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Evaluation of different immunoassays for the detection of antiphospholipid antibodies: Report of a wet workshop during the 13th International Congress on Antiphospholipid Antibodies ☆

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ABSTRACT

Background: The performance and standardization of anticardiolipin (aCL) and anti-β_2 glycoprotein I antibodies ($a\beta_2$GPI) tests for the confirmation of diagnosis of antiphospholipid syndrome (APS) remain a matter of debate and concern. We evaluated the performance of different ELISAs and other new immunoassays for the detection of aCL and $a\beta_2$GPI in a wet workshop at the 13th International Congress on Antiphospholipid Antibodies in Galveston, TX (April 13th, 2010, APLA 2010).

Methods: Aliquots of 26 un-identified APS or persistently aPL positive serum samples and 21 controls (9 from healthy individuals and 5 from patients with infectious diseases and 7 with various autoimmune diseases) were distributed to all participants/groups. All serum samples were evaluated in various aCL and $a\beta_2$GPI ELISAs, a chemiluminescent immunoassay, a fluoro-enzyme immunoassay, and in a multiplexed immunoassay system. Monoclonal and polyclonal calibrators were also evaluated.

Results: Although not all the assays reported the titers of aCL and $a\beta_2$GPI in the same units, the correlation of positive titers among the assays was good. All aCL and $a\beta_2$GPI tests showed excellent clinical sensitivities, specificities and positive predictive values and good agreement with respect to the levels of the IgG and IgM antibodies, regardless of assay type, or whether tests were done using automated or "manual" systems.

Conclusions: New methodologies for the detection of aPL look promising and comparable to currently approved ELISA tests. This study provides evidence of progress of efforts of harmonization of tests used to detect aPL.

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1. Introduction

The Antiphospholipid Syndrome (APS) is an autoimmune disease that requires clinical manifestations (venous or arterial thrombosis, and/or pregnancy-related morbidity) and laboratory tests for diagnosis [1,2]. Antibodies to a variety of phospholipids and phospholipid-binding proteins, usually detected by coagulation tests and immunology assays are referred to in general as antiphospholipid antibodies (aPL). In the last APS consensus statement, laboratory criteria were revised [3]. The revised criteria include lupus anticoagulant (LA), and high titers of IgG and/or IgM anticardiolipin (aCL) and anti-β_2 glycoprotein I antibodies ($a\beta_2$GPI). The diagnosis of aCL and $a\beta_2$GPI relies on the presence of IgG or IgM antibodies at titers >40 units or >99th percentile (for aCL) and at titers >99th percentile (for $a\beta_2$GPI). Persistent positivity of laboratory assays is important with the recent criteria suggesting an interval of at least 12 weeks between the 2 positive tests instead of 6 weeks as in the 1999 Sapporo criteria.

The diagnosis of LA relies on a set of successive phospholipid-dependent clotting tests [4]. In the aCL test, cardiolipin or a mixture of phospholipids is coated onto an ELISA plate, and in the $a\beta_2$GPI assay, β_2GPI is directly coated onto a high-binding ELISA plate. Newly

☆ In memory of Dr. Silvia Pierangeli.

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developed multiplexed or chemiluminescent immunoassays use a different method than the ELISA methods for forming the cardiolipin–β_2GPI complex on the solid phase.

The performance and standardization of ELISAs used in the confirmation of diagnosis of APS are still a matter of debate and concern. In addition, while new methodologies and new platforms (automated vs. manual) for the detection of aPL have become available, proper inter-assay simultaneous comparisons have not been carried out. One important topic of discussion is whether to use monoclonal or polyclonal antibody preparations for the calibration and/or quality control of these assays. However, the proper cross-validation and evaluation of the available preparations are lacking.

2. Materials and methods

2.1. Samples

Aliquots of 500 μl of 26 un-identified confirmed APS serum samples (diagnosis according to the Sapporo modified criteria) [3] and persistently positive aPL sera, and 21 controls (9 from healthy individuals, 7 from patients with autoimmune diseases and 5 from patients with infectious diseases) were blinded and distributed to all participants/groups. All aPL positive specimens, whether diagnosed with APS or not were LA positive, determined as recommended by the recent Standardization Subcommittee (SSC) on LA and phospholipid-dependent antibodies of the International Society on Thrombosis and Haemostasis (ISTH) guidelines [4]. All 47 samples (Tables 1A and 1B) were provided by the Antiphospholipid Standardization Laboratory, University of Texas Medical Branch, USA. Selected samples had been evaluated for aCL and aβ_2GPI. After selection, serum samples were aliquoted and stored at −70 °C. All samples were pre-tested in a blind fashion, by all groups/companies before the wet workshop in their own laboratories. A manual with all procedures and relevant information was distributed to the participants (n = 60). Identity of the samples was released after the wet workshop results had been submitted by all participants.

2.2. Standards

A variety of currently available monoclonal and polyclonal calibrators were assayed in the various aPL assays as if they were patients' samples, as follows: 1) polyclonal calibrators for aCL (calibrators G2 (for IgG) and M3 (for IgM) from a set of 8 calibrators (LAPL-GM200, Louisville APL Diagnostics, Inc.). Freeze-dried form. Units expressed in GPL and MPL units for IgG and IgM, respectively), and 2) monoclonal calibrators (chimeric "Sapporo" monoclonal antibodies, freeze-dried form. HCAL (IgG) and EY2C9 (IgM) for IgG and IgM aCL and aβ_2GPI). Center for Diseases Control and Prevention (CDC), chimeric monoclonal antibodies "Sapporo" in liquid and stabilized form [one IgG (HCAL) and one IgM (EY2C9)] (for IgG and IgM aCL and aβ_2GPI). INOVA Diagnostics, Inc., and human monoclonal for IgG, IgM, IgA (for aCL and aβ_2GPI). Liquid form. Two calibrators (AbyD05045 and AbyD03892) were tested (Phadia).

All polyclonal and monoclonal preparations were provided at no cost by the manufacturer/distributor to be used in this wet workshop. All preparations used were from the same lot for each type of calibrator and users were instructed on how to reconstitute the calibrators that were in freeze-dried form.

2.3. Methods

All serum samples were analyzed with the different assays. All kits were evaluated as per the manufacturers' instructions and results were expressed in their respective units of measurement. The workshop was open to all congress participants. Kits and instruments were made available to the wet workshop at no cost by the manufacturers.

Table 1A
Clinical and original laboratory data of serum samples tested in the workshop (healthy and disease controls).

Sample APLA#	Gender	Age (years)	Disease	LA	aCL G/M	aβ_2GPI G/M
1	F	35	Autoimmune thyroid disease; anti-TPO	N	N/N	N/N
2	F	49	Autoimmune thyroid disease; anti-TPO	N	N/N	N/N
3	M	56	Autoimmune thyroid disease; anti-TPO	N	N/N	N/N
4	F	25	SLE; ANA positive	N	N/N	N/N
5	F	31	SLE; ANA positive	N	N/N	N/N
6	F	19	SLE; ANA positive	N	N/N	N/N
7	M	32	HIV	N	P/N	P/N
8	M	54	HIV	N	N/N	N/N
9	M	41	Syphilis	N	N/N	N/N
10	M	39	Syphilis	N	N/N	N/N
11	M	36	HCV	N	N/N	N/N
12	F	33	HC	N	N/N	N/N
13	M	39	HC	N	N/N	N/N
14	F	46	HC	N	N/N	N/N
15	F	56	HC	N	N/N	N/N
16	M	43	HC	N	N/N	N/N
17	F	19	HC	N	N/N	N/N
18	M	43	HC	N	N/N	N/N
19	F	50	HC	N	N/N	N/N
20	F	42	HC	N	N/N	N/N
47	M	45	SLE; ds DNA antibodies	N	N/N	N/N

Anti-TPO: anti-thyroidperoxidase antibodies positive; dsDNA: double stranded DNA; ANA: antinuclear antibodies; SLE: systemic lupus erythematosus; HIV: human immunodeficiency virus; HCV: hepatitis C virus; N: negative; P: positive; HC: healthy control.

The following commercial kits and methods were evaluated (summary in Table 2):

a) The *APhL IgG and IgM HRP ELISA®* [Louisville APL Diagnostics (LAPL) Inc.] is an ELISA assay that utilizes a mixture of negatively charged phospholipids instead of cardiolipin. Results are expressed in standard aCL units (GPL and MPL units) for IgG and IgM isotypes, respectively. The assay was performed manually. The cut-off value of 15 GPL/MPL units is recommended. Values between 15 GPL and 27 GPL or between 15 MPL and 38 MPL units are considered to be in the "indeterminate" or low positive range for IgG and IgM, respectively.
b) The *QUANTA Lite® aCL IgG/IgM* and *aβ_2GPI IgG/IgM* (INOVA Diagnostic Inc.) ELISAs were performed manually for the detection of aCL and aβ_2GPI IgG and IgM isotypes. The aCL assay utilizes human monoclonal antibodies as calibrators (HCAL and EY2C9), and reference calibrators from the Rheumatology Lab. Seton Hall University are utilized for aβ_2GPI. The cut-off value of 20 GPL/MPL units is recommended for aCL, and 20 Standard β_2GPI IgG or IgM Units (SGU) is recommended for aβ_2GPI.
c) The *EL-aCL™ GM* and *EL-β_2GPI™ GM* (TheraTest Laboratories Inc.) ELISAs were performed manually for the detection of aCL and aβ_2GPI IgG and IgM isotypes. Both assays utilize a one-point calibrator for calculation of antibody activity. The cut-off value of 15 GPL units is recommended for aCL IgG and 10 MPL units for aCL IgM, and 25 U/ml for aβ_2GPI IgG and 4 U/ml for aβ_2GPI IgM. Equivocal values are: 16–20 for IgG and 11–13 for IgM aCL; and 26–40 for IgG and 5–10 for IgM aβ_2GPI kits. These kits were only evaluated by the manufacturer before the wet workshop.
d) The *HemosIL® AcuStar aCL IgG and IgM* and *aβ_2GPI IgG and IgM* [Instrumentation Laboratory (IL)] are novel, fully automated, random access, chemiluminescent immunoassays designed exclusively for use on the ACL AcuStar Hemostasis Testing Systems. Briefly, the assays consist of paramagnetic particles coated with cardiolipin/human β_2GPI or only β_2GPI which captures the antibodies from the sample. After incubation, magnetic separation and a wash step, a tracer consisting of an isoluminol-labeled anti-human IgG or

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