Full Length Article

Significance of fully automated tests for the diagnosis of antiphospholipid syndrome

Kenji Oku [a,*], Olga Amengual [a], Masaru Kato [a], Toshiyuki Bohgaki [a], Tetsuya Horita [a], Shinsuke Yasuda [a], Naoya Sakamoto [b], Masahiro Ieko [c], Gary L. Norman [d], Tatsuya Atsumi [a]

[a] Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[b] Department of Gastroenterology and Hepatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[c] Department of Internal Medicine, Health Science University of Hokkaido, To-betsu Cho, Japan
[d] Inova Diagnostics Inc., San Diego, CA, USA

ABSTRACT

Antiphospholipid antibodies (aPLs) can vary both immunologically and functionally, thus it is important to effectively and correctly identify their presence when diagnosing antiphospholipid syndrome. Furthermore, since many immunological/functional tests are necessary to measure aPLs, complete examinations are often not performed in many cases due to significant burden on the testing departments. To address this issue, we measured aPLs defined according to the classification criteria (anticardiolipin antibody: aCL) IgG/IgM and anti-β_2 glycoprotein I antibody (aβ_2GPI) (IgG/IgM) as well as non-criteria antibodies (aCL IgA, aβ_2GPI IgA and aβ_2GPI domain I), in a cohort of 211 patients (61 APS, 140 disease controls and 10 healthy individuals). APLs were measured using a fully automated chemiluminescent immunoassay instrument (BIO-FLASH®/ACL AcuStar®) and with conventional ELISA tests. We demonstrated that both sensitivity and accuracy of diagnosis of aCL IgG and aβ_2GPI IgG were high, in agreement with the past reports. When multiple aPLs were examined, the accuracy of diagnosis increased. The proportion of APS patients that were positive for 2 or more types of aPLs (47/61, 77%) was higher than that of patients with systemic lupus erythematosus (SLE)(3/37, 9%), those with non-SLE connective tissues diseases (1/53,2%), those with other diseases or healthy volunteers. Based on these findings, it was concluded that the fully automated chemiluminescent immunoassay instrument, which allows the simultaneous evaluation of many types of aPLs, offers clear advantages for a more complete, more rapid and less labor-intensive alternative to running multiple ELISA and could help in better diagnosis for suspected APS patients.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Antiphospholipid syndrome [1] is a condition in which the antibody group, collectively referred to as antiphospholipid antibodies (aPLs), leads to autoimmune thrombosis and pregnancy complications. Recently, there has been a proposal to revise the Sapporo international classification criteria of APS, known as the Sapporo criteria-Sydney revision [2]. APLs broadly refers to autoantibodies that bind to various phospholipids or to plasma proteins after they combine to form phospholipid complexes. However, not all types of these antibodies exhibit pathogenicity. As antiphospholipid syndrome(APS)-related aPL, or aPL with pathogenicity, anticardiolipin antibody (aCL) IgG/IgM, anti-β_2 glycoprotein I (anti-β_2GPI antibody [aβ_2GPI]) IgG/IgM, and lupus anticoagulant

have been defined, according to the classification criteria [2]. It has also been reported that in addition to IgG and IgM antibody isotypes, IgA antibodies to aCL or aβ_2GPI can be detected in some APS patients, but the pathological importance of such findings remains unclear [3]. Furthermore, it has been reported that autoantibody to a specific part (domain 1) of the β_2GPI molecule correlated significantly with APS thrombosis (anti-β2GPI domain 1 antibody: aβ_2GPI D1) [4].

The Automated Coagulation Laboratory (ACL) Acustar (Instrumentation Laboratories, USA) is an instrument (also known as the BIO-FLASH instrument) that allows quantitative measurement of autoantibodies using a chemiluminescence immunoassay (CIA) that is gaining acceptance in clinical practice [5–7]. Paramagnetic beads are coated with cardiolipin or β_2GPI and form the basis for the measurement. After incubation of the magnetic beads with blood(serum/plasma) samples, magnetic separation, and washing of the beads, a tracer is added. The tracer consists of isoluminol-labelled anti-human IgG antibody or anti-human IgM antibody which will bind to any antibodies captured on the beads. Following another incubation, an agent is then added to induce

* Corresponding author at: Division of Rheumatology, Endocrinology and Nephrology, Hokkaido University Graduate School of Medicine, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan.
E-mail address: kenoku@med.hokudai.ac.jp (K. Oku).

chemiluminescence, the amount of light emitted is measured, and a quantitative evaluation is made in relative light units (RLUs). RLU are converted to chemiluminescent units (CU)/ml using an assay-specific standard curve. The upper limit of normal (ULN) value is set at 20 CU/ml for all assays, based on the 99th-percentile of healthy volunteers in the USA.

In this study, we compared the diagnostic performance of BIO-FLASH/ACL Acustar assays to ELISA versions of these assays on specimens from a cohort of Japanese patients with collagenosis, including APS.

2. Patients and methods

2.1. Patients

We selected 61 consecutive patients with APS (including 55 patients with primary APS) from whom serum was preserved when they visited the rheumatology outpatient department of Medicine II, Hokkaido University Hospital or the outpatient Department of Internal Medicine, Health Sciences University of Hokkaido, from April 2005 to March 2013. Control subjects were selected from patients who visited the medical facilities during the same period and consisted of 37 patients with systemic lupus erythematosus (SLE) without complications of thrombotic/obstetric events, 53 patients with non-SLE connective tissue diseases (CTD: included 24 subjects with rheumatoid arthritis, 7 with scleroderma, 4 with myositis, 6 with vasculitis syndrome, 5 with Sjorgen's syndrome, and 7 with other autoimmune diseases).

Non-autoimmune patients were also selected as a control. Sixteen patients who were diagnosed as non-CTD with APS mimicking disease (non-autoimmune thrombosis, pregnancy complication) were included. Additionally, 34 outpatients of Department of Gastroenterology and Hepatology, Hokkaido University Hospital, who were diagnosed as having chronic virus hepatitis were selected. As previously reported, production of the transient aPL is often related to infections [8]. Among them, the chronic virus hepatitis are reported as prone to produce aPL [9,10].

Two hundred and one subjects in total were examined in addition to 10 healthy volunteers. There were no significant differences between the APS group and the other patient groups with respect to age and sex. The diagnosis of antiphospholipid antibody syndrome was made according to the Sydney revision of the Sapporo criteria [2] by the expert rheumatologists.

2.2. Measurement of antiphospholipid antibody

After obtaining informed consent from each subject and explaining the aim of this study, we measured aCL IgG/IgM, and $a\beta_2GPI$ IgG/IgM in the preserved serum samples using the QUANTA Flash Anti-phospholipid Assay Panel (INOVA Diagnostics, USA.; APL CIA panel), and aCL IgG/IgM, $a\beta_2GPI$ IgG/IgM with a home-made ELISA that was prepared using a standardized in-house protocol [11,12]. We also examined aCL IgA, $a\beta_2GPI$ IgA, and $a\beta_2GPI$ D1 IgG, which have not been defined in the classification criteria, using the APL CIA panel.

As noted above, the upper limit of normal (ULN) for each component of the APL CIA panel was set at 20 CU/ml. As reported previously [13], the cutoff values of the home-made ELISAs were set at 18.5 IgG phospholipid units or higher for IgG aCL and 7.0 IgM phospholipid units or higher for IgM aCL, based on the 99th percentile of 132 healthy control volunteers. Furthermore, the cutoff value of $a\beta_2GPI$ was set at 2.2 units/ml or higher for IgG and 6.0 units/ml or higher for IgM.

2.3. Analysis methods

We measured the sensitivity, specificity, likelihood ratio, and odds ratio of the each CIA-measured aPLs on APS diagnosis. The gold standard of the APS diagnosis was the expert diagnosis of the rheumatologists according to the Sapporo criteria Sydney revision. We used the Kruskal-Wallis test to the compare aPLs titers among patient groups. The concordances of the ELISA- and CIA-measured aPLs were analyzed with the Cohen's kappa test. We considered the differences as being statistically significant when the p values were 0.05 or lower. All analyses were performed using XLSTAT® (Addinsoft, France).

3. Results

3.1. Measurements with the APL CIA panel

The range of measurement values for the entire cohort obtained with the APL CIA panel was aCL IgG 177.9 (0 [minimum]–5955.7 [highest]) CU/ml, aCL IgM 15.5 (0–678.4) CU/ml, aCL IgA 10.5 (0–267.7) CU/ml, $a\beta_2GPI$ IgG 755.6 (0–52,115.1) CU/ml, $a\beta_2GPI$ IgM 21.12 (0–1471.3) CU/ml, $a\beta_2GPI$ IgA 13.8 (0–350.9) CU/ml, and $a\beta_2GPI$ DI 3.6 (0–3843.7) CU/ml. The proportions of positive findings for each antibody measurement (20 CU/ml or higher) were 51/211 subjects (24.2%), 18/211 (8.5%), 22/211 (10.4%), 59/211 (28.0%), 18/211 (8.5%), 22/211 (10.4%), and 30/211 (14.2%), respectively.

When comparing the antibody values between the APS, SLE, non-SLE collagen diseases, other diseases, and hepatic disease groups using the APL panel, the APS group exhibited significantly higher titers than other patient groups for all antibody tests (Fig. 1). The 10 healthy volunteers were negative for all aPL tests with both the homemade ELISA and the APL CIA panel.

3.2. Measurement accuracy with the APL CIA panel

Table 1 presents the sensitivity, specificity, positive and negative likelihood ratios, odds ratio and probability of correct classification of the APL CIA panel in the diagnosis of APS. Thus, the sensitivities represent the positive rates of each aPLs in APS patients and specificities represent the negative rate of aPLs in non-APS patients. The APL CIA panel showed a specificity of 90% or higher for all antibody tests. In contrast, the sensitivities for IgM aCL and IgM $a\beta_2GPI$ in the diagnosis of APS were comparatively low (25.0% and 28.3%, respectively), as were those for IgA aCL and IgA $a\beta_2GPI$ (26.2% and 27.9%, respectively) (Table 1). The APL CIA panel showed comparatively high probabilities for correct classification; 0.86 for IgG aCL and IgG $a\beta_2GPI$. When examination was performed for aPL alone, which was defined according to the classfication criteria, the findings for single positive cases in the APS group, were aCL IgG 3/61 subjects (4.9%), aCL IgM 1/61 (1.6%), $a\beta_2GPI$ IgG 4/61(6.6%), and $a\beta_2GPI$ IgM 3/61(4.9%).

The positivity for aCL IgA and $a\beta_2GPI$ IgA was similar in all patients. Additionally, all patients with positive IgA aCL and/or IgA $a\beta_2GPI$ were positive for at least two criteria-defined aPLs.

Positivity for $a\beta_2GPI$ D1 was observed in 50.8% (31/61) of the APS patients and in none of the controls. Single positivity for $a\beta_2GPI$ D1 was observed only in 1/61 APS patients.

3.3. APL CIA panel and the homemade ELISA

Positivity and titers for the aPLs were compared between the APL CIA panel and the homemade ELISA (Table 2).

When the concordance rates of positive/negative antibody related to the APS diagnosis were compared between the APLCIA panel and conventional tests, it was found that the κ value was 0.55 or higher for individual test items, suggesting that homology with conventional tests was fair (Table 3).

Similar results were confirmed when the data of patients with collagenosis (patients with APS, SLE, and non-SLE) and patients who visited the rheumatology outpatient department (patients with and without collagen diseases who visited the rheumatology outpatient department) were used as the test cohort.