THE COMPARISON OF REAL WORLD AND CORE LABORATORY ANTIPHOSPHOLIPID ANTIBODY ELISA RESULTS FROM ANTIPHOSPHOLIPID SYNDROME ALLIANCE FOR CLINICAL TRIALS & INTERNATIONAL NETWORKING (APS ACTION) CLINICAL DATABASE AND REPOSITORY ANALYSIS

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Keywords: anticardiolipin; anti-b2glycoprotein I; antiphospholipid antibodies, antiphospholipid syndrome, miscarriages, reproducibility; thrombosis,

Abstract

Background: The APS ACTION International Clinical Database and Repository includes a secure web-based data capture system storing patient information including demographics, antiphospholipid antibodies (aPL)-related medical history, and aPL tests. Despite efforts at harmonization, inter-assay variability remains a problem in aPL testing. As a clinical repository open to researchers, ensuring comparability between assays and consistency in results between APS ACTION laboratories is essential to the validity of studies emerging from this network.

Objective: To assess the level of agreement between an aPL-registry inclusion and core laboratory (core lab) anticardiolipin antibody (aCL) and anti-β_2-glycoprotein-I antibody (aβ_2GPI) ELISA testing results.

Methods: Patients are recruited from 25 international centers based on positive aPL tests at inclusion. All samples are retested at the corresponding national APS ACTION core lab to confirm aPL positivity based on standard validated protocols. We analysed the categorical agreement, degree of linear association, and correlation between inclusion (local laboratory) and core lab aPL tests. Samples were included in this study only if results of aPL testing with ELISA at baseline were available.

Results: 497 registry samples underwent confirmatory aPL tests. Categorical agreement between the inclusion and core lab values, as expressed by Cohen's kappa coefficients, ranged between 0.61 and 0.80 (as substantial agreement). The correlation between quantitative results in the aCL and aβ_2GPI was better for IgM and IgA compared to IgG (Spearman rho 0.789 and 0.666 vs. 0.600 for aCL and rho 0.892 and 0.744 vs. 0.432 for aβ_2GPI).

Conclusions: The results of inclusion for aCL and aβ_2GPI tests used for recruitment into the registry were in agreement to the results obtained by the APS ACTION core laboratories; aCL and aβ_2GPI results showed very good categorical agreement. This agreement increased when considering high titer (>40 units) samples. APS ACTION is a reliable and useful research resource for APS.

Introduction

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis and/or pregnancy morbidity, associated with persistent positivity for antiphospholipid antibodies (aPL). The AntiPhospholipid Syndrome Alliance For Clinical Trials and InternatiOnal Networking (APS ACTION) is an international research network that has been created specifically to design and conduct well-designed, large-scale, multicenter studies in persistently aPL-positive patients [1,2]. One of the major projects of APS ACTION is an international clinical database and repository ("registry"), which was created to study the natural course of disease at least over 10 years in persistently aPL-positive patients with/without other systemic autoimmune diseases. As part of the registry, blood samples are collected at inclusion and annually for aPL confirmation and future basic science studies. Five APS ACTION core laboratories (core lab), divided by region and located in Italy, Brazil, USA, Australia and UK, perform confirmatory aPL testing based on standard validated protocols.

Since clinical features of APS (thrombosis and pregnancy morbidity) are common in the general population and often related to other underlying factors, the diagnosis of APS relies on the detection of persistently positive aPL. Thus, reliable laboratory tests for aPL with good clinical performance and reproducibility are required. There is a large variety of solid phase assays available to assess aPL, but harmonization of assays is not optimal [3-5].

Given the importance of ensuring comparability between assays and uniformity in results in order to improve the interpretability of laboratory test for clinical trials and research studies, our objective was to assess the level of agreement between registry inclusion (local laboratory testing) and confirmatory core lab anticardiolipin antibody (aCL) and anti-$\beta_2$glycoprotein-I antibody (aβ_2GPI) enzyme-linked immunosorbent assay (ELISA) results.

Methods

APS ACTION Network and Registry

The network consists of 50 physician-scientists from 25 international centers[6,7,8].

APS ACTION registry inclusion criteria are moderate-to-high titer aCL and/or aβ_2GPI and/or positive lupus anticoagulant (LA) test based on the International Society on Thrombosis and Haemostasis (ISTH) recommendations[9], tested at least twice 12 weeks apart within one year prior to enrolment. Patients are followed up annually with clinical data and blood collection. A secure web-based data capture system (REDCap) is used to store patient information including demographics, clinical manifestations, and aPL data [10].

Study Design

Samples were collected between 2013 and 2016 and stored at -80°C at the core laboratories facilities. Five APS ACTION core laboratories have been set up worldwide in Sao Paulo (Brazil, South America), Sydney (Australia), Galveston (Texas, USA), Padova (Italy, Europe), and London (UK). These core laboratories are fully functional and routinely perform internal and external validation exercises as described elsewhere[6,7,8]

Inclusion samples from registry patients were tested for IgG, IgM and IgA aCL and aβ_2GPI using the QUANTA Lite® aCL and aβ_2GPI ELISA kits (Inova Diagnostics) at the regional APS ACTION core laboratory. The IgG, IgM and IgA aCL assays were reported in GPL, MPL and APL units respectively, while aβ_2GPI assays were reported in SGU, SMU and SAU units. All testing was done according to manufacturer instructions. Samples were included in this study only if results of aPL testing with ELISA at baseline were available.

Each center belonging to the APS Action Network has obtained approval by their local Ethical Committee. Every patient agreeing to participate from this registry has provided written consent.

Statistical Analysis

Agreement of categorical positive and negative aPL test results was assessed using κ-coefficients (<0.20, poor; 0.21–0.40 fair; 0.41–0.60 moderate; 0.61–0.80 good; 0.81–1.00 very good), as previously reported [11]. The degree of linear association between inclusion and core lab testing was quantified by the Spearman's correlation method. Two different cut off values were selected to perform the categorical analyses; the manufacturer cut-off at 20 corresponding units and the Sydney criteria moderate titer cut-off value at 40 corresponding units [12,13]. To assess further the agreement between inclusion and core lab measurements, the mean difference was obtained and the limits of agreement were calculated as the mean difference ± 2 standard deviations (SD) [14]. Systematic difference between the results obtained from inclusion vs. core lab values (for categorical agreement) was assessed by McNemar's test [15]. The Bland–Altman plot (difference plot) was also used for data plotting to assess the agreement between inclusion and core lab values. The 95% confidence intervals (CI) were calculated as appropriate. P-values <0.05 were considered statistically significant. All statistical analysis was performed using SPSS 19.0 (IBM Software, NY).

Results

Of 573 patients recruited for the APS ACTION repository as of June 2015, 497 (87%) were tested at the regional APS ACTION core laboratory. Table 1 shows the concordance of aPL test results between inclusion and core lab values (κ-coefficients ranging from 0.61 and 0.80, mean 0.70, as evidence of substantial agreement). Overall, when using cut off values of 20/40 units, the level of agreement between dichotomized results for both aCL and aβ_2GPI was higher for IgM and IgA, compared to IgG (aCL agreement: 82%/82% MPL and 79%/85% APL vs. 84%/91% GPL, respectively; aβ_2GPI agreement 80%/82% SMU and 86%/89% SAU vs. 82%/86% SGU, respectively). When using the 99th percentile cut-off values for anti-ß2GPI IgG and IgM assays, the level of agreement between dichotomized results was 285/349 (81.7%) and 340/389 (87.4),

respectively, with κ-coefficients of 0.77 and 0.78, as evidence of substantial agreement and almost identical of those observed using 20 GPL/MPL.

The categorical agreement for titers between inclusion and core lab results is detailed in Table 2.

No systematic difference between the proportion of ‘‘positive’’ responses from the inclusion and core lab testing was found by McNemar’s test. Only a small minority of samples with high inclusion (local laboratory) aCL titers (>80 GPL/MPL/APL) tested negative at core lab (2.2%, 0.2%, 1.8% and 4.4%, 0.9%, 1.8% when using 20 and 40 GPL/MPL/APL as cut off values, respectively). Similarly, among patients with high IgG aβ_2GPI titers at inclusion >80 SGU, only 2.5% and 4.5% showed a negative core lab result when using 20 and 40 SGU as cut off values, respectively. One patient with a high IgM aβ_2GPI titer tested negative in the core lab. All patients positive in the local laboratory for IgA aβ_2GPI >80 APL also tested positive at the core lab.

Quantitative analysis showed a good correlation between inclusion and core lab IgG aCL (R= 0.600, p=0.041), with a mean difference of 6.8±58.3 GPL (Figure 1A). A strong correlation was also found for IgM aCL (R=0.789, p=0.032; Figure 1B) and IgA aCL (R=0.666, p=0.039). The mean difference was 12.9±41.3 MPL for IgM and 8.3±28.2 APL for IgA.

A moderate correlation between inclusion and core lab was found for IgG aβ_2GPI (R= 0.432, p=0.057), with a mean difference of 7.9±84.5 SGU (Figure 1C). The correlations were stronger for IgM aβ_2GPI (R= 0.744, p=0.029; Figure 1D) and IgA aβ_2GPI (R= 0.829, p=0.031), with mean differences of 16.9±59.4 SMU and 7.9±31.1 SAU, respectively. The Bland-Altman plots (Figure 1) showed mean differences between the two measurements as high as 7 GPL/SGU for IgG, both aCL and aβ_2GPI. Mean differences between the two measurements were 13 MPL and 17 SMU for IgM aCL and aβ_2GPI, respectively. For both IgG and IgM, the error between inclusion values and core laboratory increased throughout the range of measurements.

Discussion

Our study shows that the categorical agreement (positive and negative) of aPL results obtained in local laboratories at inclusion, ranged from 80% to 90% when compared to results obtained by five APS ACTION core laboratories. Importantly, when using moderate-to-high titers of antibodies (>40 units) as cut off value, the level of agreement increased from 82% to 87% compared to lower positive titers (in the range of 20 to 40 units).

When analysing different isotypes, the correlation between quantitative results for aCL and aβ_2GPI was better for IgM and IgA, compared to IgG. One could speculate that although the strength of the correlation for IgG might suffer from some discordance for the medium-to-high titer samples, however these findings might not have an impact on the qualitative comparison between inclusion (local laboratory) values and core laboratory.

In patients presenting with thrombosis or pregnancy morbidity, a positive aPL test is the major determinant as to whether the patient is diagnosed with APS. Similarly, risk stratification based on aPL profile is critical for optimal management [16].

In this study, we have identified a small group of discordant test results. These observations are in line with previous work assessing the inter-laboratory level of agreement for aPL [17,18] or for other immuno-enzymatic assay testing [19-21]. In particular, a very limited number of samples, having high titers of aPL at inclusion were not identified as positive by the core lab testing, discrepancies not rarely seen in routine laboratory practise. Our findings are in line with a recent observation by Willis *et al*. showing that although qualitative agreements between immunoassays for both aCL and aβ_2GPI are acceptable, some kit dependent variations in term of titers might be observed [22].

As shown by the Bland-Altman plots, the measurement error between inclusion and core lab values increased throughout the range of measurements and its amplitude varied from one parameter to another (being the lowest for IgG aCL and aβ_2GPI and the highest for IgM aβ_2GPI). Nevertheless, it is important to highlight that the diagnosis of APS should not be based on one particular aCL or aβ_2GPI determination but rather on the serological history of the patients, which also includes LA

testing, as criteria for APS. In this respect, our data reinforce the need of using the full panel of criteria tests (i.e., aCL, LA, and aβ_2GPI) when suspecting APS.

Overall, the observation that local laboratories aPL testing results are consistent with those detected in core laboratories allows some considerations. The overall good level of agreement supports that using local laboratories positive aPL test results as inclusion criteria for the APS ACTION Registry is a reliable and reproducible approach. To be a useful resource, data in a medical registry must be of good quality. Our analysis in this study ascertains the validity and the reliability of the data registry. Most importantly, the level of agreement we observed reflects the efforts over the last decade to improve aPL testing worldwide in terms of reproducibility and harmonization.

Of note, IgA aPL testing, not currently recommended in the classification criteria, is undertaken in a limited number of laboratories and often performed as a home-made ("in-house") assay for both aCL and aβ_2GPI [23-25]. The use of these in-house assays bring other variables into the equation, such as coating conditions, buffers, and plastic materials, all contributing to a higher inter-assay (comparability) variability. Efforts to improve reproducibility and harmonization of IgA aPL testing are ongoing.

Our analysis presents some limitations. Firstly, the discrepancy due to intra-assay variation could not be assessed as per the study design. Secondly, it was outwith the scope of this study to compare the clinical accuracy of inclusion and core laboratory aPL testing for clinical manifestations. Thirdly, we focused our analysis on solid assays testing only and did not evaluate LA results or assay performance, which is part of a parallel ongoing study. Besides, as the extent of agreement between the baseline and core lab results increase with the magnitude of the measurement, we cannot exclude a funnel effect, at least in part attributable to the heterogeneity in the methods used for the inclusion testing (e.g. different full scale value). In fact, details about different methods used in local laboratories to test for aPL (e.g. type of immunoassay, commercial kit Vs. home-made assay) were not included among mandatory information for each patient to be entered into the registry, limiting the possibility to perform further analyses on this aspect.

Similarly, the cross-sectional design of this study does not allow any consideration on the persistence of aPL testing results over the follow-up time. However, the strength of our study lies in the large number of samples, which cover the entire range of aPL titers and isotypes, and refer to a patient population that represents both the clinical disease and the aPL profile spectrum in APS. Finally, in this study, aCL and aβ2GPI testing was performed by only one method at the APS ACTION core laboratories. Due to lack of recognized gold standard in aPL testing, further validation exercises investigating the use of different methods (e.g. chemiluminescence) are currently ongoing.

In conclusion, our findings confirm that the use of local aCL and aβ_2GPI results as inclusion data for the APS ACTION "Registry" is a solid and reproducible approach. In addition, our findings support the fact that aPL testing, at least when performed by ELISA, has now improved in term of reproducibility and harmonization, with a very low level of disagreement comparable to other ELISA tests routinely used in the field of autoimmunity [21,26]. Nevertheless, the increasing number of available techniques for aPL testing other than ELISA might still introduce new challenges to the harmonization and the comparability of autoantibody results. Systematic assessment of reference materials is currently underway, and preliminary results appear to be encouraging[27].

Acknowledgment

The authors would like to thank Joann Vega, CCRC, for her research/administrative assistance including patient recruitment and data monitoring. INOVA Diagnostics unrestrictedly provided all antiphospholipid antibody kits to APS ACTION core laboratories but had no input in the study and/or analysis of the data or results. Data management was performed using REDCAP provided by the Clinical and Translational Science Center at Weill Cornell Medicine (CTSC grant UL1 TR000457).

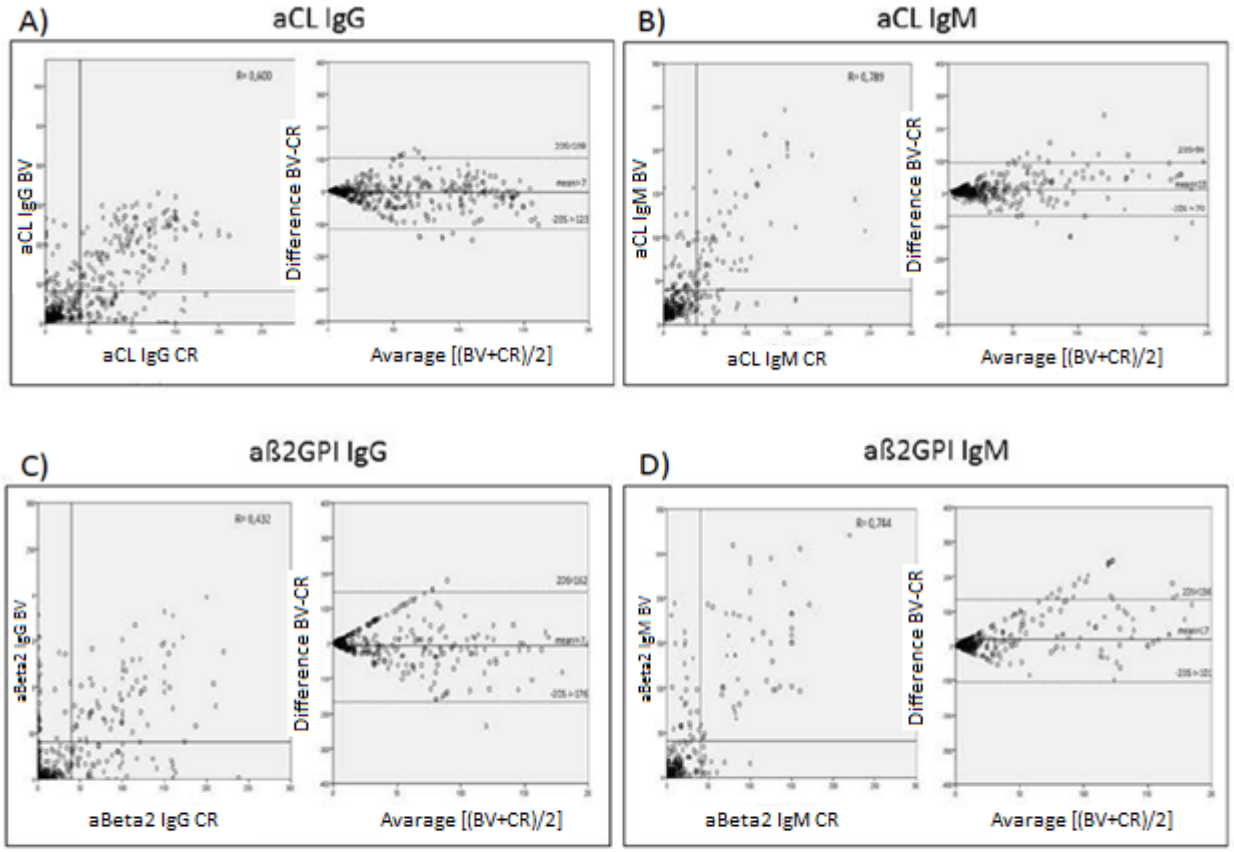
Table 1: Categorical agreement between inclusion and core laboratory anticardiolipin antibody (aCL) and anti-β_2-glycoprotein-I antibody (aβ_2GPI) results

		Cut off >40 Inclusion/Core lab	K coefficient	Cut off >20 Inclusion/core lab	K coefficient
aCL	IgG	368/450 (81.7%)	0.649	367/450 (81.6%)	0.644
	IgM	381/447 (85.2%)	0.779	354/447 (79.2%)	0.618
	IgA	99/109 (90.8%)	0.797	91/109 (83.5%)	0.765
aβ_2GPI	IgG	287/349 (82.2%)	0.773	278/349 (79.7%)	0.621
	IgM	346/389 (88.9%)	0.783	335/389 (86.1%)	0.781
	IgA	108/122 (88.5%)	0.782	100/122 (82.0%)	0.771

Table 2: Categorical Agreement for Titers Between inclusion and core laboratory anticardiolipin antibody (aCL) and anti-β_2-glycoprotein-I Antibody (aβ_2GPI) Results

Core laboratory	Inclusion			
aCL IgG	**0-19**	**20-39**	**40-79**	**> 80**
0-19	171	24	17	10
20-39	18	14	19	10
40-79	10	11	24	15
> 80	4	1	20	82
aCL IgM	**0-19**	**20-39**	**40-19**	**> 80**
0-19	232	20	8	1
20-39	43	22	14	3
40-79	15	13	18	7
> 80	6	6	24	15
aCL IgA	**0-19**	**20-39**	**40-19**	**> 80**
0-19	82	2	3	2
20-39	7	1	1	0
40-79	3	0	0	0
> 80	1	0	1	6
aβ_2GPI IgG	**0-19**	**20-39**	**40-19**	**> 80**
0-19	176	18	18	9
20-39	13	6	10	7
40-79	5	2	19	13
> 80	8	3	6	36
aβ_2GPI IgM	**0-19**	**20-39**	**40-19**	**> 80**
0-19	263	8	11	1
20-39	16	6	3	0
40-79	6	9	5	3
> 80	12	1	10	35
aβ_2GPI IgA	**0-19**	**20-39**	**40-19**	**> 80**
0-19	71	3	2	0
20-39	9	3	2	0
40-79	5	1	7	2
> 80	3	1	3	10

<u>Figure 1:</u> Correlation and Bland–Altman Plots for inclusion and core laboratory Anticardiolipin Antibody (aCL) and Anti-β_2-glycoprotein-I Antibody (aβ_2GPI) Results



Data are presented for aCL (A & B) and aβ_2GPI (C &D), separating results for IgG and IgM. For each aPL isotype, correlation (left panels) and Bland–Altman plots (right panels) are shown.

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