Clinical Immunology 194 (2018) 92–99

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Thrombotic risk assessment and analytical performance of the chemiluminescent analyzer IDS-iSYS for the detection of anti-cardiolipin and anti-beta 2 glycoprotein I autoantibodies

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ARTICLE INFO

Keywords:
Anti-cardiolipin
Anti-β2GPI
Thrombosis
Anti-phospholipid syndrome
Chemiluminescence

ABSTRACT

Patients with antiphospholipid antibodies (APLA) are predisposed to develop thrombosis, however the standardization of anti-cardiolipin (aCL) and anti-beta 2 glycoprotein I (β2-GPI) Ab assays are challenging. Therefore we decided to test the performance of a new chemiluminescent assay (CLIA), and assayed aCL and aβ2-GPI IgG/M in serum from 120 healthy individuals, 108 patients with idiopathic venous thrombosis, 78 patients with antiphospholipid syndrome (APS), and 64 non-thrombotic APLA-carriers using CLIA IDS-iSYS. Very good (aCL/aβ2-GPI IgG) to moderate (aCL/aβ2-GPI IgM) agreement with a commercial and an in house ELISA assay were observed and, in particular, CLIA demonstrated the highest sensitivity in aβ2-GPI IgG detection. Finally, aCL/aβ2-GPI Ab capacity to predict the thrombotic risk was tested showing for CLIA a significant odds ratio (OR) when considering double positivity for aCL/aβ2-GPI IgG, aCL IgG at high levels, and aβ2-GPI IgG at high levels. In conclusion, CLIA improves aβ2-GPI IgG detection and thrombotic risk assessment.

1. Introduction

Persistent anti-phospholipid antibody (APLA) detection represents a risk factor for thrombosis and, in particular, in those patients with systemic autoimmune diseases (SADs). In SADs, APLA ranges from 15 to 45% with the highest prevalence reported in systemic lupus erythematosus (SLE). Among SLE patients with APLA, the annual rate of thrombosis is 4% in contrast to 0.1% in the normal population [1]. In order to explain why vascular manifestations occur occasionally in spite of the persistent presence of APLA, a "two-hit" hypothesis was proposed. The hypothesis suggests a concomitant role of APLA on endothelial cells, platelets and activation of immune cells on one hand while, on the other hand, there is a role for precipitating events that induce thrombotic consequences *via* complement and coagulation cascade activation. Precipitating events are related to infections, oral contraceptives, surgery, immobility, and associated SAD [2–5]. APLA represent a heterogeneous family with beta2 glycoprotein I (β2-GPI)-dependent anticardiolipin (aCL) autoantibodies (Ab) and lupus anticoagulant (LAC) as the main pathogenic Ab [6–8]. By definition, the antiphospholipid syndrome (APS) links the persistent presence (> 12 weeks) of APLA with vascular thrombosis and/or pregnancy morbidity [9,10].

Although aCL and aβ2-GPI IgG/M tests are essential for APS diagnosis, their determination remains challenging since "non-thrombotic" APLA are also found in patients with recent viral infections, patients with malignancies, patients receiving drugs such as procaïnamide and chlorpromazine, and even in otherwise healthy individuals [11]. In addition, aCL and aβ2-GPI IgG/M tests are usually performed using

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https://doi.org/10.1016/j.clim.2018.07.006
Received 25 June 2018; Accepted 10 July 2018
Available online 11 July 2018

Table 1
Demographic, clinical and immunological characteristics of the patients.

Cohort characteristics	APS	APLA-carriers	Idiopathic thrombosis	Healthy controls
Number	78	64	108	120
Age (years)	49 ± 13	53 ± 17	58 ± 16	48 ± 13
Sex (F:M)	55:23	54:10	35:73	98:22
APS-I	70	0	0	0
APS-II/SAD and inflammatory diseases	8	47	0	0
Infections/Cancers/Other	0/0/0	3/2/12	0	0/0/0
Thrombosis/fetal loss	78	0	108	0
aCL IgG CLIA (GPL/mL, mean ± SD)	447 ± 887*	66 ± 167	0.31 ± 0.75	0.05 ± 0.50
aCL IgM CLIA (MPL/mL, mean ± SD)	32 ± 71	52 ± 111#	1.56 ± 3.42	1.12 ± 2.41
aβ2-GPI IgG CLIA (AU/mL, mean ± SD)	735 ± 1545*	86 ± 215	0.65 ± 1.82	0.29 ± 0.76
aβ2-GPI IgM CLIA (AU/mL, mean ± SD)	33 ± 67	77 ± 193#	1.58 ± 3.28	4.87 ± 3.18

Abbreviations: aCL: anti-cardiolipin antibodies; aβ2GPI: anti-β2 glycoprotein I antibodies; APS: antiphospholipid syndrome primary (I) or secondary (II); APLA: antiphospholipid antibodies; SAD: systemic autoimmune diseases; SD: standard deviation; CLIA: chemiluminescence immunoassay; AU: arbitrary unit; mL: milliliter. Multiple comparisons were performed using multiple one-way ANOVA and the Tukey's test was used for post-hoc corrections: significant for *APS *versus* APLA/idiopathic thrombosis/healthy control groups; #APLA *versus* idiopathic thrombosis/healthy control groups.

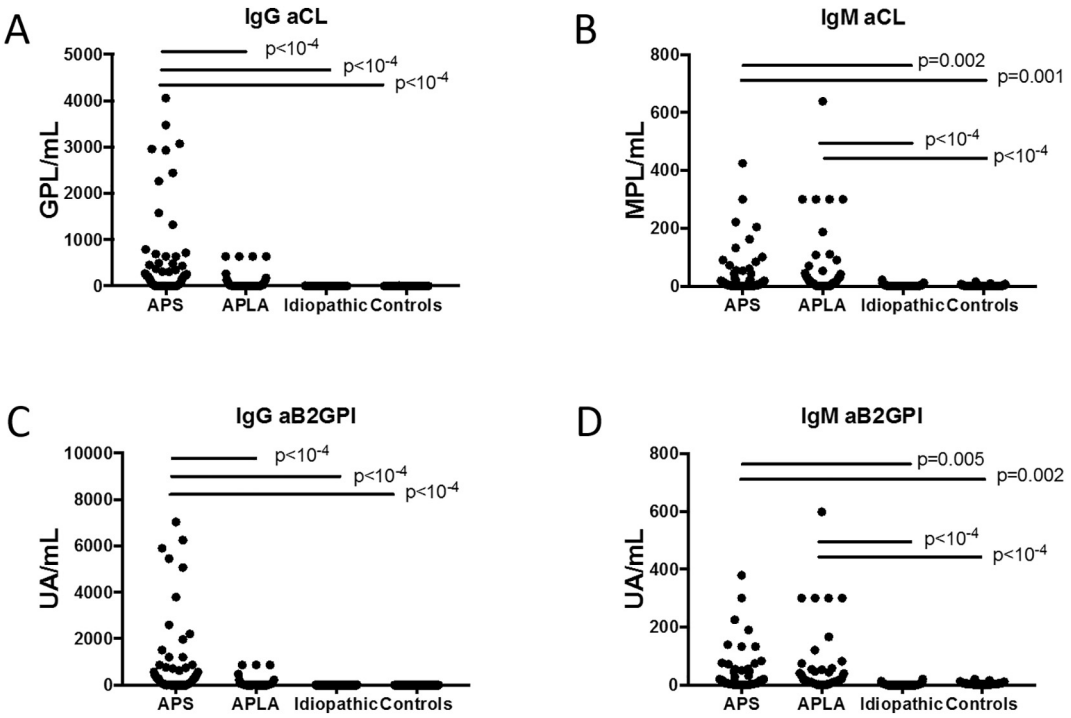

Fig. 1. Antiphospholipid antibody (APLA) determination in 78 patients with antiphospholipid syndrome (APS), 64 non-thrombotic APLA-carriers, 78 patients with idiopathic venous thrombosis (idiopathic), and 120 healthy controls (controls) using the IDS-iSYS chemiluminescence immunoassay (CLIA).

enzyme linked immunoassays (ELISA) that present major limitations in terms of robustness, reproducibility, inter-laboratory comparability and standardization although several international recommendations have been proposed [12,13]. This is particularly challenging since a lack of specificity associated with a high number of false positive results may lead to mistaken decisions that lead to an elevated risk of hemorrhaging due to prescribing unnecessary oral anticoagulants, while, in the case of false negative results, there is a risk of recurrent thrombotic events.

In order to circumvent ELISA limitations for aCL and aβ2-GPI IgG/M detection, it has been stated that chemiluminescence assays (CLIA) represent an interesting alternative with similar clinical performance and, moreover, technological advantages in terms of reproducibility, repeatability, linearity and limits of detection [14]. However, those studies were limited since cut-off levels were not re-evaluated and comparisons were made with only one ELISA, either a homemade or commercial version [15,16]. With the introduction of the IDS-iSYS system using Zenit RA reagents produced by Technogenetics, the time has come to evaluate this alternative from the technical point of view and from its capacity to predict thrombotic risk events, which were the two aims of the present study.

2. Material and methods

2.1. Patient selection

A total of 370 sera were tested in this retrospective study from the Brest Rheumatology and the Internal Medicine departments between 2014 and 2018 (Table 1), including 70 samples from patients with primary APS and 8 with secondary APS, 64 non-thrombotic patients carriers for APLA associated with SADs/inflammatory diseases (SLE $n = 26$, rheumatoid arthritis $n = 5$, idiopathic purpura thrombopathic $n = 4$, primary Sjögren's syndrome n = 2, other SAD/inflammatory diseases $n = 10$), infections ($n = 3$), cancer (n = 2) or other diseases ($n = 12$), 108 patients with idiopathic and unprovoked venous thrombosis occurring in the absence of a major risk factor as previously reported [17], and 120 healthy controls (HC). HC included subjects