Original Article

A review of 10 years of data from an external quality assurance program for antiphospholipid antibodies: no evidence for improved aCL and β2GPI assay standardization

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Background: Anticardiolipin (aCL) and anti-β2-glycoprotein I (aβ2GPI) antibodies are important markers in the diagnosis of the antiphospholipid syndrome. Previous studies have shown significant variability in results obtained from different kits and manufacturers for these antibodies. In response to this lack of homogeneity, there have been international initiatives aimed at improving the reproducibility and standardization of these assays. To assess if these standardization initiatives have led to improved consistency in routine diagnostic laboratory reporting of these antibodies, we retrospectively reviewed 10 years of data from an External Quality Assurance (EQA) program.

Methods: Data submitted by laboratories participating in the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) antiphospholipid EQA program over a ten-year period (2009–2018) for IgG and IgM aCL and IgG aβ2GPI antibodies were reviewed. Changes in assay methodologies, consensus of results against the target set by RCPAQAP, and the number of laboratories reporting semi-quantitative results were assessed.

Results: Methodologies used for the detection of aCL and aβ2GPI antibodies have changed considerably since 2009, with a steady trend towards non-ELISA based methodologies, such as chemiluminescence, fluorescence immunoassay and Luminex based techniques. Consensus in resulting (defined as ≥80% concordance in reporting "negative" or "positive" results for a sample) did not significantly change across the 10-year period for any test. There was a significant decrease in the proportion of laboratories reporting semi-qualitative results (i.e., low/medium/high positive) for IgG aCL (P=0.0036) and IgG aβ2GPI antibodies (P=0.007). No significant change was noted for IgM aCL antibodies (P>0.999).

Conclusions: Despite concerted efforts by a number of international groups to improve the standardization of aCL and aβ2GPI antibodies assays, a review of data obtained over a 10-year period of EQA testing in diagnostic laboratories demonstrated that there is no evidence to support that these efforts have translated into improvements in the consistency of IgG/IgM aCL and IgG aβ2GPI antibody results.

Keywords: Antiphospholipid antibodies; anti-cardiolipin antibodies; anti-β2-glycoprotein I antibodies; standardization; External Quality Assurance (EQA)

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Introduction

The antiphospholipid syndrome (APS) is characterized by the occurrence of vascular thromboses (arterial and/or venous) and/or pregnancy morbidity, in the presence of antiphospholipid antibodies (aPL) (1). Recently, there has been an expansion in the repertoire of novel antibodies that have been proposed to identify APS patients, including anti-prothrombin/phosphatidyl serine (aPT/PS) antibodies (2). Despite this, the mainstay of diagnostic laboratory testing and the only assays included in the latest 2006 APS Classification Criteria remains the identification of anticardiolipin (aCL) and anti-β2-glycoprotein I (aβ2GPI) antibodies, in addition to clot-based tests for lupus anticoagulant (LA) (1).

Previous attempts to ensure standardization across these assays, especially in regard to enzyme-linked immunosorbent assays (ELISAs) for aCL and aβ2GPI antibodies have included international workshops, Consensus Guidelines and the formation of Working Parties including the Australasian Anticardiolipin Working party and the College of American Pathologists Working Group (1,3-9). In addition to this, polyclonal IgG and IgM calibrators for aβ2GPI antibodies have recently been developed (10,11). Despite these initiatives, there remains ongoing issues with assay reproducibility and standardization (12-18). This variation limits the clinical utility of these assays.

The high degree of variation and subsequent requirement for interpretation of these assays are highlighted by results reported in External Quality Assurance (EQA) programs, as well as in previous publications using such data or other cross laboratory data (12-15,18). For more than 20 years the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) has been performing an EQA for IgG and IgM aCL and IgG aβ2GPI antibodies. Herein, we provide an updated review of data obtained as part of this program, which demonstrates ongoing variation in the reporting of aPL antibodies in the period 2009–2018, and thus indicates a limited impact of the current International Consensus Guidelines to improve the standardization of results reported for aCL and aβ2GPI over this time period.

Methods

For the IgG and IgM aCL and IgG aβ2GPI antibodies program, donor samples are collected from a single source (individual patient) with a clinical history consistent with the diagnosis of APS, or in the case of negative samples, collection is from patients with no history of autoimmune disease. Samples are stored at –80 ℃ before being aliquoted into 500 µL vials and shipped to participating laboratories, where they are stored at –20 ℃ before analysis. Approximately 70 laboratories participate in this program, with all reporting IgG aCL antibodies, an average of 54 reporting IgM aCL antibodies and 37 reporting IgG aβ2GPI antibodies. Results of each sample, including the methodology, kit and manufacturer were submitted to the RCPAQAP by participants through an online portal.

Each aPL EQA module consisted of 8 samples sent on an annual basis (80 samples over a 10-year period) to be tested throughout the year, and included a range of low to high samples and an average of one negative sample per year. Reported methodology, consensus of results against the target set by RCPAQAP, and the number of laboratories reporting semi-quantitative results were analysed over a ten-year period (2009–2018) to identify changes to laboratory testing and reporting procedures during this time.

Data was analysed using linear regression, one-way ANOVA and Kruskal-Wallis multiple comparison tests in Prism™ v 8.0 statistical software (GraphPad, San Diego, CA). A P value of <0.05 was considered significant.

Results

Changes in methodologies

An analysis of results reported to the RCPAQAP between 2009 and 2018 demonstrated a marked shift in the methodologies used for the detection of IgG aCL (*Figure 1A*) and IgG aβ2GPI (*Figure 1B*). In 2009, ELISA methodology was used by >90% of participants; however, over the 10-year evaluation period, there has been a steady trend for laboratories to switch to non-ELISA based methodologies, in particular to chemiluminescence, fluorescence immunoassay and Luminex based techniques. Laboratories who also reported IgM aCL antibodies always used the same methodology for both IgG and IgM aCL assays.

Concordance of qualitative results

Consensus (as defined by ≥80% concordance in reporting "negative" or "positive" for a sample) did not significantly change across the 10-year period for all tests (IgG

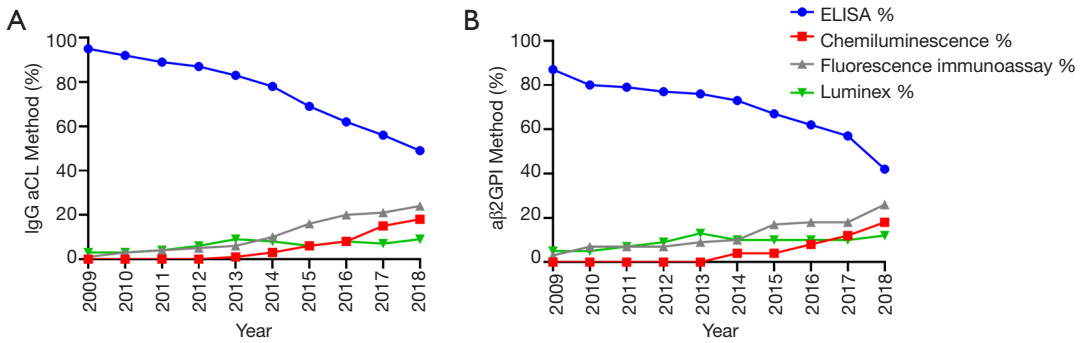

Figure 1 Reported methodologies used for aCL and aβ2GPI antibodies testing. Laboratories reporting results to the RCPAQAP stipulate the method utilised to obtain their result. ELISA (blue) was the majority method across all years for both IgG aCL (A) and IgG aβ2GPI antibodies (B); however, there was a significant increase in other methods, including chemiluminescence (red), fluorescence (grey) and Luminex (green) assays, representing a move away from ELISA assays.

and IgM aCL and IgG aβ2GPI) when analysed using linear regression (*Figure 2A*). When concordance data for individual programs was analysed using a one-way ANOVA across the years 2009–2018, the results indicate no significant variation of consensus reporting across all tests and programs (*Figure 2B,C,D*), even when accounting for the change in methods (*Figure 1*) and proportion of qualitative reporting (*Figure 3*).

Reporting of semi-quantitative results

An interesting finding from this data was the significant increase in the proportion of laboratories returning qualitative results (i.e., positive or negative) as opposed to semi quantitative for IgG aCL (*Figure 3A*) (2009=42.5±10.5, 2018=61.4±4.1, P=0.0036) and IgG aβ2GPI antibodies (*Figure 3C*) (2009=39.9±10.0, 2018=61.6±14.0, P=0.007), indicating a noteworthy shift in results reporting. No significant change was noted for IgM aCL antibodies (*Figure 3B*) (2009=43.8±7.1, 2018=43.7±3.4, P>0.999); however, there were more "negative" samples for IgM aCL antibodies over the testing period, which influenced the number of semi-quantitative results reportable.

This correlates with the change in methods technologies (*Figure 1*) and is likely related to manufacturer recommendations, which often have not been validated for semi-quantitative reporting (as defined by the 2012 International Consensus Guidelines on Anticardiolipin and Anti-Beta2-Glycoprotein Testing) (3). In addition, semi-quantitative ranges have never been clearly defined for aβ2GPI testing, and therefore any definition of these ranges would be essentially on an arbitrary basis.

Discussion

We present data submitted to the RCPAQAP antiphospholipid antibody program over a 10-year period and demonstrate a sizeable shift in methodologies away from ELISA and towards chemiluminescence, fluorescence immunoassay and Luminex for both IgG and IgM aCL and IgG aβ2GPI antibodies (*Figure 1*). This shift introduces new challenges for standardization, including the introduction of new reporting units (such as chemiluminescent units or CUs), varied detection limits (including increases in the dynamic detection range for the newer methods compared with ELISA-based methods), non-linearity in some methodologies and multiple cut-off values for the detection of 'positive' samples. This shift away from a single method of antibody detection would be expected to lead to further disparities in assay repeatability and validity between methods, although intra-assay reproducibility with newer methods may be improved compared to historical ELISA assays.

Recent findings support the comparative performance of several aCL and IgG aβ2GPI antibody detection kits and their correlation with particular APS clinical manifestations (17). However, the results presented here do not demonstrate any significant improvement in the consensus obtained for representative samples for any aPL assay. Without the uniform adoption of a validated reference standard(s) that has also been proven to be transferable across different methodologies (i.e., on ELISA, chemiluminescence, fluorescence immunoassay and Luminex methodologies), by all manufacturers, it appears unlikely that improved consensus in aPL results will be

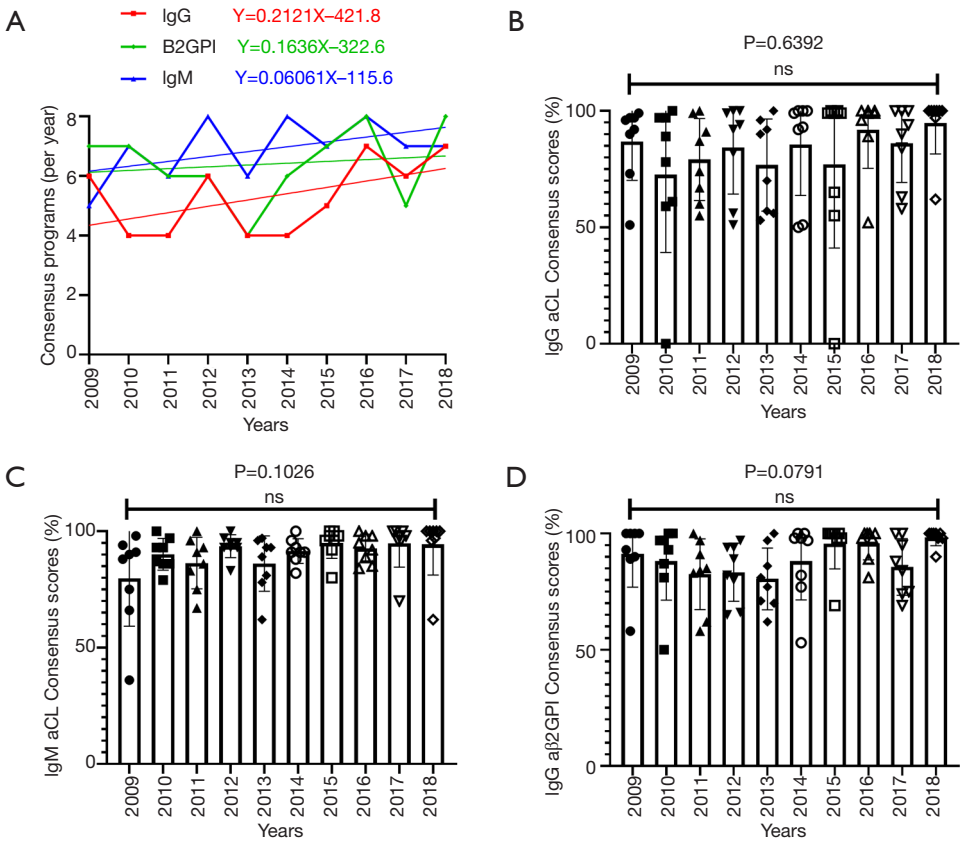

Figure 2 Concordance of results returned to the RCPAQAP from 2009–2018. Test results for each analyte were aggregated into a single data point as a percentage of results that were concordant with the target set by RCPAQAP for the 8 samples that were tested each year. Data is displayed as a line diagram (A) to show the number of programs that reached concordance (i.e., consensus equal to or greater than 80% of results designates the target) or histograms (B-D) to demonstrate the actual consensus percentage for each program for IgG aCL (B), IgM aCL (C) and aβ2GPI (D). Analysis was performed with linear regression (A) or a one-way ANOVA (B-D) and a P value of <0.05 was considered significant.

achievable.

Amongst recommendations from the 2012 International Consensus Guidelines on Anticardiolipin and Anti-β2-glycoprotein I Testing: Report from the 13th International Congress on Antiphospholipid Antibodies, was the endorsement that laboratories should use semi-quantitative (low/moderate/strong) reporting for positive results (3). This is in recognition of the high predictive value of strongly positive results (defined as >99th percentile of the reference population) and the low specificity of results close to assay cut-off values, especially for IgM assays (16,17). This analysis of EQA submissions demonstrates that more laboratories are moving away from reporting semi-quantitative results and instead reporting qualitative (positive/negative) determinations only (*Figure 3*). This is likely to be a consequence of laboratories aligning themselves with manufacturer's recommendations for result reporting, as some of the newer aCL assays have not be validated for semi-quantitative reporting, along with the absence of defined semi-quantitative ranges for aβ2GPI results.

While the limitation of the analysis presented here is the relatively small number of patient samples (80 in total), a strength is the large number of laboratories reporting on each sample, and the fact that these results are representative of "real world" diagnostic findings. In addition, samples selected for the program included a large range of values across clinically important ranges.

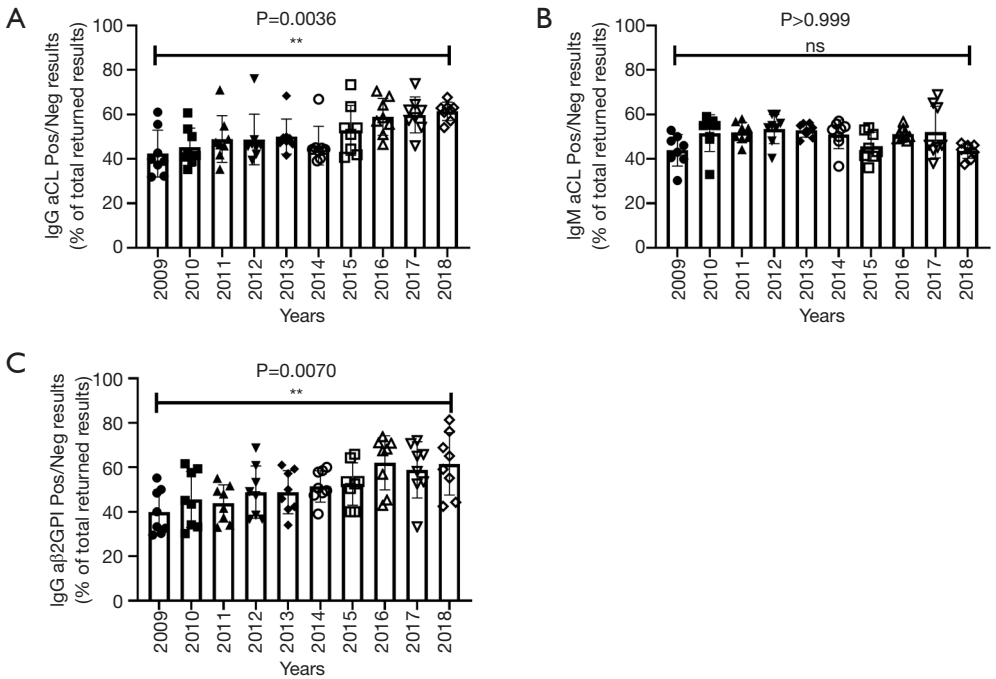

Figure 3 The proportion of reporting laboratories that return qualitative *vs.* semi-quantitative results. Depending on the method used, laboratories reported results for IgG aCL (A), IgM aCL (B) and aβ2GPI (C) antibodies as either qualitative (i.e., positive or negative) or semi-quantitative (negative/low/moderate/strong). Histogram bars represent the mean ± SD of the percentage of laboratories returning qualitative results. Statistical analysis was performed using a one-way ANOVA with Kruskal-Wallis multiple comparison test and a P value of <0.05 was considered significant.

Conclusions

This review of EQA data of IgG/IgM aCL and IgG aβ2GPI antibodies demonstrates that despite concerted efforts by a number of international groups to improve standardization across these assays, there is no evidence to support that these efforts has translated to improvements in the consistency of results from diagnostic laboratories enrolled in the RCPAQAP antiphospholipid program.

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Footnote

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