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Full Length Article

Significance of fully automated tests for the diagnosis of antiphospholipid syndrome

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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.

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

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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 BIOFLASH/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 aβ_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, aβ_2GPI IgG/IgM with a home-made ELISA that was prepared using a standardized in-house protocol [11,12]. We also examined aCL IgA, aβ_2GPI IgA, and aβ_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 aβ_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, aβ_2GPI IgG 755.6 (0–52,115.1) CU/ml, aβ_2GPI IgM 21.12 (0–1471.3) CU/ml, aβ_2GPI IgA 13.8 (0–350.9) CU/ml, and aβ_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 aβ_2GPI in the diagnosis of APS were comparatively low (25.0% and 28.3%, respectively), as were those for IgA aCL and IgA aβ_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 aβ_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%), aβ_2GPI IgG 4/61(6.6%), and aβ_2GPI IgM 3/61(4.9%).

The positivity for aCL IgA and aβ_2GPI IgA was similar in all patients. Additionally, all patients with positive IgA aCL and/or IgA aβ_2GPI were positive for at least two criteria-defined aPLs.

Positivity for aβ_2GPI D1 was observed in 50.8% (31/61) of the APS patients and in none of the controls. Single positivity for aβ_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.

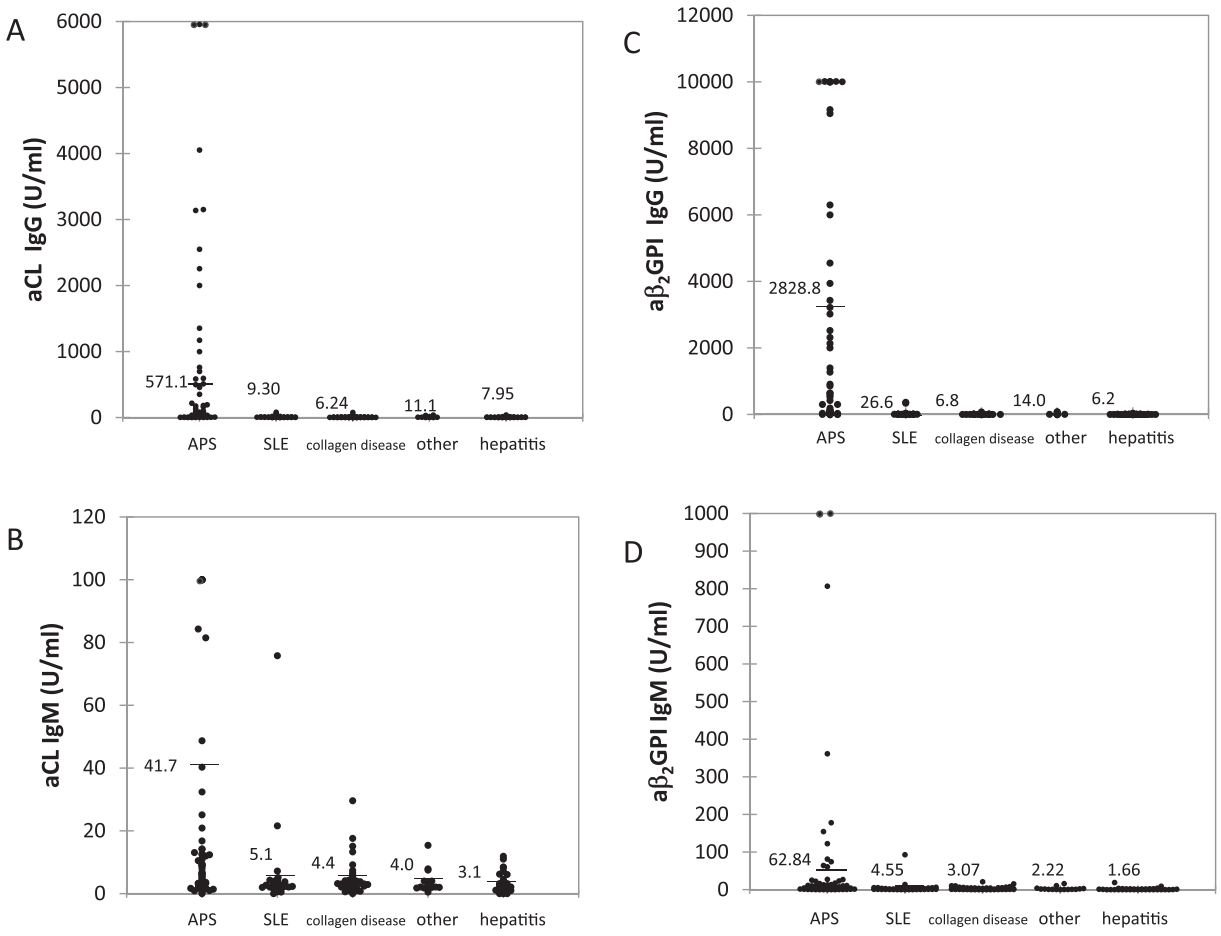

Fig. 1. Antiphospholipid antibody values calculated for individual patient groups using the APL CIA panel. We measured aCL IgG(A), aCL IgM(B), aβ_2GPI IgG(C), and aβ_2GPI IgM(D) using the APL CIA panel for patients with antiphospholipid antibody syndrome [1], systemic lupus erythematosus (SLE), non-SLE collagen disease (collagen) who visited the rheumatology outpatient department (other), and gastrointestinal disease (chronic virus hepatitis; hepatitis). The vertical axis shows the individual antibody values (unit: CU/ml). The APS groups exhibited higher values for all antiphospholipid antibodies ($p < 0.0001$, Kruskal-Wallis test) compared with the other groups. The data of aCL IgG, aCL IgM, aβ_2GPI IgG, and aβ_2GPI IgM are plotted with 6000 CU/ml, 100 CU/ml, 12,000 IU/ml, and 1000 CU/ml used as the upper limits, respectively, and the data that showed higher titers are also plotted as the upper limit.

3.4. APS diagnosis and the number of positive aPLs

The specificities for APS diagnosis were elevated and the sensitivities or probabilities of classification declined as the number of positive criteria-defined aPLs was increased in the APL panel analysis. The sensitivity and/or the probability classification of a diagnosis of APS in patients with more than one positive criteria-defined aPL was better than that of patients only a single specific aPL. The number of positive aPL antibodies detected in the various patient groups is shown in Table 4. Only ten of the total cohort of 211 patients were positive for all four criteria-defined aPLs. All ten were APS patients. Triple positivity for APL antibodies was found in 4 patients with APS, one patient with SLE, and none in any of the other clincial groups. Patients with three positive criteria-defined aPLs were more often observed in APS patients (4/61:7%) compared with the other groups (1/37:3%) in lupus patients and none in the other groups. Similarly the frequency of APS patients with two positive criteria-defined aPLs was higher (22/61:44%) than the in incidence observed in the other groups (2/37(6%) for lupus, 1/53 for non-lupus autoimmune disease, 0/34 for hepatitis and 0/10 among the healthy controls). Patients with multiple-positive aPLs were more often observed in the APS group (47/61: 77%) compared with the other groups (lupus 3/37, non-SLE CTD 1/53, non-autoimmune 1/16, hepatitis 0/34, healthy 0/10; $p < 0.0000001$ Kruskal-Wallis test).

4. Discussion

The current findings confirm that the utility of the APL panel was similar to conventional ELISA methods. Past reports suggested that IgG tests (aCL, aβ_2GPI) show higher sensitivity for the diagnosis of APS, while IgM assays show higher specificity and this was also observed in the current findings. There are recent reports that analyzed IgG/M aCL and aβ_2GPI with the APL CIA panel in primary APS compared with the non-APS control patients with APS manifestations (thrombosis and/or pregnancy morbidity) [14,15]. The studies analyzed the sensitivity and specificity of each aPLs individually and reported that the APL CIA panel has similar sensitivities and specificities to the comparable ELISA tests. In this report, the sensitivities and specificities of each CIA panel tests compared with the past two reports, had mild to moderate discrepancies possibly due to the different backgrounds of the patient cohorts.

The present study is the first to analyze the significance of the CIA panel in the real-life setting of patients with autoimmune diseases in the rheumatology clinic. Also, it is the first to clarify the significance of multiple-positivity determinations using an automated instrument for the diagnosis of APS. Additionally, a wide variety of control groups (37 SLE, 53 non-SLE CTD patients and 16 non-CTD patients with resembling manifestations) were included in our study, adding significant robustness to the specificity analysis.

Table 1
Results of measurement of patients with antiphospholipid antibody syndrome using the APL CIA panel.

	Sensitivity % (95%CI)	Specificity % (95%CI)	PLR (95%CI)	NLR (95%CI)	OR (95%CI)	Class (95%CI)
A Testing with current criteria assays only (IgA and aβ$_2$GPI D1 assays excluded)						
aCL IgG	68.3 (55.0–79.7)	93.7 (88.4–97.1)	10.9 (5.8–20.8)	0.34 (0.23–0.47)	32.1 (13.6–75.8)	0.86 (0.82–0.91)
aβ$_2$GPI IgG	75.0 (62.1–85.3)	90.2 (84.1–94.5)	7.7 (4.61–12.9)	0.28 (0.17–0.41)	27.6 (12.4–61.5)	0.86 (0.82–0.91)
aCL IgM	25.0 (14.7–37.9)	97.9 (94.0–99.6)	11.9 (3.8–37.4)	0.77 (0.64–0.86)	15.6 (4.6–52.5)	0.76 (0.70–0.82)
aβ$_2$GPI IgM	28.3 (17.5–41.4)	99.3 (96.2–100.0)	40.5 (7.1–236.0)	0.72 (0.60–0.82)	56.1 (9.1–339)	0.78 (0.72–0.84)
Positive for any item	84.0 (64.6–94.1)	90.1 (82.0–94.9)	8.49 (4.46–16.2)	0.18 (0.07–0.44)	47.83 (14.2–161)	0.88 (0.83–0.95)
Positive for the second and subsequent items	67.2% (54.3–77.9)	93.4% (87.8–96.6)	10.24 (5.31–19.7)	0.35 (0.24–0.51)	29.2 (12.4–68.5)	0.86 (0.81–0.91)
Positive for the third and subsequent items	20.7% (12.2–33.0)	99.3% (95.5–100)	28.3 (3.77–213)	0.80 (0.70–0.91)	35.5 (6.32–199)	0.76 (0.70–0.82)
Positive for the fourth item	17.2% (9.5–29.2)	100% (96.6–100)		0.83 (0.74–0.93)		0.75 (0.69–0.81)
B Testing by criteria assays and IgA aCL, IgA aβ$_2$GPI, and aβ$_2$GPI D1						
aCL IgG	68.3 (55.0–79.7)	93.7 (88.4–97.1)	10.9 (5.8–20.8)	0.34 (0.23–0.47)	32.1 (13.6–75.8)	0.86 (0.82–0.91)
aβ$_2$GPI IgG	75.0 (62.1–85.3)	90.2 (84.1–94.5)	7.7 (4.61–12.9)	0.28 (0.17–0.41)	27.6 (12.4–61.5)	0.86 (0.82–0.91)
aCL IgM	25.0 (14.7–37.9)	97.9 (94.0–99.6)	11.9 (3.8–37.4)	0.77 (0.64–0.86)	15.6 (4.6–52.5)	0.76 (0.70–0.82)
aβ$_2$GPI IgM	28.3 (17.5–41.4)	99.3 (96.2–100.0)	40.5 (7.1–236.0)	0.72 (0.60–0.82)	56.1 (9.1–339.4)	0.78 (0.72–0.84)
aCL IgA	26.2 (16.8–38.6)	96.7 (92.2–98.9)	7.92 (3.04–20.7)	0.76 (0.66–0.89)	10.4 (3.74–18.8)	0.76 (0.71–0.82)
aβ$_2$GPI IgA	27.9 (18.2–40.3)	96.7 (92.2–98.8)	8.42 (3.25–21.8)	0.75 (0.64–0.87)	11.3 (4.09–31.4)	0.77 (0.71–0.83)
aβ$_2$GPI D1	52.5 (40.0–64.7)	100 (96.9–100)		0.48 (0.36–0.62)		86.7 (82.1–91.3)
Positive for any item	84.0 (64.6–94.1)	90.1 (82.0–94.9)	8.49 (4.46–16.2)	0.18 (0.072–0.44)	47.83 (14.2–161)	0.88 (0.83–0.95)
Positive for the second and subsequent items	67.2% (54.3–77.9)	93.4% (87.8–96.6)	10.24 (5.31–19.7)	0.35 (0.24–0.51)	29.2 (12.4–68.5)	0.86 (0.81–0.91)
Positive for the third and subsequent items	20.7% (12.2–33.0)	99.3% (95.5–100)	28.3 (3.77–213)	0.80 (0.70–0.91)	35.5 (6.32–199.3)	0.76 (0.70–0.82)
Positive for the fourth and subsequent items	17.2% (9.5–29.2)	100% (96.6–100)		0.83 (0.74–0.93)		0.75 (0.69–0.81)
Positive for the fifth and subsequent items	17.2% (9.5–29.2)	100% (96.6–100)		0.83 (0.74–0.93)		0.75 (0.69–0.81)
Positive for the sixth and subsequent items	11.5% (5.4–22.2)	100% (96.9–100)		0.89 (0.81–0.97)		0.75 (0.69–0.80)
Positive for the seventh and subsequent items	11.5% (5.4–22.2)	100% (96.9–100)		0.89 (0.81–0.97)		0.75 (0.69–0.80)

aCL: anticardiolipin antibody, aβ$_2$GPI: antiβ$_2$GPI antibody, PLR: positive likelihood ratio, NLR: negative likelihood ratio, OR: odds ratio, 95%CI: 95% confidence interval, class: probability of correct classification.

Table 2
Results of measurement of patients with antiphospholipid antibody syndrome using homemade ELISAs.

Measurement	Sensitivity (%) (95%CI)	Specificity (%) (95%CI)	PLR (95%CI)	NLR (95%CI)	OR (95%CI)	Class (95%CI)
aCL IgG	71.9 (59.0–81.9)	92.2 (85.1–96.2)	9.26 (4.67–18.4)	0.30 (0.20–0.46)	30.4 (12.3–75.2)	0.85 (0.80–0.91)
aβ$_2$GPI IgG	62.5 (42.6–78.8)	94.3 (86.8–97.8)	10.88 (4.40–26.9)	0.40 (0.24–0.67)	27.3 (8.38–89.1)	0.87 (0.81–0.94)
aCL IgM	22.0 (13.3–34.3)	100.0 (95.6–100.0)		0.78 (0.68–0.89)		0.72 (0.65–0.79)
aβ$_2$GPI IgM	21.7 (9.4–42.5)	97.8 (91.7–99.8)	9.78 (2.03–47.2)	0.80 (0.64–1.00)	12.2 (2.53–59.2)	0.82 (0.75–0.89)
Positive for any item	95.5 (76.2–100)	90.1 (82.0–94.9)	9.65 (5.16–18.1)	0.05 (0.01–0.34)	191.3 (32.07–1142)	0.91 (0.86–0.96)
Positive for second and subsequent items	68.2% (47.1–83.7)	90.8% (83.2–95.2)	7.42 (3.74–14.7)	0.35 (0.19–0.65)	21.2 (7.06–18.2)	0.87 (0.81–0.93)
Positive for third and subsequent items	31.8% (16.3–52.9)	92.9% (85.7–96.7)	4.46 (1.74–11.4)	0.73 (0.55–0.98)	6.07 (1.93–19.1)	0.82 (0.75–0.89)
Positive for fourth item	4.3% (0.0–23.0)	100% (95.0–100)		0.96 (0.88–1.04)		0.81 (0.73–0.88)

aCL: anticardiolipin antibody, aβ$_2$GPI: anti-β$_2$GPI antibody, PLR: positive likelihood ratio, NLR: negative likelihood ratio, OR: odds ratio, 95%CI: 95% confidence interval class: probability of correct classification.

Table 3
The concordance rates of aPL detections between ELISAs and the APL CIA panel.

	aCL IgG	aCL IgM	aβ$_2$GPI IgG	aβ$_2$GPI IgM
Kappa value (95% CI)	0.657 (0.509–0.768)	0.562 (0.327–0.744)	0.591 (0.224–0.724)	0.558 (0.321–0.741)

Kappa value: Cohen's kappa value, 95% CI: 95% confidence interval, aCL: anticardiolipin antibody, aβ$_2$GPI: anti-β$_2$GPI antibody.

Among the 61 patients with criteria-defined APS, 6 and 8 APS patients were negative for both aCL and aβ$_2$GPI aPLs respectively using both the APL CIA panel and the homemade ELISAs. In general, the proportion of APS patients who are diagnosed with lupus anticoagulant alone varies from 20 to 40%, but the proportion was comparatively low in our cohort.

We investigated non-criteria aPLs in our study that are IgA aPLs (aCL IgA and aβ$_2$GPI IgA) and aβ$_2$GPI DI antibody. The significance of IgA aPLs remains controversial, however, there are several reports showing the moderate sensitivities of the antibodies or high hazard ratio for APS [16] [17].

In our study, IgA aPLs had high specificities while their sensitivities remain low comparable to IgM antibodies (aCl IgM and aβ$_2$GPI IgM) in accordance with the past reports. Moreover, in all cases with positive IgA antibodies, more than one criteria-defined aPLs were positive. Thus, these antibodies may have less contribution to the screening of APS, however, they possibly are beneficial for confirmation of the diagnosis.

Although aβ$_2$GPI D1 antibody was positive in just over half (52.5%) of the APS patients, it was 100% specific for APS. While most of the positive aβ$_2$GPI D1 cases were positive for aβ$_2$GPI IgG, 2/31 cases were negative for aβ$_2$GPI IgG, including 1 case that was negative for all other aPLs that were examined in this study. The antibody values for aβ$_2$GPI D1 in these 2 cases were 47.1 and 51.7 U/ml, respectively, which were higher than the ULN value. Anti-β$_2$GPI D1 functions as a confirmatory test with its high specificity and may detect autoantibodies that cannot be detected by conventional aβ$_2$GPI measurement methods.

Lupus anticoagulant is recognized as sensitive and specific for the diagnosis of APS, however it is technically very demanding to perform and there are many unclear factors that significantly influence its reliable measurement. Recently, it has been determined that prothrombin is a major target of lupus anticoagulant in addition to β$_2$GPI. It has been suggested that phosphatidylserine-dependent anti-prothrombin antibody (aPS/PT) should be considered as a specific criteria marker of APS along with aCL and aβ$_2$GPI. Furthermore, it has also been suggested that these 3 antibodies (aCL, aβ$_2$GPI and aPS/PT) might be more useful overall than the lupus anticoagulant test [18]. At present PSPT is not available on the BIO-FLASH/ACL Acustar platform however, so its inclusion in any aPL test panel will need to utilize results obtained from tests in ELISA format. In the APS patient cohort examined in our study, a single positive case with 4 individual aPLs that were measured with APL CIA panel, was confirmed. Furthermore, it was found that the sensitivity and probability for the correct classification of APS diagnosis was higher when all 4 types of aPL were examined, compared with testing for only 3 types. Indeed, in the test with the APL CIA panel, the proportion of positive aPLs in the APS group was higher than in the other groups. These findings have already been suggested in past reports, and various types of aPL with high titer are easily expressed in APS patients, compared to patients with connective tissue diseases who are positive for aPL and those who temporarily express aPL occur readily caused easily in such APS patients [19].

Based on these observations, many examinations have been performed to quantitatively assess the antibody value of aPLs. We defined antiphospholipid antibody scores through formula manipulation for the measured hazard ratios of individual aPLs and antibody values for thrombosis, using the aPL data from patients with connective tissue diseases, including the APS patients. It was clearly shown that when an appropriate cutoff value was set, the scores would significantly contribute to accurate APS diagnosis, with higher sensitivity and specificity, compared with the conventional APS classification criteria, and that high scores would represent an extremely high risk of future development of thrombosis in patients with connective tissue diseases [20]. This

Table 4
Number of positive cases in individual groups when tested with the APL CIA panel.

Positive tests	APS	SLE	CTD	Non-autoimmune	Hepatitis	Healthy
A. Criteria assays only (IgA and aβ$_2$GPI D1 assays excluded)						
0	8 (13%)	31 (84%)	45 (85%)	13 (81%)	29 (85%)	10 (100%)
1	12 (20%)	3 (8%)	7 (13%)	2 (13%)	5 (15%)	0
2	27 (44%)	2 (6%)	1 (2%)	1 (6%)	0	0
3	4 (7%)	1 (3%)	0	0	0	0
4	10 (16%)	0	0	0	0	0
Total	61	37	53	16	34	10
B. Testing by criteria assays and IgA aCL, IgA aβ$_2$GPI, and aβ$_2$GPI D1						
0	7 (11%)	30 (81%)	43 (81%)	13 (81%)	29 (85%)	10 (100%)
1	12 (20%)	3 (8%)	7 (13%)	2 (13%)	4 (12%)	0
2	6 (10%)	3 (8%)	2 (4%)	1 (6%)	0	0
3	15 (25%)	0 (0%)	1 (2%)	0	1 (3%)	0
4	5 (8%)	0	0	0	0	0
5	9 (15%)	1 (3%)	0	0	0	0
6	0 (0%)	0	0	0	0	0
7	7 (11%)	0	0	0	0	0
Total	61	37	53	16	34	10

score has been validated at other facilities [13,21]. The APL score is calculated by measuring 14 types of aPL. It was confirmed that measurement of fewer aPL would also contribute to APS diagnosis and the prediction of thrombosis (modified aPL score) [13]. It was also determined that examinations utilizing aPSPT, aCL, and aβ_2GPI would exhibit higher diagnostic capability than the present classification criteria.

5. Conclusion

The chemiluminescent assays (CIA) evaluated multiple aPLs simultaneously with equivalent accuracy to the classical ELISA. In our cohort, two or more aPLs were specifically found in APS patients, reinforcing that the multiple aPLs analysis is essential in APS diagnosis and CIA is a labor-saving technique that readily analyze multiple tests automatically.

In the future, there remains a strong possibility that concomitant use of automated measurement of aPL and a quantitative evaluation tool assessing antibody value, i.e., including the antiphospholipid antibody score, could contribute to higher diagnostic detection of APS and improved prediction capability for thrombosis.

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