SSC Subcommittee Project/Collaborative Project

Role of anti-domain I β2-glycoprotein I antibodies measured by different methods, in the diagnosis and risk stratification of antiphospholipid syndrome

Subcommittee on Lupus anticoagulant/ antiphospholipid antibodies

Person responsible (Chair / Principal Investigator): Katrien Devreese and Hilde Kelchtermans

Investigator: Dongmei Yin (PhD student)

Description Abstract

As the clinical symptoms of the antiphospholipid syndrome (APS) frequently occur irrespective of the syndrome, diagnosis predominantly depends on the laboratory assays measuring the level or function of antiphospholipid antibodies (aPLs). β2-glycoprotein I (β2GPI) is increasingly accepted as the most important target of aPLs. Anti-β2GPI antibodies (aβ2GPI) constitute a heterogeneous population, but current in vivo and in vitro evidence show that especially the first domain (DI) of β2GPI contains an important pathogenic epitope. This epitope containing Glycine40-Arginine43 (G40-R43) has proven to be cryptic and only exposed when β2GPI is in its open conformation. A previous study demonstrated a highly variable exposure of the cryptic epitope in commercial anti-β2GPI assays, with implications on correct patient classification.

So far, research assays were applied to detect anti-domain I (aD1), but recently a commercial chemiluminescence immunoassay assay (CIA) is available to detect aD1. Several studies with the CIA aD1 assay confirmed high odds ratio for thrombosis and the role of aD1 in risk stratification. Correlating to the higher risk, aD1 IgG are mainly present in triple positive patients, showing also higher levels. Although, a limited number of studies showed that aD1 had no added value to the current aPL panel. So far, aD1 measured by the only available commercial assay, should be rather considered as a confirmation of the higher thrombotic risk, rather than a candidate for replacement of the aβ2GPI.

Unexpectedly, we recently (unpublished data) revealed impaired exposure of the pathogenic epitope in the commercially available aD1 chemiluminescence immunoassay assay detecting specific antibodies directed to DI. Also, a high correlation has been illustrated between aβ2GPI and aD1 antibodies measured by the chemiluminescence method. In a previous our study, we illustrated that aβ2GPI IgG and aD1 IgG titers show an excellent qualitative agreement. In contrast to de Laat et al, who found that approximately 50% of aβ2GPI IgG binds domain 1, we found a positive agreement of 91.7% in the overall study population. In other words, probably both assays practically measure the same antibody population, which partially elucidates why aD1 IgG detection does not add diagnostic power to the aPL panel used in our study. So far, no correlation has been evaluated between the original in-house aD1 assay and the commercial one now available.

Therefore, we want to re-develop the in-house assay and compare it to the commercial aD1 assay in a large cohort of APS and non-APS patients.
Aim of our study is:

1. Compare the in-house aD1 assay with the commercial CIA assay measuring monoclonal antibodies directed against different epitopes on DI
2. Investigate the frequency and role of aD1, and compare with aβ2GPI and anti-cardiolipin antibodies in the diagnosis of APS and the correlation with thrombosis and/or pregnancy complications in a large multicenter study

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

aDI are detected by both the commercial CIA assay (Quanta Flash β2GPI DI (Inova Diagnostics, San Diego, USA) on the HemosIL AcuStar™ platform (Werfen/Instrumentation laboratories, Bedford, USA) as well as an in-house aDI assay. For the commercial aDI assay, upon confirmation in 20 healthy controls, the manufacturer’s cutoff for the classification as positive or negative is applied. For the detection of aDI using the in-house assay, DI (3 μg/mL) is coated on a hydrophobic and a hydrophilic plate. By coating DI on a hydrophobic plate, the hydrophilic epitope G40-R43 is available for binding antibodies. When domain I is coated on a negatively charged hydrophilic plate, the epitope G40-R43 is involved in the binding to the plate and not available for binding of antibodies. Both types of plates were chosen in order to have an equal density of DI coated to the surface which is checked with a monoclonal mouse aDI 3B7 that does not recognize epitope G40-R43. Plates are blocked with 3% bovine serum albumin/0.1% Tween/150 mM NaCL, pH 7.4 for 1 h. Patient samples (duplicates) are diluted 1/100 in blocking solution and incubated for 1 h, followed by incubation of an anti-IgG peroxidase-labeled antibody to detect bound antibodies. 3’,5,5’-tetramethylbenzidine is used for staining and absorbance is read at 450 nm. To determine the presence of anti-domain I antibodies, the obtained optical density (OD) from a sample on a hydrophobic plate is divided by the OD obtained from the hydrophilic plate. A ratio greater than two discriminates between aβ2GPI that recognize epitope G40-R43 and aβ2GPI that recognize other parts of β2GPI. Cut-off values are determined in at least 120 plasma samples derived from healthy volunteers. In all runs, a blank, negative control (normal pool plasma) and positive controls are included.

The project, in a broader context including also IgG and IgM antiphospholipid antibodies, was launched by Katrien Devreese at the Scientific and Standardization Committee (SSC) session ‘Lupus Anticoagulant/Phospholipid-Dependent Antibodies’ of the upcoming 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 centers agreed:
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Katrien Devreese  Ghent University Hospital, Belgium
Denis Wahl/Stéphane Zuily  Centre hospitalier universitaire de Nancy, France
Armando Tripodi  Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Università degli Studi di Milano, Italy
Gary Moore  Diagnostic Haemostasis & Thrombosis Laboratory, St Thomas’ Hospital, London, UK
Jacek Musial  Jagiellonian University Medical College, Krakow, Poland
Pierre Fontana  University Hospital Geneva, Switzerland
Jasper Remijn  Gelre hospitals, Apeldoorn, Zutphen, the Netherlands
Jean-Christophe Gris  Centre Hospitalier Universitaire, Dept of Clinical Chemistry and Hematology, Nîmes, France

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 multi-center clinical study in 2016. Since priority was given to IgG and IgM (criteria aPL), the analysis of aD1 is performed afterwards.

Correlations between the assay results and the clinical symptoms will be investigated, for all parameters and all platforms.

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, autoimmune disease without thromboembolic or pregnancy complication, diseased controls for thrombosis, diseased controls 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, like subfertility and prolonged activated partial thromboplastin time and 60 women that were diagnosed with APS without information on the specification of 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: 2018-2020 (for aD1)
- Finalization/analysis: 2020 (for aD1)
- Reporting: 2019-2020 (aD1)

Expected outcomes (ie. publications):

- Abstract ISTH 2019
- Original article on the role of aD1 in thrombotic and obstetric APS
- Depending on the outcome, also a Recommendation of the SSC

Description of project set/up and management, needed infrastructure and resources (summary):

The in-house aDI assay will be re-developed in our laboratory. Therefore, DI will be ordered in the same preparation as the originally described in-house assay. The cost for the production of new DI with the same quality attributes as in the original publication to re-develop the aDI in-house ELISA with an optimal exposure of the pathogenic DI epitope is estimated at 16,500 USD.

Upon coating, the exposure of the pathogenic G40-R43 epitope will be verified using monoclonal aDI antibodies. The assay will be technically validated using normal pooled plasma supplemented with monoclonal aDI antibodies. Subsequently, the assay will be clinically validated using patient and control samples of the multi-center study, while comparing results with the commercial aDI assay as well as the aβ2GPI. The frequency of aD1 will be compared between both aDI assays and the included aβ2GPI and aCL assays. The correlations with thrombosis and pregnancy complications will be assessed, to determine the possible added value for the diagnosis and risk stratification of APS patients.

The samples are sent by the participating centers to the Ghent University Hospital Coagulation Laboratory, samples are stored and analysed with all platforms at this location. Manufacturers provide the reagents for the commercial available assays and instruments if not available in the lab of the Ghent University Hospital.
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References:


-Dongmei Yin, Bas de Laat, Katrien M. J. Devreese, Hilde Kelchtermans. The clinical value of assays detecting antibodies against domain I of β2-glycoprotein I in the antiphospholipid syndrome. Autoimmunity Reviews 2018 accepted for publication