Thrombosis Research 136 (2015) 1033–1039

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Clinical utility of automated chemiluminescent antiphospholipid antibody assay

Teresa Iwaniec, Marcin P. Kaczor, Magdalena Celińska-Löwenhoff, Stanisław Polański, Jacek Musiał *

Department of Medicine, Jagiellonian University Medical College, Kraków, Poland

ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form 5 July 2015
Accepted 29 August 2015
Available online 11 September 2015

Keywords:
Antiphospholipid antibodies
Antiphospholipid syndrome
Chemiluminescent immunoassays
Enzyme-linked immunosorbent assay
Systemic lupus erythematosus

SUMMARY

Background: The threshold for clinically relevant levels of antiphospholipid (aPL) antibodies for the diagnosis of antiphospholipid syndrome (APS) remains a matter of debate. As new technologies for antibody detection are introduced, their performance characteristics must be clearly understood and compared to traditional assays.
Objectives: To assess the analytical performance and clinical utility of fully automated anticardiolipin (aCL) and anti-β2 glycoprotein I (aβ2GPI) chemiluminescent immunoassays (CIA) in comparison to the traditional ELISA tests.
Patients/methods: Samples from 220 autoimmune patients were studied (primary APS — 74; secondary APS — 47, systemic lupus erythematosus (SLE) without APS — 99). All samples were tested for IgG and IgM aCL and β2GPI antibodies using both CIA and ELISA, and for lupus anticoagulant (LAC).
Results: Good qualitative agreement and quantitative correlation were found between methods in regard to individual antibodies and their categories (profiles). All assays showed good clinical performance in APS, and strong correlation with APS-related clinical symptoms. Importance of determining individual laboratory 99 percentile values for a healthy population as normal cut-off values was shown. Additionally, based on a clinical approach, this study has established the low/medium threshold for QUANTA Flash aCL IgG and IgM assays.
Conclusions: This study showed good clinical performance and strong correlation of the new automated CIA aPL assays with APS clinical symptoms. It also enabled us to determine the corresponding low/medium antibody threshold for the aCL antibody methods with different unit types.

1. Introduction

The international consensus statement on the classification criteria for definite antiphospholipid syndrome (APS) specifies anticardiolipin (aCL) and anti-β_2-glycoprotein I (β_2GPI) antibodies of IgG and/or IgM isotype in medium or high titer as one of the laboratory criteria [1]. The document defines medium or high titer for aCL antibodies either as >40 IgG and IgM phospholipid (GPL or MPL) units, or >99th percentile of the values obtained on reference healthy subjects, measured by standardized enzyme-linked immunosorbent assays (ELISA). As inter-laboratory agreement between aCL measurements is known to be poor due to discrepancies in the cut-off, calibration, and other methodological issues [2–4], the committee recommends to report positive results in ranges of positivity (i.e. low-medium-high) rather than quantitative results [1;5]. However, a few issues remain unresolved. First, the 99th percentile value is often significantly different from the 40 GPL or MPL units [6]. In fact, the value depends on performance characteristics of the particular assay, statistical method, and reference population that is used to establish the cut-off. The committee overseeing the revised classification criteria pointed out the lack of suitable evidence on this issue, and specifically commented that these values are to be used "until international consensus is reached" [1]. Second, given the analytical variability of antiphospholipid (aPL) assays, the use of the same numerical value and unit type does not guarantee the same clinical utility. Finally, new technologies such as chemiluminescent immunoassays (CIA) and addressable laser bead immunoassays (ALBIA) have been increasingly replacing ELISA methods. Their characteristics and the different units used to measure antibody levels need to be taken into account when attempting to distinguish between relevant antibody titers. In fact, sensitivity of assays for the detection of aPL antibodies should be sufficient to correctly classify APS.

The consensus statement suggests to subclassify aPL positive patients into categories (profiles) depending on the number of positive tests. This is based on the evidence that multiple aPL positivity (as opposed to single positivity) is associated with an increased rate of thrombosis and more severe course of the disease, shown both by retrospective and prospective studies [6–8].

Abbreviations: APS, antiphospholipid syndrome; aCL, anticardiolipin; AMR, analytical measuring range; aβGPI, anti-beta2 glycoprotein I; CIA, chemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; ROC, receiver-operating characteristics; SLE, systemic lupus erythematosus; SjS, Sjögren's syndrome.

* Corresponding author at: Dept. of Medicine, Jagiellonian University Medical College, Ul. Skawińska 8, 31-066 Kraków, Poland.
E-mail address: mmmusia@cyf-kr.edu.pl (J. Musiał).

http://dx.doi.org/10.1016/j.thromres.2015.08.021
0049-3848/

It is important to note that 99th percentile of the values obtained on healthy subjects serving as the true threshold between analytically true positive and negative results does not necessarily determine the clinically meaningful antibody level. The choice of assays and the cut-off values may greatly affect APS diagnosis [9–11]. For this reason, following the 14th International Congress on Antiphospholipid Antibodies, a dedicated group of experts recommended that the question of low-medium-high antibody levels be approached from a clinical point of view [12]. The clinical approach examines the performance of a particular assay for its association with APS-related clinical symptoms.

The goal of this study was to evaluate analytical and clinical performance of the new antiphospholipid antibody CIA tests and to compare them with the traditional ELISA assays on a clinically-defined patient population.

2. Materials and methods

2.1. Patients

The study included 220 samples collected at the Immunological Outpatient Clinic, Department of Internal Medicine, at Jagiellonian University Medical College (Kraków, Poland) from consecutive autoimmune patients referred to the outpatient clinic with the suspicion of SLE and/or APS. Data on the presence or absence of venous thrombosis, arterial thrombosis, and obstetric complications were available for all patients. APS diagnosis was made based on the updated APS criteria [1]. SLE patients were diagnosed according to the ACR whenever at least 4 criteria were met [13]. All other diagnoses were established as described before [14]. Additionally, samples from a group of 120 sex- and age-matched healthy volunteers (84 women and 36 men; mean age 44.6 years, range 20–75 years) were used to calculate value of the 99th percentile. This study was approved by a local Ethics Committee, and informed consent was obtained from all patients according to the Declaration of Helsinki. All samples were tested for aCL and β_2GPI antibodies in both IgG and IgM classes using CIA and standard ELISA. They were also examined for the presence of lupus anticoagulant (LAC).

2.2. Sample preparation

Blood samples for aCL and aβ2GPI determination in both assays (ELISA and CIA) were collected in serum separation tubes and spun for 10 min at 3500 rpm at room temperature within 2 h from sampling. Serum samples were then stored at −80 °C for further study. For lupus anticoagulant detection blood was drawn in 3.2% (0.109 mol/l) sodium citrate tubes (one part sodium citrate to nine parts venous blood). Platelet-poor plasma was prepared by double centrifugation within 2 h (first: 10 min/3500 rpm and second: 10 min/14,000 rpm) and stored at −80 °C for further analysis.

2.3. Measurement of autoantibodies

2.3.1. CIA method

We used QUANTA Flash® (Inova Diagnostics Inc., San Diego, CA, USA) aCL and β2GPI (IgG and IgM) assays. These microparticle chemiluminescent immunoassays (CIAs) are run on the BIO-FLASH® instrument (Biokit S.A., Barcelona, Spain). The BIO-FLASH is a random access, rapid response, fully automated chemiluminescent analyzer. QUANTA Flash assays are semi-quantitative methods, using lot specific Master Curves for the quantitation of the results. Results are expressed in (arbitrary) chemiluminescent units (CU).

2.3.2. ELISA method

We used QUANTA Lite® aCL and β2GPI(IgG and IgM) (Inova Diagnostics, San Diego, CA, USA), traditional enzyme-linked immunosorbent assays (ELISAs) for the semi-quantitative determination of aCL and β2GPI antibodies in human serum. The ELISA method for aCL determination uses wells coated with purified bovine cardiolipin antigen and human B_2GPI. The QUANTA Lite aCL assays report results in GPL and MPL units, and the QUANTA Lite β2GPI assays report results in standard IgG and IgM units (SGU and SMU, respectively). All QUANTA Lite ELISAs were performed according to the manufacturer's guidelines.

2.3.3. Lupus anticoagulant detection

Lupus anticoagulant (LA) was determined in a three-step procedure according to the guidelines of the ISTH [15]. Diluted Russell's viper venom time (dRVVT; LA1-screen; Siemens, Germany) and a sensitive activated partial thromboplastin time (PTT LA; Diagnostica Stago, France) were used for screening purposes, whereas LA2-confirm (Siemens, Germany) and Staclot LA (Diagnostica Stago, France) were run as confirmatory tests. Reference values for each test were established using 99th percentile of the healthy population.

Please note that LA determined by coagulometric method was not subject to any comparisons, but served only to properly classify patients to the antiphospholipid syndrome group.

2.4. Precision and linearity of the analytical measuring range

Precision performance and linearity of the QUANTA Flash aCL and β_2GPI CIAs were verified as part of the analytical assessment. Testing was performed according to relevant Clinical and Laboratory Standards Institute (CLSI) guidelines, EP5-A2 and EP6-A. For the precision study (within run), between days and total imprecision were determined by running two samples (the Low and the High Controls) in triplicate for five days. Linearity testing was performed by serially diluting two samples (one high and one low) to span the analytical measuring range (AMR) for each assay, running the dilutions in duplicate, plotting obtained values against expected values, and analyzing the results with linear regression.

2.5. Statistical analyses

Data were statistically evaluated using Analyse-It Software (Leeds, United Kingdom) at a significance level of 0.05. Spearman's correlation and Cohen's *kappa* agreement tests were carried out to assess the quantitative correlation between unit values and agreement between portions, and p values < 0.05 were considered significant. Receiver operating characteristics (ROC) analysis was used to assess the diagnostic performance of the different immunoassays. Cut-off verification on 120 normal subjects was calculated using a non-parametric percentile method [16].

3. Results

3.1. Precision and linearity

Within-run coefficients of variation (%CV) for high and low level patient samples ranged from 1.0% to 3.5%. The between-day %CV ranged from 1.2% to 10.0%, and the total imprecision ranged from 1.5% to 10.3% for all assays. For the linearity study, results obtained on the two samples per assay were combined in the same linear regression plot, showing excellent correlation between the obtained and expected results for the various QUANTA Flash assays. QUANTA Flash aCL IgG had a regression line slope of 0.98 (95% CI 0.97 to 1.00) with a R^2) of 1.00; QUANTA Flash aCL IgM had a slope of 0.99 (95% CI 0.96 to 1.02) with an R^2 coefficient of 1.00; QUANTA Flash β2GPI IgG had a slope of 1.04 (95% CI 0.99 to 1.09) with an R^2 of 0.99; QUANTA Flash β2GPI IgM had a slope of 0.97 (95% CI 0.94 to 1.01) with an R^2 of 1.00.