Search for uniformity in reporting qualitative results as low-medium-high antibody titers for anti-cardiolipin and anti-β2glycoprotein I IgG/M based on a clinical approach, a multicenter study
Subcommittee on Lupus anticoagulant/ antiphospholipid antibodies

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Description Abstract

Classification of the anti-phospholipid syndrome (APS) predominantly relies on the detection of anti-phospholipid (aPL) antibodies. As a consequence, the quality and reliability of these assays is of utmost importance. Three different types of aPL assays are included in the official revised Sydney criteria; (1) phospholipid-dependent prolongation of coagulation (Lupus anticoagulant, LAC); (2) detection of IgG/IgM anti-β2glycoprotein I (β2GPI) antibodies and (3) detection of IgG/IgM anti-cardiolipin (aCL) antibodies [1]. Solid phase assays detecting aCL and aβ2GPI antibodies are complicated with inter-method and inter-laboratory variation [2–5]. To reduce the inter-assay and inter-laboratory variation, aCL and aβ2GPI results may be reported semi-quantitatively as negative, low, medium and high positive [2–5]. In the Sydney criteria a threshold of ≥ 40 GPL or MPL units is suggested when measured with a standardized ELISA for the definition of moderate-to-high aCL or aβ2GPI IgG and aCL or aβ2GPI IgM titers, respectively [1]. Clinicians have adopted this arbitrary cut-off value [6]. However, the use of ≥ 40 GPL or MPL units as a threshold for moderate-high aPL titers is poorly evaluated in different ELISAs and not evaluated in other (newer) systems like chemiluminescent immunoassays [1,2,6].

So far, SSC-ISTH guidelines indicate that aCL and aβ2GPI have to be reported quantitatively with the titer and indication of the local cut-off value [3, 4]. A qualitative classification is not recommended due to variability in titers between systems. Therefore, according to the SSC-ISTH guidelines each test result above the cut-off value calculated as higher than the 99th percentile, should be regarded as positive. On the other hand, a qualitative classification into low-medium-high that is interchangeable between methods could be useful for the clinician.

Therefore, we aimed for uniformity in reporting qualitative results as low-medium-high antibody titers for aCL and aβ2GPI IgG/M based on a clinical approach among three automated commercially available solid phase assay and one commercially available ELISA assay.

Aim study

1. Evaluate the use of ≥ 40 GPL or MPL units as threshold for moderate-high aPL in all four solid phase assays
SSC Subcommittee Project/Collaborative Project

2. Calculate a cut-off value for the definition of low, medium and high aPL titers for each solid phase assay based on a clinical approach

Design and methodology (Data expected to collect, sample size and statistical analysis):

This project is part of the multicenter study launched by Katrien Devreese at the Scientific and Standardization Committee (SSC) session ‘Lupus Anticoagulant/Phospholipid-Dependent Antibodies’ of the ISTH in Toronto, Canada (June 2015).

Based on the information of the External quality Control of diagnostic Assays and Tests (ECAT) the most frequently used IgG/IgM solid-phase assays in the diagnosis of APS, and based on the willingness of manufacturers for collaboration, we included 4 different platforms for IgG, IgM and IgA: BioPlex®2200 (Bio-Rad, Bio-Rad Laboratories, Hercules, USA), ImmunoCap®EliA (Thermo Fisher Scientific/Phadia, Uppsala, Sweden), ACL AcuStar® (Werfen/Instrumentation Laboratories, Bedford, USA) and QUANTA Lite ELISA® (Inova Diagnostics, San Diego, USA).

To collect a variety of samples several European centers were asked to participate and contribute in a significant number of samples 8 medical centers agreed:

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All samples were sent to and tested at one location (Coagulation Laboratory, Ghent University Hospital) on manual and automated anti-β2GPI and anti-cardiolipin assays (IgG/IgM/IgA) of
different suppliers. We started the analyses (IgG and IgM) of a multi-center clinical study in 2016 and have produced multiple publications regarding the agreement of criteria aPL and the added value of non-criteria aPL [7–11]. Here, we aim to investigate whether the agreement between aPL assays is improved if results are reported as semi-qualitative results. A threshold value of 40 and 80 GPL or MPL will be evaluated along with a threshold calculated from a clinical approach. Such cut-off values are calculated based on the sensitivity and specificity from a receiver operator curve (ROC) curve. Kappa agreement, correlations between titers and odds ratios will be calculated.

**Study population (Inclusion, exclusion, eligibility) (patient population; recruitment of participating institutions/physicians and subjects; minimum number needed; expected number):**

We aimed to collect about 250 in each patient category. We included healthy donors (HC), autoimmune disease (AID) without thromboembolic or pregnancy complication, diseased controls (DC) for thrombosis, diseased controls (DC) for pregnancy complications, thrombotic APS, obstetric APS. To calculate cut-off values, we aimed to collect normal volunteer samples (n=250 in total).

The sample collection resulted in a total of 1404 patient samples. We excluded samples with insufficient data or sample volume. Finally, we included 1168 samples from 8 European centers. Classification of APS was based on the Sydney criteria. Patients were classified by the corresponding center resulting in 259 thrombotic APS patients, 204 patients with a history of thrombosis and negative for laboratory criteria of APS, 122 obstetric APS patients, 33 patients with pregnancy complications and negative for laboratory criteria of APS, 196 patients with an autoimmune disease other than APS, 100 individuals with a normal pregnancy, 194 controls that were referred for aPL testing for other reasons than the clinical criteria of APS, including subfertility and prolonged activated partial thromboplastin time; and 60 women that were diagnosed with APS without specific information on the clinical manifestations. Centers with the indicated number of samples included Ghent (469), London (196), Nîmes (164), Nancy (114), Kraków (101), Milan (52), Geneva (50) and Apeldoorn (22). 200 samples from healthy volunteers were collected from two centers (Ghent and Krakow).

**Expected timeline:**

- Project stage/set up: (full multicenter study) 2016
- Launch: (full multicenter study) 2015
- Duration subproject on qualitative reporting: 2020-2021
- Finalization/analysis: 2021
- Reporting: 2021

**Expected outcomes (ie. publications):**

- Abstract ISTH 2021
- Original article
Description of project set/up and management, needed infrastructure and resources (summary):

The samples were sent by the participating centers to the Ghent University Hospital Coagulation Laboratory, samples are stored and analyzed with all platforms at this location. Manufacturers provided the reagents and instruments if not available in the lab of the Ghent University Hospital.

Possible references:


