Original Article

Clinical Importance of Positive Test Results for Lupus Anticoagulant and Anticardiolipin Antibodies

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• ***Objectives*: To assess the performance of 4 clotting assays for lupus anticoagulant (LA) detection, to determine the prevalence of LA and anticardiolipin antibodies (aCL), and to correlate LA and aCL prevalence with systemic disease and thrombosis.**

• ***Patients and Methods*: We studied 664 consecutive patients at the Mayo Clinic in Rochester, Minn, who were referred for laboratory testing because of a clinical suspicion of LA or thrombophilia between June 25, 1990, and July 1, 1991.**

• ***Results*: Of 664 patients tested for LA, 584 also were tested for aCL. Of patients tested for both LA and aCL, 137 (23.5%) had positive results for one or both tests (13 [9.5%], LA-positive only; 76 [55.5%], aCL-positive only; and 48 [35.0%], positive for both). The dilute Russell viper venom time (DRVVT) was the most frequently positive LA assay (74% of the 61 patients with positive results for LA). Twenty-two patients (36.1% of the 61) had positive results for all 4 LA assays, whereas 21 (34.4% of the 61) had positive results for only 1 LA assay: activated partial thromboplastin time (3 patients [4.9%]), plasma clot time (5 patients [8.2%]), kaolin clot time (5 patients [8.2%]), or DRVVT (8 patients [13.1%]). Thromboembolism prevalence was not definitely associated with positive test results (LA only, aCL only, or LA plus aCL), nor was it strongly associated with aCL isotype or titer. Furthermore, thromboembolism prevalence was not increased when all LA assays were positive, although a history of deep venous thrombosis or pulmonary embolism was nonsignificantly associated with positive results for all 4 LA tests. The likelihood of having both LA- and aCL-positive test results was higher among patients with systemic lupus erythematosus (26 [19.0%] of 137 patients with positive results for one or both tests), but they had no more thrombotic events or fetal loss than other patients in our study group.**

• ***Conclusions*: The DRVVT identified more patients with LA than the other LA tests, but more than 1 LA test was required to identify all patients with LA. Positive results were much more common for aCL than for LA. No single LA test or anticardiolipin isotype correlated with thrombosis or systemic disease in this population.**

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aCL = anticardiolipin antibodies; AI = autoimmune disease; APTT = activated partial thromboplastin time; CA = cancer; DRVVT = dilute Russell viper venom time; DVT = deep venous thrombosis; KCT = kaolin clot time; LA = lupus anticoagulants; PAE = peripheral arterial event; PCT = plasma clot time; PE = pulmonary embolus; PNP = platelet neutralization procedure; SLE = systemic lupus erythematosus; TIA = transient ischemic attack

Lupus anticoagulants (LA) and anticardiolipin antibodies (aCL) are associated with an increased risk of venous and arterial thrombotic events, recurrent fetal loss, and thrombocytopenia.[1-5] Other clinical manifestations include livedo reticularis,[6,7] superficial thrombophlebitis,[8] leg ulcers,[8] cardiac valvular disease,[9-11] migraine headache,[12,13] and chorea.[14-16] Lupus anticoagulants or aCL (or both) occur in primary antiphospholipid antibody syndrome[15] and in other diseases, especially systemic lupus erythematosus (SLE).[15,17-20] Lupus anticoagulants and aCL are separate manifestations of antiphospholipid antibodies, and one often occurs without the other.[21,22] Although the relationships of LA and aCL, as well as aCL isotype and titer, to various clinical events have been studied widely, the best testing methods for identifying LA and aCL and the best method for associating one type of antiphospholipid antibody or specific aCL isotype or titer with clinical thromboembolic events remain uncertain.[15,17,23] We undertook the present study to address some of these issues by evaluating LA and aCL testing, patients with positive results for LA or aCL or both, their associated diseases, and the frequency of thromboembolic events.

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PATIENTS AND METHODS

Study Population

We prospectively studied 664 consecutive patients at the Mayo Clinic in Rochester, Minn, who were referred

within a 12-month period (June 25, 1990, to July 1, 1991) to Mayo's Special Coagulation Laboratory for the following indications: (1) suspected thrombophilia (thrombotic diathesis), including suspected antiphospholipid antibodies; (2) unexplained prolonged clotting time; and (3) other hemostatic problems if a prolonged and inhibited phospholipid-dependent clotting time was found.

All 664 patients were tested for LA, 584 of whom were also tested for aCL. Of the 664 patients, 57% were referred for suspected thrombophilia and 35% for suspected LA; the remainder were tested for LA for other reasons such as unexplained clotting time prolongations. During the study period, 966 patients underwent specialized coagulation testing for other indications.

Measurements

Blood for coagulation testing was collected by clean venipuncture into plastic syringes and added to plastic vials containing 0.1 mol/L sodium oxalate or 3.8% sodium citrate at a ratio of 9:1. Oxalated platelet-rich plasma was obtained by centrifugation at 250 × *g* for 3 minutes. Citrated platelet-poor plasma was obtained by centrifugation at 1700 × *g* for 10 minutes. Citrated platelet-poor plasma filtered through a 0.2-μm filter (Gelman Sciences Inc, Ann Arbor, Mich) yielded platelet-free plasma. Plasma was maintained on ice until testing (within 4 hours of venipuncture). Serum for aCL testing was harvested after centrifugation of nonanticoagulated blood that was allowed to clot in glass test tubes.

All patients were screened for LA using 4 different clot-based assays. The activated partial thromboplastin time (APTT) was measured with a coagulometer (CoaScreener, LABiTec GmbH, Ahrensburg, Germany) with automated APTT reagent (bioMérieux, Durham, NC). Fifty microliters of citrated platelet-poor plasma and 50 μL of automated APTT reagent were incubated together at 37°C for 5 minutes; next, 50 μL of warmed 0.02 mol/L calcium chloride was added, and the time to clot formation was measured. The plasma clot time (PCT)[24,25] or recalcification time was measured manually (tilt tube) by adding 200 μL of 0.02 mol/L calcium chloride to 200 μL of oxalated platelet-rich plasma at 37°C, and the time to the first signs of clot formation was measured. The dilute Russell viper venom time (DRVVT)[26] was measured with the coagulometer using Russell viper venom fraction (Sigma Diagnostics, St Louis, Mo) reconstituted as advised by the manufacturer and diluted 1:200 in imidazole-buffered saline. Phospholipid was supplied by automated APTT reagent diluted 1:8 in imidazole-buffered saline. The DRVVT was measured as follows: 50 μL of citrated plasma, 50 μL of dilute Russell viper venom, and 50 μL of dilute phospholipid were incubated together for 2 minutes at 37°C; 50 μL of warmed 0.02 mol/L calcium chloride was added, and the time to clot formation was determined. The kaolin clot time (KCT)[27] was measured with the coagulometer by incubating 100 μL of filtered platelet-free plasma and 50 μL of a 2% kaolin suspension for 3 minutes at 37°C; after the addition of 100 μL of warmed 0.02 mol/L calcium chloride, the time to clot formation was determined.

Mixing studies with normal plasma used frozen-thawed, pooled normal plasma in the APTT and DRVVT tests or fresh plasma (platelet rich or platelet free) from normal daily donors in the PCT and KCT tests, respectively; these studies excluded factor deficiency and confirmed inhibition as the cause of clot time prolongation. *Inhibition* was defined as failure to correct the prolonged clotting time into the normal range of mean ± 2 SD in a 1:1 mixture of patient plasma to normal plasma. The platelet neutralization procedure (PNP)[28] was performed to evaluate any prolonged and inhibited APTT. Also, a thrombin time was measured to exclude heparin as a cause of clot time prolongation, and, when indicated, factor assays were used to exclude specific inhibitors. A prothrombin time was measured in each case.

Serum aCL were detected and measured by a standardized enzyme-linked immunosorbent assay.[29,30] Microtiter plate wells were adsorbed with cardiolipin (Sigma Chemical Co, St Louis, Mo), and alkaline phosphatase–conjugated, polyclonal antihuman immunoglobulin antibodies (anti-IgG and anti-IgM) were used to detect aCL. Each serum sample was tested at multiple dilutions, 1:4 through 1:4096.

The absorbance of each dilution of test serum was compared with identical dilutions of negative control serum pool tested simultaneously. Serums were scored as positive for IgG aCL if the ratio of absorbances was greater than 3.0, and the end point was defined as the highest dilution that gave a ratio greater than 2.0. Serums were scored as positive for IgM aCL if the ratio of absorbances was greater than 2.0, and the end point was defined as the highest dilution that gave a ratio greater than 2.0. Positive results with titers of 8 to 32 compare with results expressed in GPL or MPL (isotypes IgG or IgM antiphospholipid antibodies) units of 20 to 50, and titers of 64 to 256 compare with results expressed in GPL or MPL units of 50 to 80. Titers greater than 256 are strongly positive and compare with results expressed in GPL or MPL units of greater than 80 (H.A.H., unpublished data).

Definitions of Positive Coagulation Test Results

Lupus Anticoagulant. ***APTT/PNP.***—Prolongation of the APTT, more than 3 SDs above mean normal (≥40 seconds), failure to correct into the normal 2 SD range (≤37 seconds) when mixed 1:1 with normal plasma; and PNP

shortening of 5 seconds or more compared with the baseline APTT and buffer APTT.

***PCT.*—**Prolongation of the PCT, more than 3 SDs above mean normal (≥95 seconds), and failure to correct into the normal 2 SD range (≤90 seconds) when mixed 1:1 with normal platelet-rich plasma.

***DRVVT.*—**Prolongation of the DRVVT, more than 3 SDs above mean normal (≥37 seconds), and failure to correct into the normal 2 SD range (≤35 seconds) when mixed 1:1 with normal pooled plasma.

***KCT.*—**Prolongation of the KCT, more than 3 SDs above mean normal (≥200 seconds), and failure to correct into the normal range (≤185 seconds) when mixed 1:1 with normal platelet-free plasma.

A positive result in any of these tests was considered diagnostic of LA, provided there was no laboratory evidence of a specific coagulation factor inhibitor or heparin as a cause of prolonged clotting times.[31]

Anticardiolipin Antibodies.—IgM titers of 1:8 or greater or IgG titers of 1:8 or greater were considered positive for aCL.[30]

Patient Histories

The medical records of patients who had positive test results for LA, aCL, or both (137 of 584 patients) were reviewed for diagnoses and symptoms before and at the time of referral to the Special Coagulation Laboratory by using a protocol approved by the Mayo Foundation Institutional Review Board. Data abstracted included history of SLE, autoimmune disease (AI) other than SLE, and any form of cancer (CA). Furthermore, any history of deep venous thrombosis (DVT), pulmonary embolus (PE), cerebrovascular events (stroke and transient ischemic attack [TIA]), hepatic venous thrombosis, inferior vena cava thrombosis, fetal loss, migraine headache, chorea, myocardial infarction, leg ulcer, and peripheral arterial events (PAEs) was recorded.

Statistical Methods

Categorical data were analyzed by using either the χ^2 test or the Fisher exact test if the expected value in 1 or more cells was less than 5. The Bonferroni correction for multiple comparisons (α=.05; therefore, 0.05 divided by the number of comparisons) indicates that P<.002 should be considered statistically significant.

RESULTS

Laboratory Features

A total of 664 patients were tested for LA; 584 also were tested for aCL. Of the 584 patients tested for both LA and aCL, 137 patients (23.5%) had positive results for one or both tests. Among these 137 patients, 61 (10.4% of 584 or 44.5% of 137) had positive results for LA; 13 (21.3% of 61 or 9.5% of 137) had positive results for LA only; 76 (55.5% of 137) had positive results for aCL only; and 48 (35.0% of 137) had positive results for both LA and aCL (Table 1). Of the 124 patients with positive results for aCL (21.2% of 584 or 90.5% of 137), 54 were positive for IgM only, 47 were positive for IgG only, and 23 were positive for both IgG and IgM isotypes.

Table 1. **Summary of LA and aCL Findings in 137 Patients With Positive Results for LA, aCL, or Both***

Result	No. (%) of patients
LA positive (by 1 or more tests)	61 (44.5)
aCL positive (IgM or IgG)	124 (90.5)
LA positive only	13 (9.5)
LA + aCL positive	48 (35.0)
aCL positive only	76 (55.5)
IgM aCL positive	70 (51.1)
IgG aCL positive	77 (56.2)
IgM aCL + IgG aCL negative	13 (9.5)
IgM aCL positive only	54 (39.4)
IgG aCL positive only	47 (34.3)
IgM aCL + IgG aCL positive	23 (16.8)

*aCL = anticardiolipin antibodies; LA = lupus anticoagulants.

The distribution of LA and aCL test results for the 61 patients with LA-positive results and the 76 patients with LA-negative results is shown in Table 2. Positive aCL test results were found in 48 of the 61 patients (78.7%) who had LA-positive test results. Among those with positive results for only a single isotype, IgG aCL were more prevalent than IgM in patients with LA than in those without LA (23 of 54 vs 8 of 47; P=.005), although not significant at the Bonferroni cutoff of P<.002.

Of the 4 LA tests, the DRVVT showed positive results most often (in 45 of the 61 patients [73.8%] with positive results for LA). Twenty-one (34.4%) of the 61 patients with positive results for LA had positive results for only 1 LA test, whereas 22 (36.1%) of the 61 had positive results for all 4 LA tests. Of the latter, all but 2 had aCL-positive results. For patients with only 1 LA test with positive results, aCL test results were negative as follows: APTT/PNP = 0 of 3 patients, PCT = 5 of 5 patients, DRVVT = 0 of 8 patients, and KCT = 2 of 5 patients. Thus, 7 patients (5.1% of 137) with antiphospholipid antibodies would not have been identified had the PCT and KCT not been performed for LA testing. An additional 6 patients (4.4% of 137) with 2 or more positive LA test results had negative results for aCL; their conditions would have been undetected had LA testing not been performed. However, DRVVT testing identified all these patients.

Table 2. **Distribution of Positive and Negative Results of LA Tests and aCL Tests in 61 Patients With Positive Results for LA and 76 Patients With Negative Results for LA but Positive Results for aCL***

						No. of patients			
No. of positive LA tests	No. (%) of patients	APTT/ PNP (n=35)	PCT (n=37)	DRVVT (n=45)	KCT (n=38)	aCL-negative results (n=13)	IgG aCL– positive results (n=54)	IgM aCL– positive results (n=47)	IgG aCL– and IgM aCL– positive results (n=23)
4	22 (16.1)	+	+	+	+	2	8	4	8
3	3 (2.2)	+	+	+	–	2	0	1	0
	2 (1.5)	+	+	–	+	0	1	0	1
	2 (1.5)	+	–	+	+	0	0	0	2
	3 (2.2)	–	+	+	+	0	2	0	1
2	1 (0.7)	+	+	–	–	0	1	0	0
	2 (1.5)	+	–	+	–	1	1	0	0
	0 (0.0)	+	–	–	+	0	0	0	0
	1 (0.7)	–	+	+	–	0	1	0	0
	0 (0.0)	–	+	–	+	0	0	0	0
	4 (2.9)	–	–	+	+	1	1	0	2
1	3 (2.2)	+	–	–	–	0	3	0	0
	5 (3.6)	–	+	–	–	5	0	0	0
	8 (5.8)	–	–	+	–	0	4	2	2
	5 (3.6)	–	–	–	+	2	1	1	1
0	76 (55.5)	–	–	–	–	0	31	39	6

*aCL = anticardiolipin antibodies; APTT/PNP = activated partial thromboplastin time/platelet neutralization procedure; DRVVT = dilute Russell viper venom time; KCT = kaolin clot time; LA = lupus anticoagulants; PCT = plasma clot time.

Demographic Features

There were 83 women (60.6%) among the 137 patients, 72 (86.7%) of whom had been pregnant at least once. The overall median age was 52 years, whereas the median age for the 26 patients with SLE was 45.5 years.

Diagnosis in the Total Group of 137 Patients

Most patients (73 [53.3%]) had no SLE, AI, or CA (Table 3). Twenty-six patients (19.0%) had SLE, 23 (16.8%) had AI, and 25 (18.2%) had CA. Four patients had SLE and CA, 1 had SLE and AI, and 5 had AI and CA. Autoimmune diseases other than SLE included rheumatoid arthritis in 2 patients, scleroderma in 3, mixed connective tissue disease in 2, giant cell arteritis in 2, other systemic vasculitis in 6, myasthenia gravis in 2, idiopathic thrombocytopenic purpura in 2, Graves disease in 2, and myositis in 2.

Prevalence of Positive Results for LA Only, aCL Only, or Both in Various Subgroups of the 137 Patients

Patients with a history of SLE were much more likely to have positive test results for both LA and aCL tests than were those without SLE (Table 3). Of the SLE-only group, 71.4% had positive results for both LA and aCL, as did 60.0% of those with more than 1 diagnosis; in contrast, approximately 25% of those with AI, CA, or no disease had positive results for both LA and aCL ($P<.002$). Recall that 5 of the 10 patients with more than 1 diagnosis had SLE. Correspondingly, only 19.0% of the SLE-only group had positive results only on aCL testing compared with 64.7%, 56.2%, and 67.1% of those with AI only, CA only, or no disease, respectively.

Thromboembolic Events and Fetal Loss in the 137 Patients With Positive Results for LA, aCL, or Both

A history of 1 or more thromboembolic events was found in 94 patients (68.6%). Deep venous thrombosis, PE, or both occurred in 43 patients (31.4%); stroke, TIA, or both occurred in 46 (33.6%); inferior vena cava thrombosis occurred in 4 (2.9%); hepatic venous thrombosis occurred in 4 (2.9%); and PAEs occurred in 17 (12.4%). Among the 72 women with at least 1 pregnancy, fetal loss occurred in 19 (26.4%). Myocardial infarction occurred in 15 patients (10.9%; 10 of whom were men), migraine headache in 18 (13.1%; 10 of whom were women), and leg ulcers in 18 (13.1%).

No difference in AI and CA disease status existed between those with and those without a history of DVT/PE events or between those with and those without a history of PAE (Figure 1). Similarly, among women with at least 1 pregnancy, no difference existed in disease status between those with and those without a history of fetal loss. The frequency of stroke or TIA was higher among patients with CA only (62.5%) than among those with SLE only

Table 3. **Frequency of LA and aCL in Various Subgroups of 137 Patients***

Result	No. (%) of patients				
	SLE only (n=21, 15.3%)	AI only (n=17, 12.4%)	CA only (n=16, 11.7%)	Combination (n=10, 7.3%)	None (n=73, 53.3%)
LA positive only	2 (9.5)	2 (11.8)	3 (18.8)	1 (10.0)	5 (6.8)
LA + aCL positive	15 (71.4)	4 (23.5)	4 (25.0)	6 (60.0)	19 (26.0)
aCL positive only	4 (19.0)	11 (64.7)	9 (56.2)	3 (30.0)	49 (67.1)

*P<.002 (Bonferroni cutoff is P<.002) for test positivity vs disease subgroup. aCL = anticardiolipin antibodies; AI = autoimmune diseases other than SLE; CA = cancer; Combination = 4 patients with SLE and CA, 1 with SLE and AI, and 5 with AI and CA; LA = lupus anticoagulants; None = no SLE, AI, or CA; SLE = systemic lupus erythematosus.

(23.8%), AI only (29.4%), multiple diagnoses (10.0%), or no SLE, AI, or PAE (34.2%) (P=.06; Figure 1), although not significant at the Bonferroni cutoff of P<.002. Patients with CA only had a higher frequency of overall thromboembolic events (95.8%) than patients with SLE only (52.4%), those with a mixture of diseases (40.0%), or those with no SLE, AI, or PAE (68.5%), although patients with AI only also had a high frequency of overall thromboembolic events (82.4%) (P=.01; Figure 1).

Distribution of LA and aCL Test Results Among Patients With Thromboembolism

Of the 94 patients with thromboembolism, results for 6 (6.4%) were LA positive only, 34 (36.2%) were both LA and aCL positive, and 54 (57.4%) were aCL positive only (Table 4). Among the 137 patients with positive LA and/or aCL results, a history of DVT/PE, stroke/TIA, PAE, or fetal loss was not associated with the type of positive test (LA, aCL, or both; P>.05 for all comparisons; Table 4). Furthermore, having positive results for all 4 LA tests was not significantly associated with DVT/PE, stroke/TIA, PAE, or fetal loss, after adjusting for the Bonferroni correction. However, the following results should be noted. Of patients with a history of DVT/PE, 28% had positive results for all 4 LA tests compared with 11% of those with no history of DVT/PE (P=.01). Among the 72 women with at least 1 pregnancy, 32% of those with fetal loss had positive results for all 4 tests compared with 13% of those without fetal loss (P=.09).

Of the 124 patients with positive results for aCL, 88 had a history of thromboembolism. Of these 88, 43 (48.9%) had IgG aCL-positive only results, 26 (29.5%) had IgM aCL-positive only results, and 19 (21.6%) had both IgG aCL- and IgM aCL-positive results (Table 4). Among those with

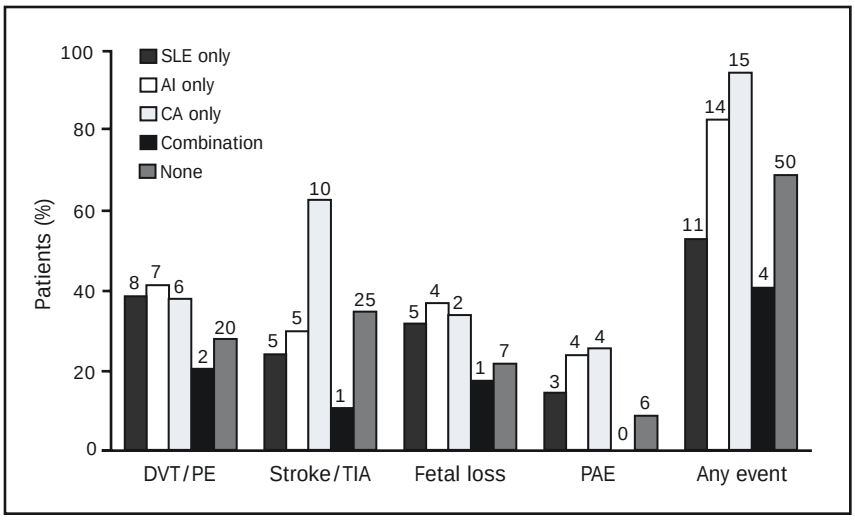

Figure 1. Percentage of patients with deep venous thrombosis/pulmonary embolus (DVT/PE), stroke/transient ischemic attack (TIA), fetal loss, peripheral arterial event (PAE), or any of these 4 events among various autoimmune status groups. Frequency in each category is shown above the corresponding column. (Note that N=72 for those with fetal loss, and N=137 for the other events.) AI = autoimmune diseases other than SLE; Any = any of the 4 events; CA = cancer; SLE = systemic lupus erythematosus.

Table 4. **Prevalence of Positive Results for LA and aCL Tests in Relation to Various Thromboembolic Events in 94 Patients***

Event	DVT/PE	Stroke/TIA	Fetal loss	PAE	Any of these events
No. (%) of patients†	43 (31.4)	46 (33.6)	19 (26.4)	17 (12.4)	94 (68.6)
	No. of patients				
Test result					
LA positive only	1	3	0	2	6
LA + aCL positive	18	20	9	4	34
aCL positive only	24	23	10	11	54
IgG positive	22	18	11	6	43
IgM positive	12	12	5	3	26
IgG + IgM positive	8	13	3	6	19

*aCL = anticardiolipin antibodies; DVT/PE = deep venous thrombosis/pulmonary embolus; LA = lupus anticoagulants; PAE = peripheral arterial event; TIA = transient ischemic attack.
†N=137 except for fetal loss (N=72).

positive results for aCL, a history of DVT/PE, fetal loss, or PAE was not associated with type of aCL test, although those with positive results for both IgG and IgM were slightly more likely to have a history of PAE (*P*=.06). Similarly, although not statistically significant, patients with positive results for both IgG and IgM were more likely to have had a history of stroke/TIA (56.5%) than were those with IgG only (33.3%) or those with IgM only (25.5%) (*P*=.04). Of note, among patients with positive results for aCL, a history of any of these events was more likely among those with positive results for IgG only (79.6%) or for both IgG and IgM (82.6%) than among those with positive results for IgM alone (55.3%) (*P*=.01; Table 4).

Correlation of LA Tests, aCL Isotype and Titer, and Thromboembolic Events

Only 33.8% of patients with low-titer IgM aCL had LA-positive test results compared with 60.0% of those with high-titer IgM and 53.7% of those with negative results for IgM aCL (*P*=.05; Table 5). Conversely, the IgG aCL titer level was significantly associated with LA-positive test results. Of those with low-titer IgG aCL, 67.4% had LA-positive test results compared with 32.4% of those with high-titer IgG and 35.0% of those with negative results for IgG aCL (*P*=.001). Despite this, the frequency of thromboembolic events did not differ with respect to lower or higher IgM or IgG titer (data not shown). A history of DVT/PE, stroke/TIA, fetal loss, or PAE was not associated with level of IgM or IgG aCL titer.

Table 5. **Lupus Anticoagulant (LA) Status in Patients With Lower and Higher Anticardiolipin Antibody (aCL) Isotype IgG (IgG aCL) and aCL Isotype IgM (IgM aCL) Titers**

Titer	No. (%) of patients (N=137)	No. of LA-positive results (% of titer level)
IgM aCL		
Negative	67 (48.9)	36 (53.7)
1:8-1:128	65 (47.4)	22 (33.8)
1:256-1:4096	5 (3.6)	3 (60.0)
IgG aCL		
Negative	60 (43.8)	21 (35.0)
1:8-1:128	43 (31.4)	29 (67.4)
1:256-1:4096	34 (24.8)	11 (32.4)

DISCUSSION

In our study population, 137 of 584 patients had positive results for LA and/or aCL (23.5%), and 48 patients (35.0%) had positive results for both. Eighty-nine patients (65.0%) were discordant for the 2 types of antibodies, despite the sensitive LA assays that were used in our study. This underscores the importance of adequate laboratory study of patients suspected of having antiphospholipid antibodies by using tests for both LA and aCL. The 21 patients with only SLE had a similar prevalence of aCL and LA (90.4% and 80.9%, respectively) and a discordance for the 2 antibodies of 28.6%. Other studies found that aCL are more frequently positive in patients with SLE than are LA,[32,33] with a discordance of about 35%.[22,34]

Comparison of the different LA tests (Table 2) shows that the APTT/PNP alone failed to detect many of the LA identified by the other tests: 26 (42.6%) of the 61 LA-positive results were detected by LA tests other than the APTT/PNP. However, our criteria for definite LA diagnosis by APTT/PNP testing were stringent. Also, other APTT reagents may be more sensitive to LA.

The test that detected the most positive results for LA was the DRVVT (73.8%). However, the more tests used, the greater was the detection. This emphasizes the importance of performing multiple (≥2) coagulation screening and diagnostic tests for the identification of LA.

The PCT identified 5 patients whose results were negative in all other LA assays, none of whom had concurrent aCL positivity but 2 of whom had thromboembolic events. These data suggest the PCT can detect clinically important antiphospholipid antibodies that would be otherwise unde-

tected. However, the PCT can be performed only with freshly obtained oxalated blood samples, precluding use of frozen samples.

The KCT has been reported as a sensitive test for LA.[27] We identified 5 patients by KCT testing with no other evidence of LA in the other tests performed. Only 3 of the 5 patients had aCL-positive results, suggesting a minor patient population whose conditions would have been undetected without performance of the KCT. However, because of the need for additional sample preparation (ie, filtering of the plasma) and difficulty in automating the test, the KCT is impractical for most laboratories.

A combination of the APTT/PNP, DRVVT, and PCT, in conjunction with aCL testing, identified 98.5% of the individuals with antiphospholipid antibodies. The APTT/PNP, DRVVT, and aCL tests together identified 94.9% of those with antiphospholipid antibodies.

Although approximately equal proportions of the 137 patients had positive test results for either IgG or IgM aCL isotype alone, the patients with both LA and aCL had a preponderance of IgG rather than IgM aCL compared with those without LA. IgG aCL titers were associated with whether or not LA test results were positive. Specifically, 67.4% of patients with lower IgG aCL titer had LA-positive results compared with 32.4% of those with higher IgG aCL titer and 35.0% of those who had negative results for IgG aCL antibodies (Table 5). We also found that patients with IgG aCL isotype tended to have more thromboembolic events than those with IgM only isotype (P=.01).

The aCL testing methods appeared to be sensitive for antiphospholipid antibodies in that aCL testing failed to identify only 9.5% of individuals with other evidence (positive LA testing) of antiphospholipid antibodies. However, because positive LA test results often are considered to be of greater clinical importance than positive aCL test results,[2-5] it is important to test for both LA and aCL. Evaluation of our aCL testing methods, subsequent to this study, suggested a possible oversensitivity of IgM testing, especially for low-titer or weakly positive antibodies.[35]

Although initially described in patients with SLE, most patients with LA or aCL do not have SLE. In a previous review,[18] 193 of 547 patients (35%) with antiphospholipid antibodies had SLE or lupuslike disease. The remainder had other AIs, CA, a drug-related anticoagulant disease, or miscellaneous diseases. We confirmed that most patients with LA or aCL did not have SLE. Indeed, about one half (53.3%) did not have SLE, AI, or CA.

Patients with CA and an antiphospholipid antibody had a nonsignificantly higher frequency of stroke or TIA (or both) than patients with other diagnoses; they also had a nonsignificantly higher tendency to have any thromboembolic event than patients with other diagnoses except non-SLE AI. Otherwise, there were no significant differences in the occurrence of thromboembolic events among the different disease subgroups. This may reflect bias in our study population; most study patients were referred to the Special Coagulation Laboratory for evaluation of suspected thrombophilia. However, our study population likely is typical of a general medical population referred for detection of antiphospholipid antibodies (LA or aCL).

Whether or not one type of antiphospholipid antibody (LA or aCL) is more strongly associated with clinical events has been addressed by different groups.[33,36-42] However, few studies have been done in which sensitive LA assays have been compared with aCL assays and correlated with clinical history. In some studies, LA testing proved to be more specifically associated with thrombosis than aCL.[5,33,37-42] However, the patient populations in these studies varied. Another study[34] reported aCL to be a better predictor of fetal distress; however, only the APTT was used to detect LA. Others, including our study, have found the APTT to be insensitive for detecting LA in approximately 50% of patients.[18]

Whether or not the presence of both types of antibodies (LA and aCL) increases the risk of thromboembolic disease compared with the presence of a single antibody also has been addressed.[21,43,44] In one study, patients with both antibodies had an increased risk of thrombosis compared with those who had LA alone.[43] Nojima et al[44] found that the prevalence of thrombosis was higher in patients with positive results for LA and aCL than in those with positive results for only LA or aCL, but another study group[22] found no increased risk. In our study, although the patients with only SLE were more likely to have positive test results for both LA and aCL, they did not have more thromboembolic events than the other groups. The findings in our study population suggest that positive results of aCL tests rather than positive results of LA tests are associated more with thromboembolic events. However, this possible association likely reflects the high prevalence of thromboembolic diseases in our study population (69%) and the much higher prevalence of aCL positivity (124 of 137 patients; 90.5%) than LA positivity (61 of 137; 44.5%).

The association of specific aCL isotypes and thromboembolic events is controversial. Several studies suggest that IgG aCL, rather than IgM aCL, is the major predictor of thrombosis and fetal loss.[45-47] However, other groups have reported associations with IgM and IgA aCL as well.[46,47] In our study, IgG singly and jointly with IgM aCL was marginally associated with any thromboembolic event (P=.01), in keeping with these earlier studies. Our observation that 26 of the 94 patients with thromboembolic events or fetal loss or both were positive for only IgM aCL suggests that some thromboembolic events may be associated with IgM aCL.

Some groups have found the highest titer of aCL, regardless of isotype, as the most predictive for thromboembolic events.[48,49] Higher-titer IgG aCL has shown a stronger association with thrombosis and fetal loss,[45,47,50] whereas lower-titer IgG aCL and IgM aCL have shown lower risk for thromboembolic events.[51] However, patients with lower titers of aCL have presented with classic manifestations of antiphospholipid antibody syndrome as well.[52,53] A recent report found that the presence of aCL was not a risk factor for venous thromboembolism in otherwise healthy adults.[54]

A complicating factor in determining whether antibody titers correlate with thromboembolic events is that antiphospholipid antibody production may be transient.[55,56] Some investigators described patients whose antiphospholipid antibody titers have decreased or become negative at the time of a thromboembolic event.[33,57] In SLE, treatment may suppress both LA and aCL levels,[49,58] whereas active lupus may increase the titers.[59] In our study, no difference was detected in frequency of thromboembolic events with respect to titer or isotype for either IgG or IgM. From our study, the titer of aCL is not a predictor of thromboembolic events. Also, patients with lupus and other AIs can have multiple risk factors for thrombosis other than antiphospholipid antibodies.[15]

CONCLUSIONS

Our study shows that the APTT/PNP, DRVVT, PCT, and KCT, when performed individually, are insensitive to the presence of all LA. Of the 4 LA tests, the DRVVT showed positive results most often, but at least 2 or more LA tests must be performed to detect essentially all LA. Moreover, additional aCL testing is required to detect essentially all antiphospholipid antibodies. Positive results were much more common for aCL than for LA. Finally, the prevalence of thromboembolism or fetal loss among patients with antiphospholipid antibodies detected by either a single LA test or a particular aCL isotype (IgG vs IgM) or titer did not differ significantly. We speculate that these tests are surrogate measures rather than true effectors of the pathologic processes causing thromboembolism or fetal loss.

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