EXTENDED REPORT

Real world experience with antiphospholipid antibody tests: how stable are results over time?

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Objective: To determine the stability and the degree of variation of antiphospholipid antibody (aPL) results over time in a large cohort of well evaluated aPL positive patients; and to analyse factors contributing to aPL variation and the validity of aPL in a real world setting in which aPL tests are done in multiple laboratories.

Methods: The clinical characteristics, drug treatment, and 1652 data points for lupus anticoagulant (LA), anticardiolipin antibodies (aCL), and anti-β_2 glycoprotein I antibodies (anti-β_2GPI) were examined in 204 aPL positive patients; 81 of these met the Sapporo criteria for antiphospholipid syndrome (APS) and 123 were asymptomatic bearers of aPL.

Results: 87% of initially positive LA results, 88% of initially negative to low positive aCL results, 75% of initially moderate to high positive aCL results, 96% of initially negative to low positive anti-β_2GPI results, and 76% of initially moderate to high positive anti-β_2GPI results subsequently remained in the same range regardless of the laboratory performing the test. Aspirin, warfarin, and hydroxychloroquine use did not differ among patients whose aCL titres significantly decreased or increased or remained stable. On same day specimens, the consistency of aCL results among suppliers ranged from 64% to 88% and the correlation ranged from 0.5 to 0.8. Agreement was moderate for aCL IgG and aCL IgM; however, for aCL IgA agreement was marginal.

Conclusions: aPL results remained stable for at least three quarters of subsequent tests, regardless of the laboratory performing the test; the small amount of variation that occurred did not appear to be caused by aspirin, warfarin, or hydroxychloroquine use.

In patients with thrombosis or pregnancy complications, a persistently positive antiphospholipid antibody (aPL) (lupus anticoagulant test (LA), anticardiolipin antibodies (aCL), and anti-β_2 glycoprotein I antibodies (anti-β_2GPI)) establishes a classification of definite antiphospholipid syndrome (APS).[1] In a clinical setting, physicians judging aPL tests face several challenges. First, aPL in individual patients may vary over time, though how much spontaneous variation occurs is unknown. Second, it is also unknown whether any variation that does occur reflects autoimmune disease activity, drug treatment, or interlaboratory differences. Third, interlaboratory correlation among aCL results is not well established—a point particularly important in the USA where, because of rapidly changing insurance coverage systems, physicians can no longer specify the laboratories in which their patients' specimens are tested.

The primary objective of this study was to determine the stability and the degree of variation of aPL results over time in a large cohort of well evaluated aPL positive patients. Second, we analysed factors we considered likely to contribute to aPL variation, and the validity of aPL in a real world setting in which aPL tests are done in multiple laboratories. The results should offer guidance to practising physicians and researchers in the management of aPL positive patients.

METHODS

We identified aPL positive patients from those entered into two databases: a national antiphospholipid syndrome collaborative registry (APSCORE)[2]; and an asymptomatic (no history of vascular or pregnancy events) aPL positive registry (APLASA).[3] Inclusion criteria were: positive aPL (aCL or LA test or both, on two occasions six weeks apart), with or without APS classification (based on the Sapporo criteria) for APSCORE; and positive aPL without APS classification for APLASA. We reviewed medical and registry records for all available aPL tests (LA test, aCL, and anti-β_2GPI) including testing dates and laboratories. In addition, we also reviewed demographic variables, definite and possible aPL related clinical manifestations (venous and arterial thrombosis, pregnancy morbidity, livedo reticularis, thrombocytopenia, and migraine), coexisting autoimmune diseases, comorbidities (hypertension requiring antihypertensive agents, diabetes mellitus requiring antidiabetic agents, hypercholesterolaemia requiring cholesterol lowering agents, and current smoking), and drug treatments.

Lupus anticoagulant tests were grouped as positive and negative, based on the guidelines of the International Society on Thrombosis and Hemostasis.[4] The aCL and anti-β_2GPI results were expressed as their immunoglobulin subclasses (aCL: IgG, IU or GPL U/ml; IgM, IU or MPL U/ml; and IgA, IU or APL U/ml; anti-β_2GPI: IgG, U/ml; IgM, U/ml; and IgA, U/ml). They were divided into four groups: 0–19 U (negative); 20–39 U (low positive); 40–80 U (moderate positive); and >80 U (high positive). We classified aPL positive patients into three groups: vascular events with or without pregnancy events (Sapporo APS classification criteria were met); pregnancy events only (Sapporo APS classification criteria were met); and asymptomatic (Sapporo APS classification criteria were not met; patients with solely non-Sapporo aPL

Abbreviations: aCL, anticardiolipin antibody; aPL, antiphospholipid antibody; APLASA, asymptomatic aPL positive registry; APS, antiphospholipid syndrome; APSCORE, antiphospholipid syndrome collaborative registry; GPI, glycoprotein I; LA, lupus anticoagulant

Table 1 Demographic and clinical characteristic of 204 antiphospholipid antibody positive patients

Characteristic	
Female	190 (93%)
Age at registry inclusion (years)*	45.9 (13.3)
Race	
White	144 (71%)
Hispanic	25 (12%)
African American	21 (10%)
Other	14 (7%)
APS as per Sapporo criteria	81
Vascular with/without pregnancy event	57
Pregnancy event only	24
Asymptomatic aPL positive patients	123

Values are n (%) or *mean (SD).
aPL, antiphospholipid antibody; APS, antiphospholipid syndrome.

Table 3 Testing laboratories and assays of antiphospholipid antibodies

aPL test	Testing laboratory and assay
LA test	In house (DVV test® and DVV confirm® by American Diagnostic Inc)
aCL test	In house (based on Harris Standards*) Quest Diagnostics (Pharmacia Diagnostics aCL kit) APSCORE (based on Harris standards*)
Anti-β_2GPI	Quest Diagnostics (The Binding Site anti-β_2GPI kit) APSCORE (Inova Diagnostics anti-β_2GPI kit)

*Harris EN, Pierangeli SS. Revisiting the anticardiolipin test and its standardization. *Lupus* 2002;**11**:269–75.
aPL, antiphospholipid antibody; APSCORE, antiphospholipid syndrome collaborative registry.

manifestations such as livedo reticularis or cardiac valve disease were also included in this group).

We arbitrarily selected the initial aPL result as the anchor. S*tability* of aPL was defined as the percentage of subsequent results in the same group; *variation* of aPL was defined as the percentage of subsequent results in different groups. The relations between clinically relevant variation (positive *v* negative for LA test, negative to low *v* moderate to high titre for aCL and anti-β_2GPI tests) and aPL related clinical manifestations or drug treatment (aspirin, warfarin, hydroxychloroquine) were analysed (χ^2 test) independently of other manifestations of autoimmune disease activity.

To examine interlaboratory variation, we identified aPL positive patients who had same specimen aCL testing from different commercial suppliers and from our immunology laboratory. Same specimen aCL results from different sources were analysed for: *consistency* (percentage of results within the same group); *agreement* between aCL groups (Cohen's κ test); and *correlation* between aCL results (Spearman rank correlation test to test the direction and strength of the relation).

RESULTS

Two hundred and four patients had positive low-medium-high titre aCL or LA test or both; table 1 gives their demographic and clinical characteristics. Fifty seven had had vascular events (21 venous only, 30 arterial only, and six venous and arterial). Table 2 gives the clinical characteristics and drug treatment of aPL positive patients who had vascular or only pregnancy event, or were asymptomatic at the time of the study entry. Patients with vascular events more often had livedo reticularis, migraine, hypertension, hypercholesterolaemia, and concomitant LA and aCL positivity ($p<0.05$).

We identified 1652 aPL tests between 1984 and 2004: 387 LA tests (61% done at our institution and 39% at other laboratories); 1097 aCL IgG/M/A tests (58% done using our in house assay, 24% at Quest Diagnostics, 12% at other

Table 2 Clinical characteristic of 204 antiphospholipid antibody positive patients based on the three patient groups

Characteristic	V ± P event	P event only	Asymptomatic
Number of patients	57	24	123
Age (years) (mean (SD))	43.5 (11.7)	43.0 (10.0)	47.6 (14.2)
No other CTD	27 (47%)[a]	8 (33%)[b]	16 (13%)[ab]
Undifferentiated CTD	7 (12%)	1 (4%)	30 (24%)
SLE*	21 (37%)	14 (58%)	56 (46%)
Livedo reticularis	11 (19%)[a]	4 (16%)	10 (8%)[a]
History of thrombocytopenia	11 (19%)	1 (4%)	11 (14%)
Migraine	23 (40%)[a]	5 (20%)	24 (20%)[a]
Hypertension	22 (39%)[a]	4 (16%)	22 (18%)[a]
Diabetes mellitus	2 (4%)	0 (0%)	4 (3%)
Hypercholesterolaemia	10 (18%)[ab]	0 (0%)[b]	6 (5%)[a]
Current smoking	3 (5%)	3 (12%)	15 (12%)
aPL tests			
LA (+) and aCL (−)	2 (4%)	3 (13%)	13 (11%)
LA (+) and aCL (+)	28 (49%)[a]	11 (46%)[b]	22 (18%)[ab]
LA (−) and aCL (+)	17 (30%)	8 (23%)	55 (45%)
LA unknown and aCL (+)	10 (18%)	2 (8%)	33 (27%)
Drug treatment†			
Warfarin	37 (65%)	0	0
Clopidogrel	4 (7%)	0	0
Aspirin/dipyridamole	1 (2%)	0	0
LMWH	5 (9%)	0	0
Aspirin	9 (16%)	24 (100%)	55 (45%)
No treatment‡	1 (2%)	0	17 (14%)

[aa, bb] Statistical significance ($p<0.05$) between groups (no statistical test was applied to the drug treatment section).
*Based on the American College of Rheumatology criteria.
†Excluding patients who were randomised to receive daily aspirin 81 mg or placebo.
‡No anticoagulant or antiplatelet agents.
aCL, anticardiolipin antibody; aPL, antiphospholipid antibody; CTD, connective tissue disorder; LA, lupus anticoagulant; LMWH, low molecular weight heparin; P, pregnancy; SLE, systemic lupus erythematosus; V, vascular.

Table 4 The number of patients based on the highest initial anticardiolipin antibody (aCL) isotype, the change in the groups based on the final aCL tests, and the stability of aCL tests over time

	0–19 U	20–39 U	40–80 U	⩾80 U
Highest initial aCL result (No of patients)	42	51	39	47
Analysis based on the final aCL results				
Final aCL in the same group*	29 (69%)	16 (31%)	9 (23%)	31 (66%)
Final aCL in a lower group*	–	21 (41%)	21 (54%)†	16 (34%)‡
Final aCL in a higher group*	13 (31%)	14 (28%)	9 (23%)	–
Analysis based on total aCL results				
Total number of aCL tests	226	302	241	338
Mean number of aCL tests	5.4	5.9	6.2	7.2
Total subsequent aCL results remaining in same group (stability, based on four groups)	59%	53%	50%	74%
	0–39 U		**⩾40 U**	
Analysis based on total aCL results				
Total number of aCL tests	528		579	
Mean number of aCL tests	5.7		6.7	
Total number of subsequent aCL tests	435		493	
Patients in whom all of the subsequent aCL results were in the same range	66 (71%)		53 (62%)	
Total subsequent aCL results remaining in same group (stability, based on two groups)§	88%		75%	

Values are n, n (%), or %.
*The number and percentage of patients in the same group, a lower group, and a higher group based on a comparison between the initial and final aCL test result. The highest initial isotype was different than the highest last isotype but were in the same group (<40 *v* ⩾40 U) in 13 patients.
†8/21 final aCL tests were in the negative and 13/21 tests were in the 20–39 U range.
‡2/16 final aCL tests were in the negative range, 6/16 tests were in the 20–39 U, and 8/16 test were in the 40–80 U range.
§19/528 (3.6%) of the aCL test results that switched from ''low to medium'' to ''medium to high'' titres were in the range of 40–45 U; and 21/579 (3.6%) of the aCL test results that switched from ''medium to high'' to ''low to medium'' titres were in the range of 34–39 U.
aCL, anticardiolipin antibody.

laboratories, and 6% by the APSCORE assay); and 168 anti-β_2GPI IgG/M/A tests (54% done at Quest Diagnostics, 37% by the APSCORE assay, and 9% at other laboratories) (table 3).

Of 159 patients tested for LA, 96 (60%) had more than one LA test (total / mean (SD) / median number of LA tests in 96 patients: 324 / 3.5 (1.8) / 3; mean follow up time, 2.4 years). Fifty one initial tests were positive, of which 37 (73%) remained positive based on the final test (mean follow up, 2.1 years; mean number of repeat tests, 2.2); 39 of 51 patients (77%) with an initial positive LA test had persistent LA positivity and 82 of 94 subsequent tests (87%) from 51 patients were positive. Forty five tests were initially negative, of which 37 (82%) remained negative based on the final test (mean follow up, 2.7 years; mean number of repeat tests, 2.7); 40 of 45 patients (89%) with an initially negative LA test had persistent LA negativity, and 121 of 134 subsequent tests (90%) from 45 patients were negative.

One hundred and seventy nine of 204 patients (88%) had more than one aCL test (total / mean (SD) / median number of aCL tests in 179 patients: 1072 / 6.2 (4.7) / 4; mean follow up time, 3.5 years). The highest isotype of the initial test was IgG, IgM, and IgA for 106, 54, and 19 persons, respectively (IgG and IgM for three (analysed as IgG), and IgM and IgA for two (analysed as IgM)). Nine patients had isolated aCL IgA positivity (LA status unknown in four). Table 4 shows the distribution of patients based on the highest initial aCL test; the change in the aCL groups based on the final aCL test; and the stability of aCL tests over time. The combined stability of aCL was 88% for negative to low positive and 75% for moderate to high positive groups. Furthermore, 71% of patients with an initial negative to low positive aCL result and 62% of patients with an initial medium to high positive aCL result had all of the subsequent aCL results in the same range.

One hundred and nine persons had anti-β_2GPI tests but only 27 (13%) had more than one (total / mean (SD) / median number of anti-β_2GPI tests in 27 patients: 87 / 3.2 (1.5) / 2; mean follow up time, 1.0 years). The highest isotype of the initial test was IgG, IgM, and IgA for 11, 9, and 1 persons, respectively (six were reported as negative for all the isotypes). Table 5 shows the change in the anti-β_2GPI for patients with more than one test. Ninety six per cent of subsequent tests in patients with an initial anti-β_2GPI titre of <40 U/ml and 76% of subsequent tests in patients with an initial anti-β_2GPI titre of ⩾40 U/ml remained in the same group. Furthermore, 83% of patients with an initial negative to low positive anti-β_2GPI result and 67% of patients with an initial medium to high positive anti-β_2GPI result had all of the subsequent anti-β_2GPI results in the same range.

Excluding asymptomatic patients participating in a trial testing low dose aspirin *v* placebo, aspirin, warfarin, and hydroxychloroquine use did not differ between persons whose aCL titres decreased from medium to high to negative

Table 5 The number of patients based on the initial anti-β_2GPI test, the change in the groups based on the final anti-β_2GPI tests, and the stability of anti-β_2GPI tests over time

	0–39 U	⩾40 U
The highest initial anti-β_2GPI result (No of patients)	12	15
Analysis based on the final anti-β_2GPI results		
Final anti-β_2GPI in the same group*	11 (92%)	11 (73%)
Analysis based on total anti-β_2GPI results		
Total number of anti-β_2GPI tests	30	57
Mean number of anti-β_2GPI tests	2.5	3.8
Total number of subsequent anti-β_2GPI tests	18	42
Patients in whom all subsequent anti-β_2GPI results were in the same range	10 (83%)	10 (67%)
Total subsequent anti-$\beta_2$2GPI results remaining in same group (stability)	96%	76%

Values are n, n (%), or %.
*The number and percentage of patients in the same group based on a comparison between the initial and final anti-β_2GPI test results.
β_2GPI, β_2 glycoprotein I.

Table 6 The consistency, agreement, and correlation between our in house assay and other aCL kits

aCL kits	n	Consistency	Agreement	Correlation
IHA *v* APSCORE IgG	31	81%	0.66	0.8
IHA *v* APSCORE IgM	31	81%	0.46	0.8
IHA *v* APSCORE IgA	31	68%	0.15	0.5
IHA *v* Diamedix IgG	75	81%	0.4	0.6
IHA *v* Diamedix IgM	75	88%	0.4	0.8
IHA *v* Diamedix IgA	75	88%	0.2	0.6
IHA *v* Inova IgG	77	78%	0.5	0.8
IHA *v* Inova IgM	77	64%	0.3	0.8
IHA *v* Inova IgA	77	71%	0.2	0.7

APSCORE, antiphospholipid syndrome collaborative registry; IHA, in house assay.

to low (n = 26), increased from negative to low to medium to high (n = 13), or remained stable (n = 65) (p = 0.93, p = 0.13, and p = 0.83, respectively). Similarly, definite and possible aPL related clinical manifestations were not statistically different between the patients whose aCL titres decreased, increased, or remained stable. The diagnosis of SLE was present in 35%, 62%, and 41% of patients whose aCL titres decreased, increased, or remained stable, respectively (p = 0.2). When 91 SLE patients were compared with 51 patients with no connective tissue disorders, there was no statistical difference between the number of patients whose aCL titres decreased (12% *v* 22%, p = 0.25), increased (12% *v* 6%, p = 0.34), or remained stable (76% *v* 72%, p = 0.72).

Thirty one patients had same specimen aCL tests by our institution's in house assay and APSCORE; 75 by our in house assay and Diamedix Diagnostics aCL kit; and 77 by our in house assay and Inova Diagnostics aCL kit. Table 6 shows the consistency, agreement, and correlation between our institution's in house assay and other aCL kits. The consistency ranged from 64% to 88% and the correlation ranged from 0.5 to 0.8. Agreement was moderate for aCL IgG and aCL IgM; agreement for aCL IgA was marginal.

DISCUSSION

Our data show that aPL results remain stable for at least three quarters of subsequent tests during a mean follow up of 2.4 years for the LA test, 3.5 years for the aCL test, and 1.0 year for the anti-β_2GPI test. The small amount of variation that occurs does not appear to result from aspirin, warfarin, or hydroxychloroquine use.

The Sapporo criteria require, without definition, medium to high titre aCL is to make the diagnosis of APS.[1] Different clinical laboratories define ''medium to high titre aCL'' as more than 15–20 international units (IU), 2.0–2.5 times the median, or the 99th centile of normal population titres. In the absence of consensus among laboratories[5] or APS experts[6] about what constitutes medium to high titre aCL, several studies concluded that more than 40 IU or GPL U/ml is more predictive of thrombotic events[7–11] than lower titres. We used a cut off aCL limit of 40 U for ''medium to high titre aCL'' and found that, regardless of the laboratory carrying out the test, if the initial aCL result was less than 40 U, the probability of obtaining a repeat study within the same range in the next 3.5 years was 88%. If the initial aCL result was equal to or more than 40 U, the probability that a second test will also be in the same range was 75%. Antiphospholipid antibodies bind primarily to the negatively charged phospholipids through the phospholipid binding plasma protein β_2GPI, and some APS patients may have only anti-β_2GPI antibodies.[12–13] Despite the small number of patients, we also found that anti-β_2GPI results of less than or more than 40 U/ml were stable in at least three quarters of subsequent tests.

Similarly, at least three quarters of LA tests stayed either positive or negative. Although our study was not primarily designed to test this question, we found that concurrent positivity of LA and aCL tests, traditional thrombosis risk factors, livedo reticularis, and migraine were more common in APS patients with vascular events than in aPL positive asymptomatic persons. These results are consistent with other studies showing that a positive LA test is more commonly associated with thrombosis than is aCL,[14] and that dual positivity of aCL and LA test is more commonly associated with thrombosis than is single positivity of aCL or LA test.[15]

Infection induced transient aPL positivity is common in the general population and usually not pathogenic. Vila *et al* analysed 552 healthy donors for the prevalence of aPL and found that 86% of positive IgG aCL and 97% of positive IgM aCL tests were negative in one year.[16] As our study population derives from registries that require two positive aPL results as inclusion criteria, our results do not apply to patients with single aPL positivity.

Although McCarty *et al* reported that aPL decreased in patients treated with hydroxychloroquine 200 mg twice daily and aspirin 81 mg once daily,[17] we did not observe any relation between aspirin, warfarin, or hydroxychloroquine treatment and change in aCL titre. The retrospective nature of the study did not allow us to analyse corticosteroid or immunosuppressive drug use accurately.

In order to determine the possible contribution of inter-laboratory differences to aPL variation, we analysed the degree of variation between aCL kits. Based on same day specimens, the consistency of aCL results among different suppliers ranged from 64% to 88%, with moderate agreement for IgG and IgM. The agreement for aCL IgA was marginal. Although interlaboratory agreement is better when positive aCL results are compared in semiquantitative measures (ranges of positivity),[18] others also found medium agreement between kits especially for IgG aCL.[19] We did not analyse interlaboratory variations between anti-β_2GPI antibody kits; however, Reber *et al* showed that anti-β_2GPI antibody kits are also poorly standardised, especially at lower titres, and the agreement is better with medium to high titre IgG anti-β_2GPI.[20] Thus physicians should be conservative in their interpretation of aCL and anti-β_2GPI levels in individual patients when results from multiple laboratories are available, a common scenario in the USA. The standardisation of aCL and anti-β_2GPI assays can still be improved to provide better agreement, a point also noted by others.[21] Nonetheless, although interlaboratory differences might have contributed to the degree of aPL variation over time, we found that at least three quarters of aPL results were stable in the range of negative to low and moderate to high over time.

Our study has several limitations. It could not evaluate the effect of SLE activity on aPL variation, a point argued by some investigators[22] but contradicted by others.[23] Second, our titre groupings were arbitrary but consistent with clinical experience. Within these ranges, aPL variation was low. However, the clinical significance of our cut off points requires further verification. Third, aPL tests from multiple laboratories were included in our analysis; however, this was intentional in order to simulate a real world experience in which multiple and visit to visit comparisons must rely on studies done in different laboratories owing to rapidly changing insurance coverage systems. Lastly, the study did not evaluate aPL variation beyond three to four years, and it is possible that more variation may be observed with longer follow up.

Conclusions

Repeat aPL results remain stable for at least three quarters of subsequent tests regardless of the laboratory carrying out the test. The variation that occurs does not appear to result from aspirin, warfarin, or hydroxychloroquine use. Although it is unknown if and what level of aPL variation has clinical significance, our findings should offer guidance to practising physicians and researchers in the management of aPL positive patients.

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