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Clinical significance and correlations between anti-β_2 glycoprotein I IgA assays in antiphospholipid syndrome and/or systemic lupus erythematosus

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ABSTRACT

Background: The objective of this investigation was to examine the clinical significance of IgA anti-β_2 glycoprotein I (anti-β_2GPI) antibodies and the inter-assay relationships between kits for their determination.
Methods: Serum samples from 269 patients with clinical diagnoses of systemic lupus erythematosus (SLE) and/or antiphospholipid syndrome (APS), individuals positive for antiphospholipid antibodies (aPL) with or without APS or SLE, and 182 controls were tested for anti-β_2GPI IgA antibodies using kits from four manufacturers.
Results: The positivity rates for the different IgA anti-β_2GPI antibody kits varied in the disease groups; 7.8–14.7% (SLE only), 12.0–15.7% (SLE and APS/aPL), 14.7–58.8% (APS only), and 17.4–52.2% (aPL only). Kappa agreements between any 2 kits within disease groups were also variable and ranged from 0.25–1.00 (SLE), 0.18–1.00 (SLE and APS/aPL), 0.22–0.94 (APS only), and 0.32–0.91 (aPL only). Univariate analyses also showed variable relative risks for specific APS clinical manifestations with the different kits evaluated. Overall, diagnostic and predictive values for IgA anti-β_2GPI antibodies are kit-dependent; therefore results are not interchangeable. While all 4 kits seem able to predict venous thrombosis tolerably well, there was a variable performance in predicting pregnancy related morbidity.
Conclusions: Efforts to standardize these assays are highly needed prior to their formal adoption in routine clinical evaluation.

1. Introduction

Testing for IgA antibodies to $\beta_2$glycoprotein I (anti-β_2GPI) has not been part of the classification criteria for the diagnosis of antiphospholipid syndrome (APS) [1]. Recently, isolated IgA anti-β_2GPI antibody positivity was reported to be associated with increased risk for thrombosis in APS irrespective of certain risk factors [2]. Of pathologic relevance, IgA antibodies to β_2GPI induced thrombogenicity as well as upregulated tissue factor (TF) in an experimental model for APS [2]. Furthermore, some investigators have highlighted an association between IgA anti-β_2GPI antibody positivity and clinical manifestations such as thrombosis and pregnancy-related morbidity (PRM) in APS and/or APS associated with systemic lupus erythematosus (SLE) [2–10]. Fanopoulos et al. showed that IgA anti-β_2GPI positivity occurred more frequently and at higher titers in SLE patients with APS manifestations [3]. These antibodies were also seen to be more common in women who experience unexplained recurrent spontaneous abortion and unexplained fetal death compared to fertile control subjects in the absence of lupus anticoagulant (LAC) and IgG anti-cardiolipin (aCL) antibodies [5]. In other studies, IgA anti-β_2GPI antibodies were more strongly associated with deep venous thrombosis (DVT) and stroke than the IgG and/or IgM isotypes [6–10]. In addition, it has been suggested that these antibodies may recognize epitopes in domain IV/V of β_2GPI associated with certain manifestations of APS [2,7]. Recently, Cousins et al. reported the presence of IgA anti-β_2GPI antibodies in a cohort of 'seronegative' APS patients [11]. However, there exist studies in predominantly APS cohorts that fail to show any increased diagnostic performance for IgA anti-β_2GPI antibodies in addition to the criteria

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antiphospholipid (aPL) antibody tests [12–14]. In these studies, the presence of IgA anti-β_2GPI antibodies did not occur independently of the other isotype aPL antibody tests for APS.

For IgA anti-β_2GPI antibody testing to be of clinical relevance, it would be optimal for the available laboratory tests to have comparable diagnostic outcomes. Recently, Martínez-Flores et al. reported that some commercial kits for detection of IgA antibodies to β_2GPI display analytical and clinical heterogeneity using a commonly available diagnostic test as a reference method [15]. Although the finding of this study is important, in the absence of reliable and/or acceptable reference reagents and informative clinical criteria to identify patients there are inherent limitations in the interpretation of the results obtained.

2. Methods

2.1. Study population

For this study a total of 269 serum samples obtained from 2 independent cohorts were analyzed. Of these, 179 were obtained from the Hopkins (Johns Hopkins University) lupus cohort and 90 from the PROMISSE (Predictors of pRegnancy Outcome: bioMarkers in antiphospholipid antibody Syndrome and Systemic lupus Erythematosus) study group. The Hopkins lupus cohort is a longitudinal study of lupus activity, organ damage, and quality of life of SLE patients. In addition to the SLE classification criteria, patients in this cohort were also evaluated based on the presence of classic APS as defined by the revised Sapporo consensus statement [1]. The PROMISSE cohort is an ongoing, prospective observational study that follows over 700 pregnant patients who are grouped and analyzed according to the presence or absence of aPL antibodies, preexisting SLE, and several pro-inflammatory mediators in an attempt to identify mechanisms and predictors of poor fetal outcome [16]. In addition to these patients, serum samples from 162 healthy women recruited at the University of Utah Health Sciences Hospital in Salt Lake City, UT were used to verify and/or establish that reference ranges of the different assays based on the 99th percentile where appropriate. Analytical specificities of the different anti-β_2GPI kits were also evaluated in patients with known serum IgA reactivity ($n = 10$). The study protocol was approved by the institutional review boards of each site and informed consent was obtained from all subjects.

2.2. Detection of antiphospholipid antibodies

In the Hopkins cohort, a modified automated Russell's viper venom time (RVVT) test was used to screen for LAC. RVVT results of >37 s were considered positive (or high), a mixing study and platelet neutralization procedure were used to confirm the presence of LAC as previously described [17–18]. Both IgG and IgM aCL were measured by ELISA (INOVA Diagnostics) according to manufacturer's instructions. For the PROMISSE cohort, LAC was detected using a panel of three tests including the dilute Russell's viper venom time (dRVVT), a lupus anticoagulant-sensitive activated partial thromboplastin time (aPTT), and the dilute prothrombin time (dPT) as previously described.[16] IgG and IgM aCL were determined using an in-house ELISA method as previously described [16]. Cut-off values (IgG aCL: 20.0 u/ml and IgM aCL: 10.0 u/ml) were determined by calculating 3 standard deviations (SD) above the mean value of a panel of normal controls.

In the current investigation, IgG, IgM, and IgA antibodies to β_2GPI antibodies were determined in all patients and controls with 3 ELISA [Bio-Rad Laboratories, Corgenix and INOVA Diagnostics] and 1 fluoroenzyme immunoassay [Phadia, currently Thermo Fisher Scientific Inc.,] commercial kits following the manufacturers' instructions. The Bio-Rad and Corgenix assays have identical characteristics with respect to units, method (ELISA), human-derived calibration material and number of calibration points ($n = 3$), and cut-off values. The INOVA assays have a unique unit for each isotype, and use the same method as the Bio-Rad and Corgenix assays but employ monoclonal (IgG and IgM) and polyclonal (IgA) calibrators with 5 calibration points and a very slight difference in reported reference ranges. The Phadia assays have different units, are based on a fluorometric method, and like the Corgenix and Bio-Rad assays use a human-derived calibrator based on 6 calibration points. Phadia assays are unique in having different cut-off values with an equivocal range. All ELISAs report the use of goat anti-human horseradish peroxidase (HRP). All kits were provided at no cost and testing performed at ARUP Laboratories by experienced technologists blinded to original test results. Results were interpreted based on the 99th percentile of the different assays for the 162 healthy controls unless otherwise stated.

2.3. Detection of serum IgA antibodies

Serum IgA levels were detected by quantitative nephelometry (BN II System) using the N antiserum to human IgA kit (Siemens Healthcare Diagnostics Inc.). IgA serum levels between 68 and 378 mg/dl were considered normal. Levels >500 mg/dl in the absence of monoclonal IgA proteins are considered polyclonal, otherwise monoclonal. Testing was performed by an experienced investigator according to manufacturer's recommended instructions.

2.4. Statistical analyses

Paired *t*-test statistical analysis was used to compare anti-β_2GPI antibody frequencies between any 2 kits. The Shapiro-Wilk test along with Q-Q plots were used to determine normality of distributions. Inter-rater reliability (Cohen K coefficient), simple positive, negative and total percentage agreement between any 2 IgA anti-β_2GPI antibody tests in the different cohorts was also determined. The relative risks with their 95% CIs were used to assess the strength of the association between specific IgA anti-β_2GPI antibody kit positivity and the lifetime history of venous and/or arterial thrombosis as well as specific and/or any pregnancy-related morbidity. Analyses were performed using SAS software, ver 9.3 of the SAS System and results were considered statistically significant if $p < 0.05$.

3. Results

3.1. Demographics and characteristics of study participants

The demographic and clinical characteristics of patients in the study are presented in Table 1. The Hopkins lupus cohort consisted predominantly of female patients (94.4%) of mainly Caucasian and African-American descent with SLE (39.7%) or SLE and APS (60.3%). The patients in the PROMISSE registry are mainly Caucasian women of child-bearing age with or without aPL antibodies. Unlike the Hopkins cohort with either SLE or SLE and APS patients, the PROMISSE cohort is clinically heterogeneous with four main disease groups, including a subset of women with neither APS nor SLE but aPL antibody-positive. Of the Hopkins patients with SLE and APS, the most common APS clinical manifestations were miscarriage (50.7%) and arterial thrombosis (35.2%). Compared to the Hopkins cohort, the PROMISSE group had more patients with PRM than thrombosis.

In addition to the patients described in Table 1, self-proclaimed subjects as well as individuals with polyclonal or monoclonal IgA were also investigated (data not shown). The healthy control group made up of predominantly Caucasian women of child-bearing age [mean age 28.7 years, range 18–42 years] was used to verify and/or establish the 99th percentile cut-off values for all kits. Patients with polyclonal or monoclonal serum IgA had a mean age 72.1 years [range 46–90 years].