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ORIGINAL ARTICLE

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Diagnostic performance of anti-β2 glycoprotein I and anticardiolipin assays for clinical manifestations of the antiphospholipid syndrome

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Abstract The objective of the present study was to analyse the performance of the tests for detection of anti-β2 glycoprotein I (β2 GP I) and anticardiolipin (aCL) antibodies for identification of clinical manifestations of the antiphospholipid syndrome (APS). Patients with systemic lupus erythematosus (SLE) as well as carriers of infectious diseases such as Kala-azar, syphilis and leptospirosis were studied. Particular interest was given to the presence of clinical complications related to APS. Anticardiolipin and anti-β2 GP I antibodies were searched using an enzyme-linked immunosorbent assay (ELISA) assay. Clinical manifestations of APS were observed in 34 of the 152 patients (22.3%) with SLE and no patient with infectious disease had such manifestations. Antibodies to cardiolipin in moderate or high levels and anti-β2 GP I were detected in 55 of 152 (36.1%) and 36 of 152 (23.6%) patients with SLE, respectively, and in 2 of 30 (6.6%) and 16 of 30 (53.3%) patients with Kala-azar, in 9 of 39 (23%) and 6 of 34 (17.6%) patients with leptospirosis, and 14 of 74 (18.9%) and 8 of 70 (11.4%) cases of syphilis, respectively. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio (LR) of the anti-β2 GP I test for the identification of the clinical manifestation of APS were, respectively, 29% [95% confidence interval (CI) = 24%–34%], 78% (95% CI = 73–83%), 15% (95% CI = 11–19%), 89% (95%CI = 85–93%) and 1.38. Regarding the aCL assay, the figure was 29% (95% CI = 24–34%), 76% (95% CI = 71–81%), 14% (95% CI = 10–18%), 89% (95% CI = 86–92%) and 1.26. As the validity and performance of the anti-β2 GP I assay were similar to the aCL in demonstrating the presence of clinical phenomena associated with APS and due to the difficulties in performing as well as the lack of standardisation of the anti-β2 GP I test, we suggest that the test for aCL should continue to be the first one performed when the presence of APS is suspected.

Keywords Anticardiolipin · Antiphospholipid · Diagnostic test · Systemic lupus erythematosus

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Introduction

The development of the Wassermann reaction for the diagnosis of syphilis [1] and the posterior characterisation of the antigen as cardiolipin [2] were the first steps for the study of the antiphospholipid antibodies (aPL). Also very important was the observation that some individuals with a false positive serologic test for syphilis could develop autoimmune diseases such as systemic lupus erythematosus (SLE) [3], establishing a connection between the aPL antibodies and immunological disorders. Another landmark in the study of these antibodies

was the description of two patients with SLE who showed a coagulation inhibitor [4] later called lupus anticoagulant [5] that was demonstrated to be an antibody against phospholipid of the coagulation cascade [6].

In 1983, Harris et al. [7] described a technique for the detection of antibodies specific to cardiolipin and noted an association of these antibodies with the presence of thrombotic phenomena, obstetric morbidity and thrombocytopenia in patients with SLE. This association was confirmed by many others, leading to the recognition of the antiphospholipid syndrome (APS) [8] that recently had its classification criteria defined in an international symposium [9].

In 1990, three groups of researchers independently drew attention to the role of a protein—β2 glycoprotein I or apolipoprotein H (β2 GP I)—as a cofactor for the anticardiolipin antibodies [10, 11, 12]. More recently there have been studies revealing the existence of antibodies directly against the protein β2 GP I without cardiolipin, and curiously, demonstrating a strong association with the presence of typical complications of APS [13, 14, 15, 16].

The objective of the present study was to analyse the validity and performance of the tests for the detection of anti-β2 GP I and anticardiolipin antibodies in the identification of the clinical manifestations of APS, that is, thrombotic phenomena and obstetric complications.

Material and methods

Patients

Two groups of patients were studied:

1. Patients with a diagnosis of systemic lupus erythematosus defined by the American College of Rheumatology criteria [17], consecutively followed at the Lupus Unit of the Hospital Santa Izabel in Salvador, Bahia, Brazil from 1996 to 1998. This population corresponds to the total number of cases followed at this unit at the time of the study. Each patient was submitted to a complete clinical assessment that included history, physical examination and routine tests. Special emphasis was given to the investigation of thrombotic complications and obstetric morbidity that represent the clinical criteria for the classification of APS [9].
2. Patients with infectious diseases, divided into three categories: (1) patients with diagnosis of Kala-azar, based on clinical features, serologic tests and myelogram, (2) patients with a clinical and serologic diagnosis of leptospirosis in acute phase, and (3) patients with primary or secondary syphilis followed at the Sexually Transmitted Diseases Unit. All patients had positive Venereal Disease Research Laboratory (VDRL) and fluorescent treponemal antibody absorbed (FTA-ABS) tests.

Methods

β2 GP I purification

Human β2 GP I was purified by perchloric acid treatment of normal serum followed by heparin-sepharose affinity purification as described previously [18].

Anti-β2 GP I assay

This assay was performed as described previously by Roubey et al. [19] with some modifications. Microtitration high-binding plates (Costar 3590) were coated with 50 µl/well of β2 GP I in phosphate-buffered solution (PBS) (10 µg/ml) and left overnight at 4°C. The plates were blocked (100 µl/well) for 1 h with 2% ovalbumin (Sigma Chemicals, St. Louis, Mo., USA) in PBS. After washing with PBS the samples were diluted 1:100 in 1% ovalbumin, 0.5% Tween 20 were added in duplicate (50 µl) to the plate and incubated for 2 h at room temperature. The alkaline phosphatase conjugated goat anti-human IgG diluted 1:5000 in diluent solution was added (50 µl/well) and incubated for 1 h. The reaction was developed using substrate p-nitrophenyl phosphate diluted in diethanolamine buffer and the optical density at 405 nm was read using a microplate reader. Furthermore, a positive control (patient with high aCL titre and APS) was used in each plate at three different dilutions: 1/100, 1/200 and 1/400. The colour reaction in each plate was stopped when OD of the 1/100 dilution of the positive control reached 1.0 OD (this was approximately 15–20 min under the experimental conditions we used for this assay). The results were considered positive when the OD obtained exceeded that of the mean value plus 3 standard deviations of 50 sera from normal healthy individuals. Different runs were normalised by using ten normal sera in each plate of anti-β2 GP I enzyme-linked immunosorbent assay (ELISA). In order to express the results in units, the OD of each sample was subtracted from the cutoff and multiplied by 100.

Anticardiolipin assay

The ELISA for anticardiolipin was performed as described elsewhere [20]. International calibrators (Louisville APL Diagnostics, Doraville, Ga., USA) were used to construct a calibrator curve and to express the results in GPL units. Degrees of positivity were considered as follows: high positive >80 GPL/units, medium positive $\geq$20–80 units, low positive $\geq$10, <20 units.

Statistical analysis

The association between qualitative variables was determined by chi-square test or Fisher's exact test when necessary. Spearman's correlation coefficient was used

when appropriate. The results were considered statistically significant when $p < 0.05$.

Results

SLE patients

The present study included 152 patients with a diagnosis of SLE, 147 females and 5 males, with a mean age of 33 years. Of these patients, deep venous thrombosis was observed in three (1.9%) and arterial thrombosis in seven patients (4.6%). Twenty-seven patients showed obstetric complications that culminated with foetal losses. The presence of one or more of the clinical features of APS was observed in 34 of the 152 patients (22.3%). The demographic data and main clinical and laboratory characteristics of the SLE patients are showed in Table 1.

Moderate to high levels of IgG aCL antibodies were detected in 55 of 152 patients (36.1%) and anti-β2 GP I in 36 of 152 patients (23.6%). No significant association between the presence of aCL antibodies in moderate to high levels and arterial ($p = 0.25$) or venous thrombosis ($p = 0.29$) was found. When we analysed the patients who became pregnant, we found a trend for a higher frequency of these antibodies in the patients with obstetric morbidity, with a marginal statistical significance ($p = 0.055$). Likewise, when we analysed the features of APS as a group we also found no statistically significant association with these antibodies ($p = 0.35$). The presence of anti-β2 GP I antibodies was also not associated with arterial thrombosis ($p = 0.35$), venous thrombosis ($p = 1.0$), obstetric morbidity ($p = 0.30$) or with these clinical complications as a whole ($p = 0.17$). In this group of SLE patients we observed a positive correlation between aCL and anti-β2 GP I antibody level ($r = 0.26$, $p < 0.002$).

Table 1 Clinical and laboratory features of the 152 SLE patients. *OTC* obstetric and/or thrombotic complications

Age (mean ± SD)	33 ± 12 years
Gender (female/male)	147/5 (96.7/3.3%)
Arthralgia/arthritis	148 (97.3%)
Fever	117 (76.9%)
Discoid lesion	26 (17.1%)
Photosensitivity	101 (66.4%)
Malar rash	80 (52.6%)
Mucous ulcers	63 (41.4%)
Raynaud	71 (46.7%)
Leucopenia[a]	41 (26.9%)
Hemolytic anemia	7 (4.6%)
Thrombocytopenia[b]	12 (7.8%)
Proteinuria[c]	43 (28.2%)
Casts	18 (11.8%)
Renal disfunction[d]	21 (13.8%)
Seizures	17 (11.1%)
Psychosis	7 (4.6%)
Pleuritis	39 (25.6%)
Pericarditis	19 (12.5%)
Ascites	8 (5.2%)
Obstetric morbidity[e]	27/88 (30.6%)
Venous thrombosis	3/152 (1.9%)
Arterial thrombosis	7/152 (4.6%)
OTC	34/152 (22.3%)
ANA (IFI)	150/152 (98.6%)
LE cells	38/127 (29.9%)
Anti-DNAn (IFI)	14/103 (13.5%)
Anti-Sm	14/103 (13.5%)
Anti-RNP	45/100(45%)
Anti-SSA	20/103 (19.4%)
Anti-SSB	1/100 (1%)
Rheumatoid factor	14/111 (12.6%)

[a]WBC $< 4000/mm^3$
[b]Platelet $< 100.000/mm3$
[c]Proteinuria + + + + or > 500 mg/24 h
[d]Creatinine higher than reference value
[e]Only 88 patients had been pregnant

Patients with infectious diseases

A total of 143 patients with infectious diseases were studied, distributed into three groups: 30 patients with a diagnosis of Kala-azar, 39 patients with leptospirosis and 74 with primary or secondary syphilis. IgG aCL was detected in the serum of two patients with Kala-azar (6.6%), one in low level and another in moderate level. Nine patients with leptospirosis (23%) had IgG aCL, three in low, five in moderate and one in high level, respectively. Amongst the patients with syphilis, IgG aCL was detected in 14 (18.9%), 4 in low level, 9 in moderate level and 1 in high level. If we consider only the values of aCL moderate or high, the frequencies of these antibodies in Kala-azar, leptospirosis and syphilis would be, respectively, 3.3% (1 of 30), 15.3% (6 of 39) and 13.5% (10 of 74).

Anti-β2 GP I antibodies of the IgG class were found in the serum of 16 of 30 patients with Kala-azar (53.3%), 6 of 34 patients with leptospirosis (17.6%) and 8 of 70 patients with syphilis (11.4%). There was no correlation among the levels of aCL and anti-β2 GP I antibodies in the serum of these patients ($r = 0{,}12$, $p = 0.17$), different from that observed in the serum of the patients with SLE. None of the patients in the infectious diseases group had a history of thrombosis or obstetric complications.

Performance of the aCL and anti-β2 GP I assays for identification of clinical manifestations of APS

It was observed that in the whole population studied (295 patients), only 34 patients, all of them SLE patients, showed clinical manifestations of APS (arterial or venous thrombosis, obstetric morbidity), i.e. a prevalence of 11%. On the other hand, the prevalence of anti-β2 GP I and aCL antibodies in moderate to high levels in the whole population was 23% (66 of 286) and 24% (72 of 295). The sensitivity, specificity, predictive positive value (PPV), predictive negative value (PNV) and likelihood ratio (LR) for a positive test of anti-β2 GP I for identification of the clinical manifestation of APS

were the following: sensitivity of 29% [95% confidence interval (CI) = 24–34%], specificity of 78% (95% CI = 73–83%), PPV of 15% (95% CI = 11–19%), PNV of 89% (95% CI = 85–93%) and LR of 1.38, which means that the test was not much more positive in patients with the clinical manifestations of APS than in those without such manifestations. Regarding the performance of the anticardiolipin test, a sensitivity of 29% (95% CI = 24–34%), specificity of 76% (95% CI = 71–81%), PPV of 14% (95% CI = 10–18%), PNV of 89% (95% CI = 86–92%) and LR for a positive test of 1.26 were observed.

Discussion

The development of assays for the detection of antiphospholipid antibodies constituted a historical landmark in the study of autoantibodies in rheumatology. A great deal of studies has been reported on its association with thrombotic episodes and foetal losses, particularly in patients with connective tissue diseases. In addition, great effort has been expended in the attempt to explain the pathogenic mechanism of these antibodies.

Although the majority of authors has demonstrated a high sensitivity of the aPL tests as markers for those clinical complications, the demonstration of the presence of aCL antibodies in patients with infectious diseases without any evidence of thrombosis tends to decrease the specificity of these tests.

In the last few years, an ELISA was developed for the detection of antibodies against purified β2 GP I, and recently published data have suggested that this test would be superior in specificity to anticardiolipin assay as a marker for thrombotic and obstetric complications [13, 14, 15, 16]. Guerin et al. [16] observed high levels of anti-β2 GP I antibodies in patients with putative APS and only marginal levels of these antibodies in patients with different clinical situations. In the experience of these authors the sensitivity and specificity of anti-β2 GP I for the identification of APS were of 83% and 95%, respectively. Moreover, Sanmarco et al. [21] demonstrated a prevalence of anti-β2 GP I in APS patients of 54% in contrast to 0.7% in patients with different infectious diseases.

In the present study, the prevalence of anti-β2 GP I and aCL in the patients with SLE was 23.6% and 36.1%, respectively, but no association with the clinical manifestations of the APS was found. Curiously, previous studies performed in different areas of Brazil have also not shown an association between aCL antibodies and clinical features of APS [22, 23]. It should be emphasised, however, that these results should be interpreted cautiously, since in addition to arterial and venous thrombosis, the APS criteria include rigorous data on obstetric morbidity that demand information not always easily obtained from the patients when performing a cross-sectional study.

Regarding the performance of these tests, the sensitivity, specificity, PPV and NPP of the anti-β2 GP I assay were 29%, 78%, 15% and 89%, while the figures for the aCL test were 29%, 76%, 14% and 89%, respectively. Based on these results, as the validity and performance of anti-β2 GP I assay were similar to the aCL in demonstrating the presence of clinical phenomena associated to APS and due to the difficulties in performing as well as the lack of standardisation of the anti-β2 GP I test, we suggest that the test for aCL should continue to be the first one performed when the presence of APS is suspected.

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