPL. aPL mediate several procoagulant mechanisms and have a direct pathogenic role in APS. β−2 glycoprotein I (β2GPI) dependent aPL, the most important subset of these antibodies, mediate thrombosis by reactivity with β2GPI bound to the membrane of cells that participate in the coagulation cascade.[2–4] It is thought that reactivity of anti-cardiolipin antibodies (aCL) is mediated by β2GPI antibodies (aβ2GPI) that bind β2GPI complexed with cardiolipin.[5,6] The lupus anticoagulant, aCL and aβ2GPI of IgM and IgG isotypes are part of the laboratory criteria for APS in the revised Sapporo classification criteria and the recent 2023 American College of Rheumatology/ European Alliance of Associations for Rheumatology (ACR/EULAR) Antiphospholipid Syndrome Classification Criteria.[7,8]

The enzyme-linked immunosorbent assay (ELISA) is the standard solid phase assay used for detection of aPL since the 1980s. The test involves a plate coated with cardiolipin or β2GPI, the addition of patient's serum or plasma, followed by the application of a secondary labeled antibody to quantify the bound IgG or IgM.

More recently, other methods for antibody detection have been developed, such as the chemiluminescent immunoassays (CLIA).[9] This two-phased assay consists of paramagnetic particles coated with cardiolipin and/or β2GPI, which capture aPL from the patient's serum or plasma. Reagents that trigger the chemiluminescence reaction are added and the emitted light is measured and reported in relative light units (RLUs), which are directly proportional to the concentration of aPL. CLIA has several advantages over ELISA due to its several fold greater sensitivity thereby allowing measurement of extremely low levels of analyte, wider dynamic range allowing measurement of high concentrations, and absence of interfering emission conferring high specificity.[10] By now, CLIA is a well-established methodology in use by many instrument manufacturers and laboratories.[11]

CLIA has been shown to have comparable performance to ELISA for aPL detection.[12–19] Furthermore, CLIA is fully automated, less labor intensive and can be performed more rapidly. However, the 2023 ACR/EULAR Antiphospholipid Syndrome Classification Criteria consensus recommendation stated that for research purposes, ELISA should be used for APS classification, as there is as yet lack of standardization between equivalent CLIA levels corresponding to moderate and high ELISA titers. This has led to debate over whether CLIA is an acceptable alternative to ELISA for APS diagnosis in routine clinical practice, contributed in part by confusion between the definitions of classification criteria and diagnostic criteria.[11,20] As the intention of classification criteria is to identify a homogenous patient cohort for inclusion in trials and observational studies, they tend to be highly specific and capture a narrower range of disease. Hence, classification criteria do not necessarily equate to the only criteria for diagnosis. Diagnosis, on the other hand, is made by evaluating signs, symptoms and supporting tests of the individual patient, and therefore, patients diagnosed with disease may or may not fulfill classification criteria.

On this background, this study aimed to clarify the qualitative and quantitative differences between ELISA and CLIA for the detection of aPL, and to correlate the results of aPL detected by each method to the clinical manifestations of APS.

Methods

This study is a diagnostic study conducted in a single tertiary hospital. The study was conducted in accordance with the Declaration of Helsinki. Ethics approval for the study was granted by the institution review board. Patient samples referred to the laboratory for detection of aPL between April 2022 to May 2023 were included in a performance evaluation of CLIA versus existing ELISA prior to implementation. Consecutive samples positive for at least one aPL by the routine ELISA method were tested concurrently using the CLIA method for aCL IgG and IgM and aβ2GPI IgG and IgM.

Blood samples were collected in serum tubes (BD Vacutainer®, Becton Dickinson and Company, Franklin Lakes, NJ, USA) and centrifuged at 4000 RPM. Serum was stored at −25 °C until ELISA and CLIA testing. ELISA was performed using the QUANTA Lite® assay (Inova Diagnostics, San Diego, CA, USA) on the Evolis® ELISA system (BioRad, Marnes-La-Coquette, France). In the ELISA, antibodies present in serum samples bind to the immobilized purified cardiolipin and bovine β2GPI antigen on the microwell plate. Anti-human IgG or IgM conjugate is added after the first wash, binding to any antibodies attached to the microwells. A chromogenic substrate is added after the second wash, and the intensity of the color is measured and compared to a five-point calibration curve. Results are expressed in phospholipid units (GPL/MPL) or standard IgG β2GPI units (SGU/SMU). The laboratory ELISA positive cut-off was ≥20 units, which was validated within the laboratory

to correspond to >99th percentile of a normal population. ELISA moderate/high titers (ie ≥ 40 units) is recommended for classification of APS, hence this threshold was also incorporated into the analysis.[7]

CLIA was performed on the ACL AcuStar® platform or (Werfen, Barcelona, Spain) as per manufacturer's instructions. CLIA uses paramagnetic beads coated with bovine cardiolipin and human β2GPI. After incubation of the magnetic beads with serum samples, magnetic separation, and washing of the beads, a tracer consisting of isoluminol-labeled anti-human IgG or IgM antibody is added. The tracer binds to antibodies captured on the beads. After another incubation, magnetic separation and wash cycle, reagents are added to induce luminescence reaction. The emitted light is measured as RLUs, which are directly proportional to the aCL and aβ2GPI present in the sample. Results are expressed in U/mL (IgG or IgM Arbitrary Units). The CLIA cut-off for positive was ≥20 U/ml, as per manufacturer's recommendations.

Lupus anticoagulant assays were performed according to the recommendations of the International Society on Thrombosis and Haemostasis (ISTH), using screening, confirmation and mixing steps. In the screening tests, diluted Russell's viper venom test (dRVVT) (LA1 Screening reagent, Siemens Healthineers, Marburg, Germany) and lupus anticoagulant-sensitive activated partial thromboplastin time (APTT) (Dade Actin FSL, Siemens Healthineers) were measured on CS-5100® (Sysmex Corp. Japan). A positive dRVVT screening test was followed by a confirmatory dRVVT (LA2 Confirmation reagent, Siemens Healthineers) measured on CS-5100®. LA1 and LA2 mixing steps were performed according to the laboratory's workflow. A positive lupus anticoagulant-sensitive APTT screening test was followed by a confirmatory test using hexagonal phase phospholipid neutralization (Staclot® LA, Diagnostica Stago, Asniere-sur-Seine, France) performed on STart® 4 Haemostasis analyzer (Diagnostica Stago). Lupus anticoagulant was present when dRVVT results expressed as normalized ratio was >1.15 or Staclot® LA clotting time difference was ≥ 8 s.

Clinical data for the patients were extracted retrospectively from electronic medical records. These were (1) patient demographics (age, gender, race), (2) history of venous or arterial thrombosis, (3) provoking risk factors for venous thromboembolism (VTE), (4) arterial thrombosis risk factors such as obesity, smoking status, hypertension, hyperlipidemia and diabetes mellitus, (5) history of pregnancy complications ie early (<10 weeks) fetal loss, premature births before the 34th week of gestation because of eclampsia or placental insufficiency and spontaneous abortions before the 10th week of gestation, as well as (6) non-criteria manifestations such as heart valve disease, livedo reticularis, thrombocytopenia, nephropathy, and neurological manifestations. Other clinical information such as the presence of other autoimmune disease, previous diagnosis of APS, type of anticoagulant or antiplatelet agent given, previous aPL results were obtained. The revised Sapporo classification criteria was used to assign whether a patient met clinical criteria for APS.[7]

Statistical analysis was performed on SPSS version 28.0 (IBM SPSS, Chicago, USA) and GraphPad Prism version 9.4 (GraphPad, Boston, USA). Normality testing was performed with the Kolmogorov-Smirnov test. Qualitative comparison of concordance between the two methods was performed using 2×2 contingency tables. Cohen's kappa value for inter-rater agreement was calculated. Quantitative comparison between titers obtained by the two methods was performed using Spearman rank order correlation for non-parametrically distributed data. Receiver operating characteristics (ROC) was performed to evaluate the sensitivity and specificity of CLIA for a dichotomous ELISA positive/negative result, recognizing that although ELISA is not a true gold standard, it is the incumbent against which the newer method is compared. A related samples McNemar test was performed to determine if the frequency of clinical criteria differed by each method. McNemar test evaluates for any difference in the proportion of clinical outcome in the subset of discordant cases. A $p < 0.05$ was considered statistically significant.

Results

Demographic and Clinical Data

A total of 107 patients were included in this study, of whom 67 were females (63%). The median age was 48 years (range 11-91 years). Seventy-two patients (67%) fulfilled at least one revised Sapporo clinical criteria. Sixty-six patients (62%) had thrombosis, and nine patients (8%) had obstetric criteria. Twenty-five patients (23%) experienced VTE, for a total of 29 VTE events. Out of the VTE events, 17 (59%) were unprovoked. Forty-six patients (43%) had arterial thrombosis, for a total of 56 arterial thrombosis events. Twenty-eight patients (26%) had primary APS, and 10 (9%) had secondary APS associated with systemic lupus erythematosus and other systemic autoimmune disease.

Concurrent ELISA and CLIA testing were performed in 31 patient samples for aCL IgG, 67 samples for aCL IgM, 30 samples for aβ2GPI IgG and 33 samples for aβ2GPI IgM. The relatively higher number for aCL IgM was due to the higher number of samples for aCL IgM measurement received during the study period.

ELISA Versus CLIA Comparison

(I) Concordance Rate. The concordance rate between ELISA and CLIA was calculated using laboratory positive cut-off (ELISA titer ≥20, CLIA ≥20 U/ml), as well as a moderate/high ELISA titer of ≥40. The results are shown

Table 1. Concordance Rate of Antiphospholipid Antibodies Detected by ELISA and CLIA.

		aCL IgG		aCL IgM		aβ2GPI IgG		aβ2GPI IgM	
		$n=31$		$n=67$		$n=30$		$n=33$	
		CLIA+	CLIA-	CLIA+	CLIA-	CLIA+	CLIA-	CLIA+	CLIA-
(A)	ELISA + (≥20), *n*	14	5	10	26	14	10	11	5
	ELISA- (<20), *n*	2	10	1	30	4	2	0	17
	Positive concordance	73.7%		27.8%		58.3%		68.8%	
	Negative concordance	83.3%		96.8%		33.3%		100.0%	
	Total concordance	77.4%		59.7%		53.3%		84.9%	
	Kappa	0.545		0.223		−0.061		0.694	
(B)	ELISA + (≥40), *n*	9	0	6	7	6	7	10	1
	ELISA- (<40), *n*	7	15	5	49	12	5	1	21
	Positive concordance	100.0%		46.2%		46.2%		90.9%	
	Negative concordance	68.2%		96.6%		29.4%		95.5%	
	Total concordance	77.4%		82.1%		36.7%		93.9%	
	Kappa	0.554		0.392		−0.234		0.864	

Abbreviations: ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent assay; aCL, anticardiolipin antibody; aβ2GPI, β2-glycoprotein I antibody; IgG, immunoglobulin G; IgM, immunoglobulin M

in Table 1. The concordance rate was higher when CLIA was compared with an ELISA titer of ≥40 than with the lower cut-off of ≥20. The concordance rate was above 77% for aCL IgG, aCL IgM and aβ2GPI IgM, but was only 36.7% for aβ2GPI IgG. Cohen's kappa coefficient was fair to excellent for aCL IgG, aCL IgM and aβ2GPI IgM when ELISA cut-off was ≥40, but was poor for aβ2GPI IgG (Kappa coefficients = 0.55, 0.39, 0.86, −0.23 respectively). Cohen's kappa coefficients dropped when ELISA ≥20 was compared with CLIA (Table 1).

(II) Quantitative Correlation Between ELISA and CLIA. The correlation between ELISA titer and CLIA units was investigated using Spearman rank correlation. Spearman correlation showed moderate to strong positive correlation between ELISA titer and CLIA units for aCL IgG ($r=0.806$, 95% confidence interval [CI] 0.625-0.905, $p<0.0001$), aCL IgM ($r=0.607$, 95% CI 0.424-0.743, $p<0.0001$) and aβ2GPI IgM ($r=0.808$, 95% CI 0.637-0.903, $p<0.0001$). However, there was no correlation between ELISA titer and CLIA units for aβ2GPI IgG ($r=0.062$, 95% CI −0.316-0.422, $p=0.746$). The scatter plots of the four pairwise correlations showed a non-linear relationship, as shown in Figure 1.

(III) Receiver Operating Characteristics of CLIA. Sensitivity and specificity of CLIA quantitative results for a positive ELISA of ≥40 units were assessed by ROC analysis (Figure 2). The area under the ROC curve (AUROC) was 0.934 for CLIA aCL IgG (95% CI 0.848-1.000, $p<0.001$), 0.806 for CLIA aCL IgM (95% CI 0.680-0.933, $p<0.001$), and 0.913 for CLIA aβ2GPI IgM (95% CI 0.771-1.000, $p<0.001$). For CLIA aβ2GPI IgG, AUROC was 0.543 (95% CI 0.313-0.773, $p=0.691$). Using the CLIA manufacturer's positive cut-off of ≥20 U/ml and other potential cut-off levels (10, 30, 40 U/ml), the sensitivity and specificity of CLIA for a positive ELISA were obtained from the ROC table and shown in Table 2.

Association with APS Clinical Criteria

McNemar test was applied to evaluate for any difference in the incidence of clinical manifestations of thrombosis and obstetric complications between cases with ELISA positive status (≥40 units) versus CLIA positive status (≥20 U/ml). The McNemar test showed that there was no statistically significant difference in the occurrence of combined thrombosis and obstetric manifestations, and that of combined venous and arterial thrombosis, in the ELISA positive group versus the CLIA positive group. The occurrence of obstetric complications alone was not evaluated due to small numbers (<5 cases). The summary of the comparison is presented in Table 3.

Due to the large discordance in aβ2GPI IgG results, we reviewed the clinical details of these cases, and the clinical correlation is presented in Table 4. The patients who were aβ2GPI IgG CLIA positive had a higher proportion who also had lupus anticoagulant detected, were double aPL positive (any two of the three aPL subtypes – lupus anticoagulant, aCL or aβ2GPI) or triple aPL positive (all three aPL subtypes), had co-existing autoimmune disease or clinical thrombosis, than the group who were positive for aβ2GPI IgG ELISA alone.

Discussion

From our study, CLIA for aCL IgG, aCL IgM and aβ2GPI IgM showed acceptable analytical performance compared

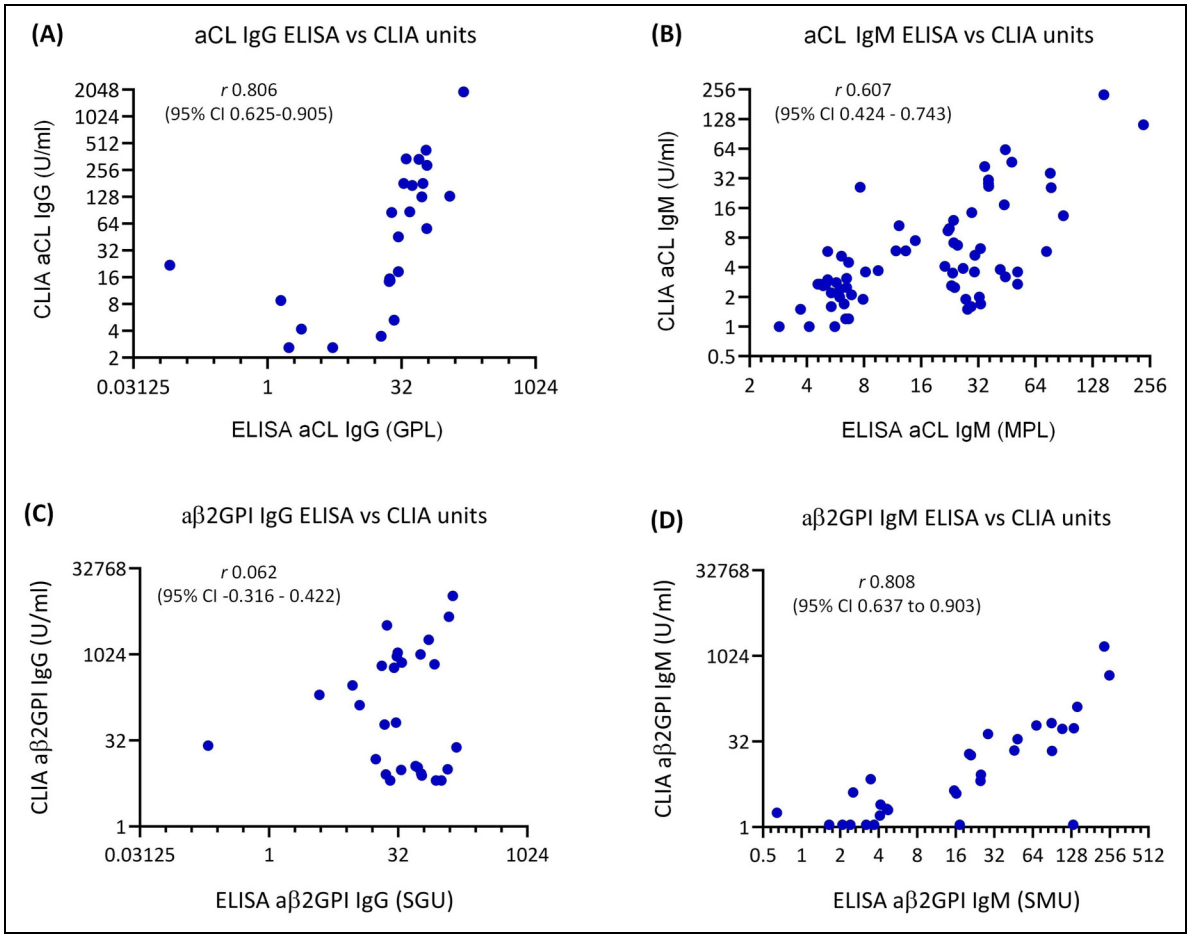

Figure 1. Correlation between quantitative units measured by ELISA and CLIA. Abbreviations: ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent assay; aCL, anticardiolipin antibody; aβ2GPI, β2-glycoprotein I antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; *r,* Spearman rank correlation coefficient; CI, confidence interval.

with ELISA assay. This was evident from the excellent AUROC of CLIA, good concordance rate with ELISA (total concordance rate of 77.4%, 82.1% and 93.9% respectively) and fair to excellent kappa coefficients for agreement. For example, CLIA aCL IgG and aβ2GPI IgM positivity showed excellent sensitivity/specificity for the corresponding ELISA moderate/high titer positivity (100%/68.2% and 90.9%/95.5%). For aCL IgM, CLIA had excellent specificity (90.7%) but lower sensitivity (46.2%) for positive ELISA, but sensitivity could be moderately improved to 61.5% if CLIA positive cut-off was lowered to 10 U/ml, but at the cost of specificity which would decline to 85.2%. Moreover, CLIA positivity had better concordance with ELISA ≥40 units than with ELISA ≥20 units, which supports that CLIA positivity correlated well to moderate/high ELISA positivity.

Compared to the ELISA assay, the CLIA assay is less time-consuming and labor-intensive and allows for more flexibility. The assay set-up time per 20 samples for the CLIA is 20 min as compared with 40 min for the ELISA. According to the manufacturer, calibration is needed only during every lot change for the CLIA as compared to every batch run for the ELISA. For the fully automated CLIA, reagents are packaged in self-contained cartridges which eliminates reagent handling. For the semi-automated ELISA, calibrators and reagents require manual set-up for each run. The CLIA gives room for greater flexibility as it allows random access and continuous loading, whereas ELISA is configured for only batch testing. The time-to-result of 30 min for the CLIA is significantly shorter than the 90–120 min for the ELISA.

It is recommended that laboratories establish in-house cut-off values for ELISA and CLIA using the 99th percentile of at least 50 to 120 healthy normal individuals.[21,22] The study by Persijn found that in-house CLIA cut-offs were significantly lower than the manufacturer's cut-off but using in-house cut-offs increased the false positive rate, leading to lower specificity.[19] In a study by Li et al, the in-house cut-off of the ACL AcuStar® CLIA was close to the 20 U/ml manufacturer's cut-off.[14] In another study by De Moerloose et al evaluating the ACL AcuStar® assay, the value of 20 U/ml corresponded well to the 99th

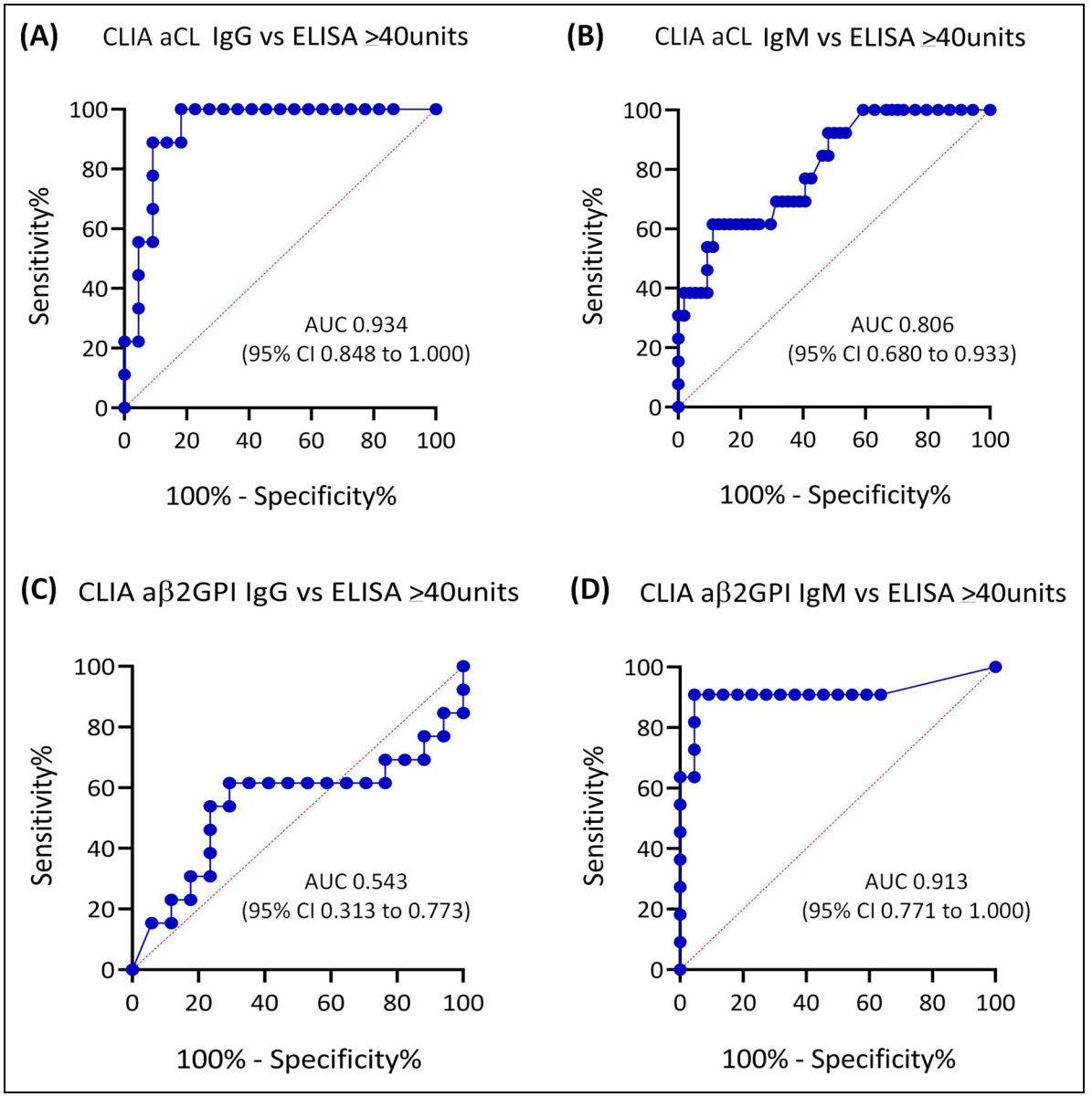

Figure 2. Receiver operating characteristics of CLIA levels for moderate/high ELISA titer of 40 units and above. Abbreviations: ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent assay; aCL, anticardiolipin antibody; aβ2GPI, β2-glycoprotein I antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; AUC, area under the curve; CI, confidence interval.

Table 2. Sensitivity and Specificity of CLIA for a Moderate/High Positive ELISA of ≥ 40 Units.

		CLIA cut-off ≥10 U/ml		CLIA cut-off ≥20 U/ml		CLIA cut-off ≥30 U/ml		CLIA cut-off ≥40 U/ml	
	n	Sen	Spe	Sen	Spe	Sen	Spe	Sen	Spe
aCL IgG	31	100%	45.5%	*100%*	*68.2%*	100%	77.3%	100%	81.8%
aCL IgM	67	61.5%	85.2%	*46.2%*	*90.7%*	38.5%	98.1%	30.8%	100%
aβ2GPI IgG	30	69.2%	23.5%	*46.2%*	*29.4%*	38.5%	29.4%	38.5%	35.3%
aβ2GPI IgM	33	90.9%	86.4%	*90.9%*	*95.5%*	72.7%	95.5%	63.6%	95.5%

Abbreviations: ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent assay; aCL, anticardiolipin antibody; aβ2GPI, β2-glycoprotein I antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; Sen, sensitivity; Spe, specificity. 20 U/ml is the manufacturer and laboratory cut-off for positivity. Italicized columns indicate sensitivity and specificity at manufacturer's positive cutoff.

Table 3. Association Between Antiphospholipid Syndrome Clinical Criteria and aPL Detected by ELISA or CLIA.

	aCL IgG			aCL IgM			aβ2GPI IgG			aβ2GPI IgM		
Total number with criteria	$n=20$			$n=46$			$n=24$			$n=25$		
Any APS clinical criterion present[#], no. (%)	**ELISA** + 8 (40.0%)	**CLIA** + 13 (65.0%)	***p*** 0.063	**ELISA** + 10 (21.7%)	**CLIA** + 10 (21.7%)	***p*** 1.000	**ELISA** + 11 (45.8%)	**CLIA** + 15 (62.5%)	***p*** 0.454	**ELISA** + 7 (28.0%)	**CLIA** + 7 (28.0%)	***p*** 1.000
Total number with criteria	$n=18$			$n=40$			$n=24$			$n=24$		
Any combined venous and/or arterial thrombosis present, no. (%)	**ELISA** + 8 (44.4%)	**CLIA** + 12 (66.7%)	***p*** 0.125	**ELISA** + 10 (25.0%)	**CLIA** + 9 (22.5%)	***p*** 1.000	**ELISA** + 11 (45.8%)	**CLIA** + 15 (62.5%)	***p*** 0.454	**ELISA** + 7 (29.2%)	**CLIA** + 7 (29.2%)	***p*** 1.000

[#]Any APS clinical criterion refers to presence of any venous and/or arterial thrombosis and/or obstetric complications according to revised Sapporo classification. Exact *p* value from related samples McNemar test is computed based on binomial distribution.
Abbreviations: aPL, antiphospholipid antibody; ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent assay; aCL, anticardiolipin antibody; aβ2GPI, β2-glycoprotein I antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; ELISA+, ELISA ≥40 units; CLIA+, CLIA ≥20 U/ml

percentile of normal and was used in the comparison with three established ELISA assays.[13] Therefore, we used the manufacturer's CLIA cut-off in this study. This underscores the importance of careful establishment of reference ranges as it can affect the diagnosis of APS.

Even though these was moderate quantitative correlation between ELISA and CLIA (*r* coefficients 0.6–0.8), this correlation was less than the coefficient of 0.95 typically required for goodness of fit in laboratory method comparison, and the degree of variability and non-linear relationship was evident on scatter plots. Therefore, ELISA units are not interchangeable with CLIA units. The thresholds of <40, 40–80 and >80 units for low, moderate and high positivity apply only for ELISA and should not be used interchangeably with CLIA. This conclusion is supported by published literature. For example, the review by Vandevelde and Devreese stated that there is limited numerical agreement between different assay methods and direct comparison of values across assays should not be made.[23] A study by Vandevelde et al demonstrated poor agreement between ELISA versus CLIA or multiplex flow immunoassay for classifying positive samples as low, moderate, or high positive. Specifically, there was no agreement observed for aβ2GPI IgG, weak agreement for aCL IgG and only moderate agreement for aCL IgM and aβ2GPI IgM. Use of standard reference calibration material produced slight improvement in agreement, yet none showed strong agreement.[24] All in, our results suggest that CLIA positivity relates well to moderate/high ELISA positivity, but apart from this, direct translation of antibody titers should not be made.

In terms of test result correlation with clinical manifestations, there were no observed or statistically significant differences between assays in the proportion of cases with criteria manifestations. Hence, CLIA is likely able to provide the same degree of clinical predictive value as ELISA in the diagnosis of APS. This conclusion is concordant with that of extant studies that reported CLIA being equivalent if not having better sensitivity for APS than ELISA. Bettacchioli et al reviewed eight studies in a meta-analysis comparing ELISA with CLIA and found that the odds ratio for clinical APS manifestations when aβ2GPI IgG was present by CLIA was higher than the odds ratio when present by ELISA (Odds ratio for CLIA of 16.4, 95% CI 3.1-34.9 vs odds ratio for ELISA of 8.8, 95% CI 2.9-27.9). The odds ratio for aβ2GPI IgM present by CLIA was also higher (Odds ratio for CLIA of 4.5, 95% CI 1.1-50.9 vs odds ratio for ELISA of 2.4, 95% CI 0.9-8.7).[25]

As the diagnosis of APS relies heavily on laboratory criteria, it is crucial that tests have high sensitivity and specificity for the disease. We performed a comprehensive review of the English language literature for studies evaluating CLIA versus ELISA for aPL detection and summarize these in Table 5A and B. These studies suggest that CLIA provides excellent analytical performance in terms of precision, reproducibility and linearity. In addition, these studies and ours demonstrate that CLIA has acceptable concordance and agreement with ELISA and provides strong clinical predictive value for APS. Some studies suggest that CLIA may have greater sensitivity than a number of ELISA platforms, particularly for aβ2GPI.[12,16–18,25,26] A compelling explanation is better exposure of cryptic epitopes that are required for binding of pathogenic aβ2GPI such as G40-R43 on domain I.

To further understand the basis for the discordance between aβ2GPI IgG CLIA and ELISA in our study, we reviewed the underpinnings of aPL. Pathogenic aPL target

Table 4. Clinical Details of Patients with Anti-β2 Glycoprotein-I IgG Detected by ELISA or CLIA.

Table 4(A): Patients with positive ELISA aβ2GPI IgG but negative CLIA aβ2GPI IgG

Patient	Age (years)	Gender	ELISA (units)	ELISA Other* (units)	CLIA (U/mL)	Lupus Anticoagulant^	Other positive aPL (ELISA and/or CLIA)#	Event(s) leading to screening	Sapporo Criteria Events on 1-year follow up
1	47	Male	54.0	62.9 (After) Positive	10.9	Not Detected	NIL	I) CVA	Started on aspirin (declined warfarin). No further events.
2	52	Female	34.7	43.5 (Prior) Positive	9.8	Not Detected	NIL	I) CVA	Started on aspirin. Developed AMI (NSTEMI) and subsequently passed on.
3	59	Male	60.3	NIL	7.8	Not Detected	NIL	I) CVA	No follow up information available
4	76	Male	51.1	NIL	11.5	Not performed	• aCL IgM • aβ2GPI IgM	(I)) Provoked pulmonary embolism (II)) Left ventricular thrombosis on background of ischemic cardiomyopathy	Started on warfarin. Developed AMI (NSTEMI).
5	40	Male	88.3	115.7 (After) Positive	<6.4	Not Detected	NIL	(I)) CVA (II)) CVA	Started on warfarin. No further events.
6	66	Female	25.8	NIL	<6.4	Not Detected	NIL	I) Recurrent dialysis access failure	No further events.
7	12	Female	101.9	91.7 (After) Positive	<6.4	Not Detected	NIL	I) Underlying SLE	No further events.
8	51	Female	120.6	>150 (After) Positive	10.1	Not Detected	NIL	I) CVA	No further events.
9	84	Female	59.4	NIL	8.5	Not Detected	NIL	I) Sagittal sinus thrombosis	Kept on apixaban. No further events.
10	20	Female	22.9	32.8 (After) Positive	8.2	Not Detected	NIL	I) Underlying SLE	No further events.

Table 4(B): Patients with positive CLIA aβ2GPI IgG but negative ELISA aβ2GPI IgG.

Patient	Age (years)	Gender	ELISA (units)	ELISA Other* (units)	CLIA (U/mL)	Lupus Anticoagulant^	Other positive aPL (ELISA and/or CLIA)#	Event(s) leading to screening	Sapporo Criteria Events on 1-year follow up
1	22	Male	30.8	71.2 (After) Positive	951.4	Present	• aCL IgG	I) AMI	Started on warfarin, no further events.
2	47	Female	31.6	47.1 (Prior) Positive	1102.4	Not detected	• aCL IgG	I) CVA	Started on warfarin, no further events.
3	21	Female	9.4	NIL	295.8	Present	• aCL IgM • aCL IgG • aβ2GPI IgM	I) Unprovoked PE and DVT	Started on warfarin, no further events.
4	68	Female	23.6	59.6 (After) Positive	3308.6	Present	• aCL IgG	I) Unprovoked DVT	Started on warfarin, no further events.
5	51	Male	28.6	52.0 (After) Positive	602.6	Not detected	• aCL IgG	I) Provoked DVT	Started on apixaban, no further events.
6	38	Male	0.2	NIL	26.1	Not performed	NIL	I) Provoked DVT	Completed 3 months of anti-coagulation. No further events.
7	42	Male	30.1	<9.4 (After)	66.0	Not detected	• aCL IgM • aCL IgG • aβ2GPI IgM	I) Cerebral vein thrombosis provoked by varicella infection	No events after 6 months of anticoagulation.
8	19	Female	22.1	23.0 (After) Positive	61.0	Present	• aCL IgM • aβ2GPI IgM	I) SLE with popliteal artery thrombosis	Already on warfarin, no further events.
9	80	Female	35.3	NIL	737.8	Not performed	• aCL IgM • aCL IgG • aβ2GPI IgM	I) Underlying SLE	On warfarin for pAF. No further events.
10	35	Male	11.4	NIL	132.6	Present	• aCL IgM	I) SLE with right anterior tibial artery stenosis	Started on warfarin. No further events.
11	18	Female	3.8	9.6 (Prior)	201.7	Present	• aCL IgM • aCL IgG • aβ2GPI IgM	I) Underlying SLE	On aspirin. No further events.
12	49	Male	20.6	NIL	651.4	Not Detected	• aCL IgG	I) Recurrent TIA	Started on antiplatelets for TIA. No further events.

Abbreviations: ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent assay; aCL, anticardiolipin antibody; aβ2GPI, β2-glycoprotein I antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; CVA, cerebrovascular accident; AMI, Acute myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; PE, pulmonary embolism; DVT, deep vein thrombosis; SLE, systemic lupus erythematosus; pAF, paroxysmal atrial fibrillation; TIA, transient ischemic attack

* aβ2GPI IgG by ELISA performed at another setting

#aβ2GPI IgM, aCL IgG or aCL IgM by ELISA performed at the same setting

^ performed at the same setting

Table 5. Summary of Studies Evaluating Antiphospholipid Antibody Testing by CLIA Compared with ELISA.

Table 5(A): Laboratory method comparison

Paper (Year)	Comparison	*n*	Qualitative agreement	Quantitative agreement
Zhang, et al (2015)[12]	CLIA (QUANTA Flash) versus ELISA (QUANTA Lite)	227	Good overall agreement for IgG/IgM/IgA aCL and IgM/IgA aβ2GPI (>90%) and moderate overall agreement for IgG aβ2GPI (76.7%)	Significant quantitative correlations between ELISA and CLIA assays in IgG/IgM/IgA aCL and IgG/IgM/IgA aβ2GPI ($P < 0.001$)
De Moerloose, et al (2010)[13]	CLIA (AcuStar) versus 3 commercial ELISA assays (QUANTA Lite, REAADS and VARELISA)	321	CLIA showed high agreement with all 3 ELISA kits (82%–96%). Kappa coefficient (0.44-0.82), with highest agreement with QUANTA Lite.	Correlation slopes of 0.9–1.1 and *r* coefficient >0.997.
Van Hoecke, et al (2012)[15]	CLIA (Zenit RA and Acustar) versus ELISA (Bindazyme)	124	Good total agreement for all assays ranging from between 93.5% and 97.6%.	N/A
Wan, et al (2020)[18]	CLIA (QUANTA Flash) versus ELISA (QUANTA Lite)	505	Good total agreement between ELISA and CLIA (>80%). In the APS population, total agreement using manufacturer's cut-off values was good. Kappa coefficients for the four antibodies ranged from moderate to substantial (0.61-0.75).	aβ2GPI and aCL IgG assays showed the highest Spearman's rho coefficients (aβ2GPI IgG = 0.742, aCL IgG = 0.715).
Iwaniec, et al (2015)[27]	CLIA (QUANTA Flash) versus ELISA (QUANTA Lite)	220	Good qualitative agreement ranging 81.8% to 90.9%. Kappa coefficient ranged from 0.56 to 0.81.	Quantitative results showed strong, significant correlation between methods. Spearman's rho ranging from 0.75 to 0.83 ($p < 0.0001$).
Noubouossie, et al (2014)[28]	Detection of aCL IgG using CLIA (Liaison) versus ELISA assay (REAADS)	87	Moderate overall agreement of 56.3%, positive agreement 49.3%, negative agreement 77.2%. When CLIA was compared to repeated ELISA, the agreement improved to good agreement (overall agreement 82.1%). Most discordant cases were positive with ELISA and negative with CLIA.	N/A
Persijn, et al (2011)[19]	CLIA (Zenit RA) versus ELISA (Bindazyme) using manufacturer's and in-house cut off values	314	Overall agreement using manufacturer's cut-offs was high for aCL and aβ2GPI IgG and IgM (93-96%). Kappa values was moderate, ranging from 0.48–0.56. Overall agreement was low when using in-house cut-offs for both IgG (60-76%), with Kappa values of 0.14–0.52. In the APS population, there was substantial agreement in aCL and fair to moderate agreement in aβ2GPI.	N/A
Li, et al (2015)[14]	CLIA (AcuStar) versus ELISA (QUANTA Lite)	204	Agreement was comparable between in-house and manufacturer's cut-offs. Total agreement ranged from 82.6% to 95.7%. Kappa coefficient ranging from fair to good while using both in-house and manufacturer's cut-offs (0.36-0.65). Overall agreement between AcuStar and ELISA was better for aCL and aβ2GPI IgG than IgM.	N/A

(continued)

Table 5. Continued.

Table 5(A): Laboratory method comparison

Paper (Year)	Comparison	*n*	Qualitative agreement	Quantitative agreement
Janek, et al (2016)[16]	CLIA (AcuStar) versus ELISA (AIDA)	122	Excluding aβ2GPI (Kappa 0.21), there is moderate agreement between CLIA and ELISA methods using manufacturer cut-off (Kappa values ranging 0.53 - 0.94). Increasing the CLIA cut-offs increased agreement for all parameters.	N/A

Table 5(B): Correlation with clinical manifestations of antiphospholipid syndrome

Paper (Year)	Comparison	*n*	Clinical Correlation
Zhang, et al (2015)[12]	CLIA (QUANTA Flash) versus ELISA (QUANTA Lite)	227	aβ2GPI IgG detection by CLIA demonstrated the highest sensitivity for APS, while aβ2GPI IgG ELISA had the lowest sensitivity. Both CLIA and ELISA demonstrated high specificity (>90%) and odds ratio for predicting APS. aβ2GPI IgG CLIA was able to best predict thrombotic events in patients with APS.
De Moerloose, et al (2010)[13]	CLIA (AcuStar) versus 3 commercial ELISA assays (QUANTA Lite, REAADS and VARELISA)	321	All 4 assays had comparable sensitivity, specificity, and agreement for predicting APS diagnosis. All 4 assays had comparable odds ratio for predicting thrombotic and obstetric events.
Van Hoecke, et al (2012)[15]	CLIA (Zenit RA and Acustar) versus ELISA (Bindazyme)	124	CLIA aCL IgG had comparable sensitivity for the diagnosis of APS, but sensitivity was significantly lower for other antibodies. Specificity was high (> 96%) for all assays without significant differences.
Wan, et al (2020)[18]	CLIA (QUANTA Flash) versus ELISA (QUANTA Lite)	505	CLIA had higher sensitivity for the diagnosis of APS for aCL IgG and aβ2GPI IgG. Sensitivity was comparable for aCL IgM and higher in ELISA for aβ2GPI IgM. Both methods had high specificity (91%–99%) and comparable odds ratio for predicting APS. By ROC, aβ2GPI IgG CLIA had the best discriminating power with the area under the curve of 0.92.
Iwaniec, et al (2015)[27]	CLIA (QUANTA Flash) versus ELISA (QUANTA Lite)	220	When single antibody positivity was considered, both methods for aCL IgG and aβ2GPI IgM showed comparable sensitivity for predicting thrombosis and/or obstetric complications, but slightly lower sensitivity of aCL IgM and higher sensitivity of aβ2GPI IgG CLIA. By ROC analysis, both methods showed good AUC of 0.7–0.8. Discrepancies mostly occurred at low antibody range. Both assays identified almost identical number of patients with high-risk profile.
Noubouossie, et al (2014)[28]	Detection of aCL IgG using CLIA (Liaison) versus ELISA assay (REAADS)	87	CLIA showed lower sensitivity (46.4% vs 71.4%), higher specificity (72.3% vs 29.7%) and higher likelihood ratio (1.67 vs 1.01) as compared to first ELISA. When compared to repeated ELISA test, sensitivity (66.6%), specificity (62.5%) and likelihood ratio (1.77) for APS were similar for CLIA and ELISA.
Persijn, et al (2011)[19]	CLIA (Zenit RA) versus ELISA (Bindazyme) using manufacturer's and in-house cut off values	314	When taken in isolation, the sensitivity of each antibody for APS was low regardless of the method. However, specificity was high (>90%) for APS and comparable for aCL and aβ2GPI using ELISA or CLIA.
Hu, et al (2021)[26]	2 CLIA systems (iFlash, QUANTA Flash) versus 2 ELISA (QUANTA Lite, AESKULISA) assays	303	For diagnosing APS and thrombosis, the accuracy of aCL and aβ2GPI IgG or IgM on one CLIA system was statistically significantly better than the other CLIA and ELISA ($p < 0.05$).

Abbreviations: ELISA, enzyme linked immunosorbent assay; CLIA, chemiluminescent assay; aCL, anticardiolipin antibody; aβ2GPI, β2-glycoprotein I antibody; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; APS, antiphospholipid syndrome; ROC, receiver operating characteristics

anionic phospholipids via interaction with phospholipid binding proteins, specifically β2GPI, whereas incidental aPL, found in 1%–5% of asymptomatic individuals, bind directly to neutral and anionic phospholipids.[4,6] β2GPI is a serum protein that comprises five domains (I – V). The N-terminal domain (Domain I) has been identified as the major binding site of pathogenic aβ2GPI.[29–31] A conformational change in β2GPI is necessary for aPL to recognize

domain I. This conformational change is induced by β2GPI binding to a negatively charged anionic surface. When this binding occurs, the cryptic epitope at position 40–43 (G40-R43) in domain I is exposed, allowing aβ2GPI exert its effect on coagulation.[30–35] Dimerization of β2GPI induced by antibody binding also increases the affinity towards interaction with anionic phospholipids.[36] In the aCL solid phase assay, cardiolipin is used as the binding surface for β2GPI and therefore the assay is effectively an assay for aβ2GPI. As the affinity of β2GPI to cardiolipin is high, dimerization of β2GPI is not essential and only the exposure of the cryptic epitope is essential in this assay.[37]

If ELISA plates are unable to induce the conformation change required to expose the cryptic epitope, or have low affinity for β2GPI, the consequence could be an inability to detect aβ2GPI.[33,38,39] Pelkmans et al, using a monoclonal antibody that reacts specifically with G40-R43 in the open conformation and another that reacts with β2GPI irrespective of conformation, showed that five different commercial aβ2GPI IgG ELISA assays differed considerably in the detection of anti-G40-R43 antibodies due to decreased exposure of the epitope.[40] The source of β2GPI may also affect the ability to bind aβ2GPI, as recombinant β2GPI shows better binding than plasma purified β2GPI due to differences in glycosylation.[33]

Domain I specific aβ2GPI is believed to be largely responsible for the clinical complications of thrombosis and late pregnancy morbidity in APS, while aβ2GPI that recognizes other domains (domains IV/V) are thought to be non-pathogenic.[41–46] Andreoli et al showed that asymptomatic aPL carriers displayed higher frequency of anti-domain IV/V, while anti-domain I antibodies were more frequent and present at higher titers in triple aPL positive APS.[45] In the study by de Laat et al, of patients with antibodies to whole β2GPI, 55% had anti-domain I antibodies which was associated with thrombosis (odds ratio 3.5, 95% CI 2.3-5.4) and obstetric complications (odds ratio 2.4, 95% CI 1.4-4.3).[46] Transient and non-pathogenic aβ2GPI are described in a multitude of infections but the majority do not cause thrombosis or APS.[47] The differences in the ability to detect pathogenic versus non-pathogenic aPL is likely to explain discrepancies in sensitivity and specificity and the clinical predictive value of each method.

Among ELISA assays, imperfect agreement exists between ELISA kits from different manufacturers, particularly in low and medium positive samples.[48] A study from the APS ACTION International Clinical Database found agreement in only 80%–90% when aPL was measured by ELISA at core versus non-core laboratories, with a small number of high titer samples showing discordance.[49] Hence, slight inter-laboratory variation is unavoidable even with modern day assay design and use of reference calibrators, and can be attributed to differences in assay components such as microtiter plates, phospholipids, blocking agents and source of β2GPI.[39] As a result, the ISTH recommends validation of new assays not only on analytical criteria but also on clinical criteria, ie association between assay results and clinical complications, whenever feasible.[22]

Therefore, we hypothesize that a major contributory factor to the discordance between CLIA and ELISA for aβ2GPI IgG is the different configuration of the β2GPI molecule. The ACL AcuStar® CLIA contains the open configuration of β2GPI molecule thereby more efficiently detects pathogenic domain I antibodies, whereas the ELISA has the β2GPI molecule in close configuration (personal communication from manufacturers). We note a study by Chayoua et al that supports this. Using two sets of monoclonal antibodies that either recognize β2GPI G40-R43 in domain I or β2GPI irrespective of conformation, they showed that ACL AcuStar® CLIA and other solid phase assays demonstrated variable sensitivity to different antibody concentrations. ACL AcuStar® CLIA detected both types of monoclonal antibodies at much lower concentrations compared with QUANTA Lite® ELISA.[50] Clinically, the patients in our study who were aβ2GPI IgG CLIA positive were more likely to have had thrombosis than those who were ELISA positive (62.5% vs 45.8%), and more likely to be lupus anticoagulant positive, double or triple aPL positive and have co-existing autoimmune disease. The ability to detect more patients with aβ2GPI has significant implications for the diagnostic accuracy of APS.

Nonetheless, due to incomplete concordance between ELISA and CLIA, there is a possibility that APS may be classified differently in 10%–20% of cases using different platforms. Although higher sensitivity means that CLIA may detect more clinically relevant antibodies, it can also lead to more false positives leading to overdiagnosis. Fortunately, the impact of a single false negative or false positive test result will be lessened given that lupus anticoagulant and other aPL are often performed together, and persistence of aPL has to be demonstrated to diagnose APS.[50] Discrepancies between assays are more likely to be of low/medium titers, whereas higher titer aPL tend to be concordant across platforms.[50] This is within the context that isolated low titer aPL is increasingly recognized to carry less clinical significance, in contrast to the clinical importance of high titer aPL, lupus anticoagulant positivity or triple aPL positivity.[1,51] Furthermore, Iwaniec et al found that the ability to identify triple aPL positive patients was similar irrespective of the solid phase assay platform used, and that APS diagnosis was concordant when the full aPL profile, instead of individual antibodies was assessed.[52]

The main limitations of our study are the modest number of cases in the cohort, and the retrospective nature of the data collection for clinical outcomes which may mean missing or incomplete data. We used the revised

Sapporo APS classification criteria to identify clinical manifestations but acknowledge that future studies could evaluate clinical manifestations defined using the 2023 ACR/EULAR updated classification criteria. The revised Sapporo APS clinical criteria for thrombosis did not distinguish between thrombosis with or without provoking risk factors, and hence provoked and unprovoked thrombosis were considered together in our study. The third limitation is the single time point of evaluation which can overestimate the clinical significance of a positive result. Finally, this study evaluates only one ELISA system and one CLIA system and may not be fully applicable to other comparators.

Conclusions

Detection of antiphospholipid antibodies by CLIA shows a close but incomplete concordance with ELISA. Nevertheless, CLIA can detect clinically relevant aPL and may even be more sensitive than some ELISA platforms for APS, depending on the ability to detect antibodies against relevant epitopes on the β2GPI molecule. CLIA positivity on the ACL AcuStar® platform correlated well with moderate/high titer on ELISA, but apart from this, antibody titers should not be directly compared across systems. CLIA is an acceptable alternative to ELISA in the routine non-research setting for the diagnosis of APS.

Abbreviations

aPL	Antiphospholipid antibodies
aβ2GPI	β2GPI antibody
aCL	Anti-cardiolipin antibody
APS	Antiphospholipid syndrome
CI	Confidence interval
CLIA	chemiluminescent assay
ELISA	enzyme-linked immunosorbent assay
IgM	Immunoglobulin M
IgG	Immunoglobulin G
ROC	Receiver operating characteristics

Abstract Presentation

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Author Contributions

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Data Availability

Data sets used in the study are available from the corresponding author on reasonable request

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Approval

This study involves human participants and was conducted in accordance with the Declaration of Helsinki. The Domain Specific Review Board of the National Healthcare Group approved this study and granted exemption from informed consent for this retrospective study (DSRB number 2023/00711).

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