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Antiphospholipid Antibody Profile Stability Over Time: Prospective Results From the APS ACTION Clinical Database and Repository

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ABSTRACT. ***Objective.*** The APS ACTION Registry studies long-term outcomes in persistently antiphospholipid antibody (aPL)-positive patients. Our primary objective was to determine whether clinically meaningful aPL profiles at baseline remain stable over time. Our secondary objectives were to determine (1) whether baseline characteristics differ between patients with stable and unstable aPL profiles, and (2) predictors of unstable aPL profiles over time.

Methods. A clinically meaningful aPL profile was defined as positive lupus anticoagulant (LAC) test and/or anticardiolipin (aCL)/anti-β_2 glycoprotein-I (anti–β_2-GPI) IgG/M ≥ 40 U. Stable aPL profile was defined as a clinically meaningful aPL profile in at least two-thirds of follow-up measurements. Generalized linear mixed models with logit link were used for primary objective analysis.

Results. Of 472 patients with clinically meaningful aPL profile at baseline (median follow-up 5.1 yrs), 366/472 (78%) patients had stable aPL profiles over time, 54 (11%) unstable, and 52 (11%) inconclusive. Time did not significantly affect odds of maintaining a clinically meaningful aPL profile at follow-up in univariate ($P = 0.906$) and multivariable analysis ($P = 0.790$). Baseline triple aPL positivity decreased (OR 0.25, 95% CI 0.10–0.64, $P = 0.004$) and isolated LAC test positivity increased (OR 3.3, 95% CI 1.53–7.13, $P = 0.002$) the odds of an unstable aPL profile over time.

Conclusion. Approximately 80% of our international cohort patients with clinically meaningful aPL profiles at baseline remain stable at a median follow-up of 5 years; triple aPL-positivity increase the odds of a stable aPL profile. These results will guide future validation studies of stored blood samples through APS ACTION Core Laboratories.

Key Indexing Terms: anticardiolipin antibodies, antiphospholipid antibodies, antiphospholipid syndrome

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Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by thrombosis and pregnancy morbidity in patients with persistently positive antiphospholipid antibodies (aPL). aPL that are used for APS classification include lupus anticoagulant (LAC) test, anticardiolipin antibodies (aCL), and anti-β_2 glycoprotein-I antibodies (anti–β_2-GPI)[1].

The assessment of aPL profiles upon evaluation of aPL-positive patients is critical. Persistently positive aPL are more likely to have important clinical implications, while transiently positive aPL, especially of low titer, may be a result of infections or medications. Certain aPL profiles, such as LAC positivity, high titer aCL/anti–β_2-GPI, or triple aPL positivity, are more strongly associated with aPL-related clinical events, although traditional risk factors also need to be taken into account while evaluating aPL-positive patients[2]. The course of aPL positivity over time is also important in the risk stratification and management of patients; however, there are limited prospective data on the course of aPL tests over time.

Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION) is an international network created to design and conduct large-scale multicenter studies and clinical trials in persistently aPL-positive patients. The APS ACTION clinical database and repository ("Registry") was created to study the natural course of persistently aPL-positive patients with or without autoimmune disorders over at least 10 years; the Registry allows us to perform large-scale cross-sectional and prospective analyses, which will eventually help us better understand the clinical characteristics of patients with APS.

In this analysis of the APS ACTION Registry, our primary objective was to determine whether clinically meaningful aPL profiles (defined as a positive LAC test and/or aCL/anti–β_2-GPI IgG/M ≥ 40 U) at baseline remain stable over time in persistently (on 2 or more occasions at least 12 weeks apart) aPL-positive patients. Our secondary objectives were to determine (1) whether demographic, clinical, and laboratory characteristics at baseline differ between patients with stable and unstable aPL profiles over time, and (2) predictors of unstable aPL profiles over time.

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MATERIALS AND METHODS

APS-ACTION Registry. The APS-ACTION Registry is a Web-based data capture system developed in Research Electronic Data Capture (REDCap) that includes patients with persistently positive aPL with or without other systemic autoimmune disease. Inclusion criteria are positive aPL, based on the Updated Sapporo APS Classification Criteria[1], tested at least twice within 1 year prior to enrollment. Patients are followed every 12 ± 3 months with clinical and laboratory data, and blood collection. Each participating center has ethics board approval (Hospital for Special Surgery Institutional Review Board ID 2014-252, lead coordinating center), and all patients have provided written informed consent that allows publication of this material.

Study cohort. As of January 2019, there were 796 patients enrolled in APS ACTION Registry from 26 centers worldwide; 472 patients with baseline clinically meaningful aPL profiles and follow-up visits with available aPL tests were included in this analysis (Figure 1).

Data collection. For this retrospective and prospective Registry analysis, we retrieved clinical and laboratory data at baseline and follow-up. The clinical data included information on demographics (age, sex, race, and ethnicity), concomitant systemic autoimmune diseases, aPL-related history (thrombotic and obstetric), noncriteria aPL manifestations (i.e., thrombocytopenia, autoimmune hemolytic anemia, cardiac valve disease, livedo reticularis/racemosa, skin ulcers, aPL nephropathy, and cognitive dysfunction), and medications. All available standard-of-care measurements (retrospective and prospective) for LAC, aCL IgG/M, and anti–β_2-GPI IgG/M from the Registry were utilized. For the baseline visit, we used the most recent aPL profile (LAC, aCL/anti–β_2-GPI IgG/M). At each annual follow-up visit, we used the first available aPL profile that was reported for that time period. High aCL/anti–β_2-GPI titers reported as "greater than x" units (e.g., > 80 U) were converted to "x" units (e.g., 80 U) to facilitate the statistical analysis.

Definitions. We defined a *clinically meaningful profile* as a positive LAC test and/or aCL/anti–β_2-GPI IgG/M ≥ 40 U, and a *stable clinically meaningful aPL profile* if it remained stable in at least two-thirds of follow-up aPL measurements. We defined aCL/anti–β_2-GPI IgG/M as positive when the reported titer was ≥ 40 U. We selected a cutoff positivity of 40 U for aCL/anti–β_2-GPI, given that (1) low aPL titers are generally transient and clinically less significant, and (2) there is limited literature demonstrating that titers > 40 U are more likely to be associated with aPL-related manifestations[1,3,4] and therefore are considered more effective in clinical care of aPL-positive patients. Inconclusive aPL profile during the follow-up was defined as (1) missing determinant aPL test result(s) (those used to determine the baseline clinically meaningful aPL profile) with no other positive aPL tests, or (2) negative determinant aPL test result(s) with missing other aPL test result(s).

Statistical analysis. We hypothesized that a clinically meaningful aPL profile at baseline remains stable over time. Univariate and multivariable generalized linear mixed models (GLMM) with logit link were used to assess the effect of time and other variables of interest on odds of clinically meaningful aPL profiles over time. A GLMM framework allowed us to introduce random effects to account for within-subject correlation due to repeated measures of aPL profiles across follow-up. *T*-test (for normally distributed variables), Wilcoxon rank-sum (for abnormally distributed variables), and Fisher exact tests (for categorical variables) were employed to compare clinical characteristics of patients with stable versus unstable aPL profiles. Univariate and multivariable logistic regression were used to examine predictors of unstable aPL profiles (negative LAC and aCL/anti–β2-GPI IgG/M < 40 U) over time.

RESULTS

At baseline, 472 patients had a clinically meaningful aPL profile. The median age of these patients was 49 years (IQR 39–59) and 349 (74%) were female. The median follow-up was 5.1 years

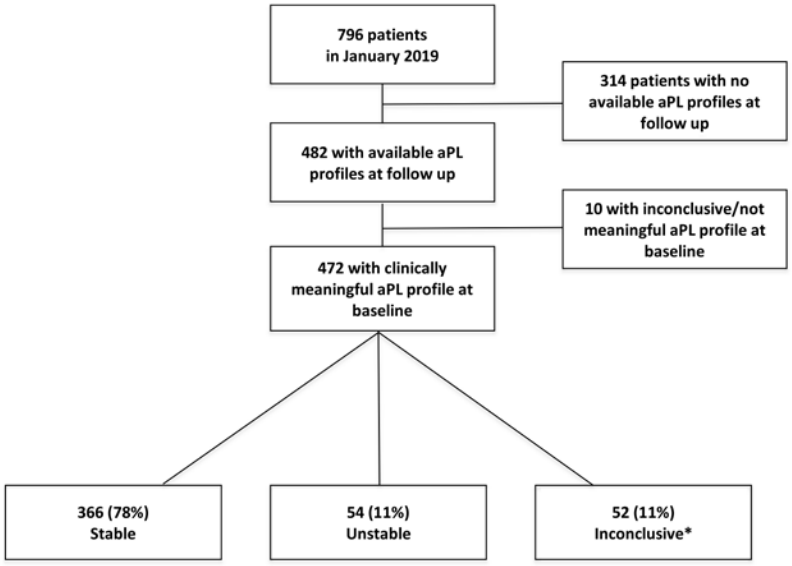

Figure 1. aPL profiles over time (N = 482). * Reasons for inconclusive follow-up aPL profile: (1) missing determinant aPL test(s) (those used to determine the baseline clinically meaningful aPL profile) with no other positive aPL tests (n = 28), and (2) negative determinant aPL test result(s) with missing other aPL test result(s) (n = 24). aPL: antiphospholipid antibody.

(IQR 4.3–5.8), and the median number of follow-up visits with aPL tests was 2 (IQR 1–3). Based on the different number of available aPL tests at each year of follow-up, 254 (73%) had clinically meaningful aPL profiles at 1-year follow-up, 216 (72%) at 2-year, 177 (72%) at 3-year, 135 (73%) at 4-year, and 61 (70%) at 5-year (Figure 1, Table 1).

aPL profile stability over time. Three hundred and sixty-six of 472 (78%) patients had stable and 54/472 (11%) had unstable aPL profiles over a median follow-up of 5 years. One hundred and fifty-one (32%) patients contributed to the stability analysis with 1 follow-up visit, 99 (21%) with 2, 105 (22%) with 3, 87 (18%) with 4, and 27 (6%) with 5. In 52/472 (11%) patients, the assessment was inconclusive; thus, these patients were excluded from further analysis (Figure 1). A univariate GLMM demonstrated that time across follow-up did not significantly affect odds of maintaining a stable clinically meaningful aPL profile over time ($P = 0.906$). Similar results were observed when the model was adjusted for age, active smoking, concomitant autoimmune disease, and hydroxychloroquine (HCQ) use at baseline ($P = 0.790$).

Demographic, clinical, and laboratory characteristics differences between stable versus unstable aPL profile status. Table 2 describes baseline demographic, clinical, and laboratory characteristics of the 420 patients who had stable and unstable clinically meaningful aPL profiles at follow-up. LAC, aCL IgM, anti–β_2-GPI IgG positivity, and positivity on ≥ 2 aPL tests at baseline were associated with a stable aPL profile ($P < 0.001$, $P = 0.004$, $P = 0.005$, and $P < 0.001$, respectively). While aCL IgG or anti–β_2-GPI IgM positivity was not associated with a stable aPL profile ($P = 0.06$ for both), a larger proportion of patients with a stable aPL profile were aCL IgG (50% vs 35%) and anti–β_2-GPI IgM positive (21% vs 9%) at baseline. In addition, patients with stable clinically meaningful aPL profiles, compared to those with unstable aPL profiles, were more likely to have higher aCL IgG (median 46 U vs 16 U) and anti–β_2-GPI IgG (median 22 U vs 3 U) titers at baseline, and triple aPL positivity (46% vs 13%), while they were less likely to have isolated LAC test positivity (17% vs 41%) or isolated anti–β_2-GPI IgG/M positivity (2% vs 9%). No differences were noted between patients with or without concomitant autoimmune disease at baseline.

Table 1. aPL profiles over time (N = 482).

	Baseline	0–12 M	12–24 M	24–36 M	36–48 M	48–60 M
No. patients with follow-up	N/A	452	398	357	282	138
No. patients with aPL results	482	348	302	245	184	87
Meaningful aPL profile[a]	472 (98)	254 (73)	216 (72)	177 (72)	135 (73)	61 (70)
Not meaningful aPL profile[b]	3 (1)	31 (9)	29 (10)	24 (10)	14 (8)	7 (8)
Inconclusive aPL profile[c]	7 (1)	63 (18)	57 (19)	44 (18)	35 (19)	19 (22)

Values are n (%) unless otherwise indicated. [a] Positive LAC test and/or aCL/anti–β_2-GPI IgG/M ≥ 40 U. [b] Negative LAC test and aCL/anti–β_2-GPI IgG/M < 40 U. [c] Missing determinant aPL test result(s) (those used to determine the baseline clinically meaningful aPL profile) with no other positive aPL tests, or negative determinant aPL test result(s) with missing other aPL test result(s). aCL: anticardiolipin antibody; anti–β_2-GPI: anti-β_2 glycoprotein I antibody; aPL: antiphospholipid antibody; LAC; lupus anticoagulant; M: months.

Table 2. Baseline clinical and laboratory characteristics of patients (N = 420) with stable or unstable clinically meaningful aPL profiles at follow-up.

	Total, n = 420	Clinically Meaningful aPL Profile		*P*
		Stable, n = 366	Unstable, n = 54	
Female	305 (73)	267 (73)	38 (70)	0.74
Age, yrs, median (IQR)	48.9 (48.1–50.4)	48.6 (47.9–49.4)	48.6 (48–50)	0.09
White	279 (78)	238 (77)	41 (87)	0.30
Non–Latin American	165 (39)	137 (37)	28 (52)	0.46
Autoimmune disease				0.76
aPL/APS only	278 (66)	244 (67)	34 (63)	
Other SAID	148 (35)	128 (35)	20 (37)	
aPL-related history				
Vascular event[a] (any)	285 (68)	245 (67)	40 (74)	0.35
Venous event (any)	183 (64)	153 (62)	30 (75)	0.16
Arterial event (any)	125 (44)	115 (47)	10 (25)	**0.01**
TIA (any)	38 (9)	37 (10)	1 (2)	**0.04**
Pregnancy morbidity[b], n	136	119	17	0.83
Spontaneous abortions[c], n	13	10	3	0.21
Premature birth[d], n	37	34	3	0.56
Unexplained fetal death[e], n	76	67	9	0.80
aPL tests				
LAC[f] (+)	319 (80)	288 (83)	31 (58)	**< 0.001**
aCL IgG ≥ 40 U	202 (48)	183 (50)	19 (35)	0.06
aCL IgM ≥ 40 U	93 (22)	89 (24)	4 (7)	**0.004**
anti–β_2-GPI IgG ≥ 40 U	139 (33)	130 (36)	9 (17)	**0.005**
anti–β_2-GPI IgM ≥ 40 U	81 (19)	76 (21)	5 (9)	0.06
≥ 2 positive aPL tests	244 (58)	226 (62)	18 (33)	**< 0.001**
aPL titers, U, median (IQR)				
aCL IgG	36 (10–93)	46 (13–100)	16 (4–56)	**< 0.001**
aCL IgM	12 (5–39)	13 (5–42)	8.5 (2–15.5)	**0.006**
anti–β_2-GPI IgG	19 (3–74)	22 (3–83)	3 (1–30)	**< 0.001**
anti–β_2-GPI IgM	9 (2–33)	10 (2–39)	4 (1–20)	0.04
aPL profiles				
Triple aPL positivity	174 (41)	167 (46)	7 (13)[g]	**< 0.0001**
Double aPL positivity[h]	120 (29)	106 (29)	13 (26)	0.75
Isolated LAC test positivity	84 (20)	62 (17)	22 (41)	**0.0002**
Isolated aCL IgG/M positivity	29 (7)	23 (6)	6 (11)	0.24
Isolated anti–β_2-GPI IgG/M positivity	13 (3)	8 (2)	5 (9)	**0.02**
Medications				
Aspirin	201 (48)	187 (51)	14 (26)	**< 0.001**
Warfarin	223 (53)	192 (52)	31 (57)	0.68
Hydroxychloroquine	194 (46)	168 (46)	26 (48)	0.82

Values are n (%) unless otherwise indicated. [a] During an average follow-up of 5 years, new thrombosis occurred in 30 (7%) of 420 patients (24 with history of baseline thrombosis, and 6 patients without), 29/30 had a stable clinically meaningful aPL profile at follow-up. [b] Out of 207 patients with history of pregnancy (with or without morbidity). [c] 3 consecutive unexplained spontaneous abortions before 10th week. [d] Premature birth before 34th week due to eclampsia, pre-eclampsia, or placental insufficiency. [e] Unexplained fetal death at or beyond 10th week. [f] LAC test was reported by each center as positive or negative (screening by dilute Russell Viper Venom Time [dRVVT] and activated Partial Thromboplastin Time [aPTT] in 55% of patients, dRVVT in 28%, aPTT in 9%, other methods in 5%, and nonreported methods in 2%). [g] Seven triple aPL-positive patients with unstable aPL profile were mostly on warfarin with fluctuating LAC test status, and relatively low level aPL ELISA at baseline (2 patients only had IgM isotype). [h] Any combination of 2 positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria. aCL: anticardiolipin antibody; anti–β_2GPI: anti-β_2 glycoprotein I antibody; aPL: antiphospholipid antibody; APS: antiphospholipid syndrome; LAC: lupus anticoagulant; SAID: systemic autoimmune diseases; TIA: transient ischemic attack.

Predictors of an unstable aPL profile over time. In a univariate unadjusted logistic model with unstable aPL profile as the outcome, triple aPL positivity at baseline was associated with a 75% decreased likelihood for unstable aPL profiles at follow-up (OR 0.25, 95% CI 0.1–0.6, P = 0.004; Table 3). Further, (1) patients with isolated LAC test positivity at baseline had 3.3-times higher odds for unstable aPL profiles (OR 3.3, 95% CI 1.5–7.1, P = 0.002), and (2) aCL or anti–β_2-GPI IgG/M ≥ 40 U, but without any aCL/anti–β_2-GPI positivity, was associated with lower odds of unstable aPL profiles over time. In a multivariable logistic model adjusted for age, sex, active smoking, concomitant autoimmune disease, and HCQ use at baseline: (1) triple aPL positivity at baseline was associated with lower odds of unstable aPL profiles (OR 0.17, 95% CI 0.1–0.4, P < 0.0001), and (2) isolated LAC test positivity and isolated anti–β_2-GPI positivity at baseline were associated with higher odds

Table 3. Baseline predictors of unstable aPL profile at follow-up.

Univariate (Unadjusted)	OR (95% CI)	*P*
Baseline aPL profile		
Triple aPL positive	0.25 (0.10–0.64)	**0.004**
Double aPL positive[a]	0.67 (0.31–1.46)	0.32
Isolated LAC test positivity	3.30 (1.53–7.13)	**0.002**
Isolated aCL IgG/M positivity[b,c]	2.13 (0.71–6.37)	0.18
Isolated anti–β_2-GPI IgG/M positivity[b,c]	2.31 (0.49–10.75)	0.29
Baseline individual aPL tests		
LAC test positivity	0.21 (0.11–0.41)	**< 0.001**
aCL IgG ≥ 40 U	0.21 (0.10–0.45)	**< 0.001**
aCL IgM ≥ 40 U	0.26 (0.09–0.73)	**0.01**
anti–β_2-GPI IgG ≥ 40 U	0.26 (0.11–0.65)	**0.004**
anti–β_2-GPI IgM ≥ 40 U	0.28 (0.08–0.93)	**0.04**
Age	1.27 (0.98–1.63)	0.06
Sex, male	0.70 (0.33–1.48)	0.35
HCQ use	0.94 (0.47–1.87)	0.85
Autoimmune disease	1.27 (0.60–2.67)	0.54
Active smoking	1.56 (0.32–7.53)	0.58
Multivariable (Adjusted)	**OR (95% CI)**	***P***
Baseline aPL profile		
Triple aPL positive[d]	0.17 (0.07–0.39)	**< 0.0001**
Isolated LAC test positivity[d]	3.65 (1.94–6.84)	**< 0.0001**
Isolated anti–β_2-GPI positivity[d]	4.17 (1.24–14.1)	**0.02**
Baseline individual aPL tests		
LAC test positivity[e]	0.26 (0.10–0.66)	**0.005**
aCL IgG ≥ 40 U[f]	0.24 (0.09–0.66)	**0.006**

Values in bold are statistically significant. [a] Any combination of 2 positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria. [b] Any titer above normal range. [c] The small number of patients in isolated aCL and anti–β_2-GPI aPL profile categories precluded from further analysis of isolated IgG or IgM isotype for these groups. [d] **A**djusted for age, sex, active smoking, concomitant autoimmune disease, and HCQ use at baseline. [e] **A**djusted for age, sex, active smoking, concomitant autoimmune disease, HCQ use at baseline, clinically meaningful (≥ 40 U) aCL IgG and IgM at baseline, and clinically meaningful (≥ 40 U) anti–β_2-GPI IgG and IgM at baseline. [f] Adjusted for age, sex, active smoking, concomitant autoimmune disease, HCQ use at baseline, LAC test result at baseline, clinically meaningful (≥ 40 U) aCL IgM at baseline, and clinically meaningful (≥ 40 U) anti–β_2-GPI IgG and IgM at baseline. aCL: anticardiolipin antibody; anti–β_2-GPI: anti-β_2 glycoprotein I antibody; aPL: antiphospholipid antibody; APS: antiphospholipid syndrome; HCQ: hydroxychloroquine; LAC: lupus anticoagulant.

of unstable aPL profiles (OR 3.65, 95% CI 1.9–6.8, $P < 0.0001$; OR 4.17, 95% CI 1.2–14.1, $P = 0.02$, respectively). When the multivariate model was further adjusted for individual aPL tests, (1) baseline LAC test positivity (OR 0.26, 95% CI 0.1–0.7, $P = 0.005$) and (2) baseline aCL IgG ≥ 40 U (OR 0.24, 95% CI 0.1–0.7, $P = 0.006$) were associated with lower odds of unstable aPL profiles over time.

Individual aPL result stability over time. Table 4 describes the course of aCL and anti–β_2-GPI IgG/M titers over time based on their assignments to 1 of the following categories at baseline and follow-up: 0–19 U, 20–39 U, 40–79 U, and ≥ 80 U. Approximately 90% and 60–80% of follow-up tests in patients with a baseline titer of 0–19 U and ≥ 80 U, respectively, remained in the same category. For baseline titers of 20–39 U and 40–79 U, during the follow-up, 23–30% and 19–33% remained in the same range, 36–60% and 41–65% decreased to a lower category, and 17–36% and 16–28% increased to a higher category, respectively. With respect to the LAC tests, 88% of patients with baseline isolated LAC positivity receiving no anticoagulation had a stable clinically meaningful profile at follow-up, compared to 52% on anticoagulation (OR 6.9, $P = 0.009$).

DISCUSSION

Our large-scale analysis of persistently positive aPL patients demonstrated that a clinically meaningful aPL profile, defined as a positive LAC test and/or aCL/anti–β_2-GPI IgG/M ≥ 40 U, remains stable during a median follow-up of 5 years independent of age, active smoking, concomitant systemic autoimmune disease, and HCQ use at baseline. Triple aPL positivity increases and isolated LAC positivity decreases the odds of a stable aPL profile.

In patients with triple aPL positivity (LAC, aCL, and anti–β_2-GPI of the same isotype) at initial aPL testing, 98% have been shown to have confirmed persistence of titers at 12 weeks, compared to 84% for double aPL positivity (aCL and anti–β_2-GPI of the same isotype) and 40% for isolated aPL test positivity (LAC, aCL, or anti–β_2-GPI)[5]. Based on a limited number of studies, 70–90% of patients with persistently positive aPL profiles remain positive during follow-up, ranging from 2 to 10 years[6,7,8]. In contrast, 1 study of 105 women with persistently positive aPL tests (49 with primary APS) found that in 59% of patients, the aPL profile become negative within approximately 10 years of follow-up[9]. The limitations of these studies include retrospective study designs with varying follow-up times and frequency of aPL tests, different cutoff levels used to define aPL positivity (≥ 20 or 40 U, or > 99th percentile of controls), and incomplete analysis of aPL profiles. Using a large, multicenter, international database of patients with persistently positive aPL profiles, we demonstrated that clinically meaningful aPL profiles remain stable over time at a median follow-up of 5 years. Our results are based on explicit and clinically relevant definitions of aPL profile positivity, and prospectively collected clinical and laboratory data.

Interpretation of aPL tests should be done cautiously since not every positive test is clinically important. Triple aPL positivity[10,11] or LAC positivity[12] is known to confer a higher risk for aPL-related clinical events compared to aCL and anti–β_2-GPI positivity. Additionally, IgG aCL and anti–β_2-GPI are more likely to be associated with clinical events compared to IgM[13]. The clinical significance of low titer aPL (20–39 U) should be interpreted carefully since it may be transient and associated with infectious triggers. Persistence of aPL positivity (when tested at least 12 weeks apart) and medium to high titers of aCL and anti–β_2-GPI, as defined by the Updated Sapporo APS Classification Criteria[1], are more likely to be associated with APS. To that point, our present study shows that patients who maintain a stable clinically meaningful aPL profile at 5 years of follow-up are more likely to have, at baseline, LAC test positivity, 2 or more positive aPL tests (including triple aPL positivity), and higher ELISA titers for aCL IgG that are clinically meaningful.

In our analysis, patients with a stable clinically meaningful

Table 4. Individual aPL course over time based on titers at baseline.

	Baseline Titer, U	No. Patients at Baseline	No. Patients Follow-up aPL	aPL Titer at Follow-up, U, % 0–19	20–39	40–79	≥ 80
aCL IgG	0–19	195	420	89	9	2	1
aCL IgM		281	652	91	5	4	1
Anti–β_2-GPI IgG		159	375	90	5	3	2
Anti–β_2-GPI IgM		206	477	94	3	1	1
aCL IgG	20–39	53	145	41	23	24	12
aCL IgM		54	140	51	24	18	7
Anti–β_2-GPI IgG		34	83	36	30	19	14
Anti–β_2-GPI IgM		31	72	60	24	7	10
aCL IgG	40–79	74	199	25	22	33	21
aCL IgM		49	113	29	14	33	24
Anti–β_2-GPI IgG		41	90	20	21	31	28
Anti–β_2-GPI IgM		17	37	51	14	19	16
aCL IgG	≥ 80	111	255	10	7	18	65
aCL IgM		41	104	6	4	29	62
Anti–β_2-GPI IgG		68	139	6	5	9	79
Anti–β_2-GPI IgM		40	90	11	8	11	70

aPL: antiphospholipid antibody, aCL: anticardiolipin antibody; anti–β_2-GPI: anti-β_2 glycoprotein I antibody.

aPL profile over time had more frequent history of arterial events ($P = 0.01$) or transient ischemic attacks ($P = 0.04$) at baseline and were more frequently taking aspirin ($P < 0.001$). One potential explanation could be the higher frequency of a triple positive aPL profile in patients with a stable aPL profile over time compared to those with unstable profiles (46% and 13%, respectively). Yet, venous events at baseline did not show a similar trend despite the triple positive aPL profile. This finding, if not occurring due to a relatively small number of patients in the unstable group, is hypothesis-generating and should be explored in future studies.

When determining predictors for an unstable aPL profile over time, we adjusted for various factors that have been implicated in maintenance of aPL test positivity. First, the use of HCQ was considered a potentially contributing factor, as a retrospective study has demonstrated that patients with systemic lupus erythematosus (SLE) and persistently positive aPL profiles (positive LAC and/or an aCL/anti–β_2-GPI ≥ 40 U) were less likely to be taking HCQ compared to patients with transiently positive or negative profiles[14]; HCQ may also decrease aCL IgG/M levels and dRVVT (dilute Russell Viper Venom Time) prolongation[15]. Second, smoking was implicated in triggering aPL production, yet interpretation of relevant studies is difficult since smoking is a risk factor for thrombosis along with aPL[16]. Finally, we speculated that presence of concomitant autoimmune disease (such as SLE) may be associated with stable aPL tests since SLE is characterized by aberrant autoantibody production; a small study has supported that SLE activity was higher in patients with persistently positive aPL tests (LAC and aCL)[17]. Therefore, even after adjusting for age, sex, active smoking, concomitant autoimmune disease (mainly SLE), and HCQ use at baseline, triple aPL positivity was still 83% less likely to be associated with an unstable aPL profile.

The LAC test, when persistently positive, is highly associated with obstetric and thrombotic events. Despite guidelines, LAC results among laboratories may be discrepant due to lack of standardization and use of different screening tests. In addition, LAC results may be unreliable when tested on anticoagulation including direct oral anticoagulants (DOAC)[18,19]. An exercise among 4 different laboratories demonstrated that discordant or inconclusive LAC test results occur in 45% of patients with history of thrombosis or suspected APS, which increases to 75% when only patients on vitamin K antagonists are examined[20]. In our cohort, isolated LAC test positivity had significantly higher odds of being associated with an unstable aPL profile; we speculate that this finding was due to a relatively high number of anticoagulated patients. For more accurate assessment, future APS-ACTION studies will be completed using Core Laboratory LAC test results, which have been performed using methods with minimal interference with anticoagulation.

Our study has several limitations. First, we have missing follow-up data, as aPL testing was based on the discretion of the treating physician; however, we plan to reassess the aPL profiles in future studies using stored blood samples from each patient visit. Second, we could not assess the aPL profile stability in 11% of patients who had inconclusive aPL profiles in our cohort. A portion of these patients (24/52) could potentially have been added to the unstable aPL group; however, we wanted to avoid basing our results on the assumption that the rest of the aPL tests remained negative when no data were available. Third, median aCL/anti–β_2-GPI titers may have been underestimated, as (1) for titers reported as "greater than x units" we used the upper limit, and (2) we used all available titers irrespective of positivity. Fourth, the association between stable aPL profile over time and aPL-related clinical events at follow-up was not formally explored as this was beyond the primary objective of the study, yet the available descriptive data show that among patients with thrombotic events at follow-up, 97% (29/30) had a stable, clinically meaningful aPL profile. Future analyses of the Registry will specifically address predictors of first and recurrent events

in aPL-positive patients. Finally, referral bias may influence the generalizability of our findings.

Despite these limitations, the APS ACTION Registry comprises patients from tertiary referral centers across the world, and we believe that the large number of patient data provide a better understanding of aPL profile changes over time. The findings of this study are expected to inform and serve as a comparator for future validation studies of aPL profiles in stored blood samples of patients in the APS ACTION Registry, bypassing issues of assay and protocol heterogeneity among different laboratories across the world, interference of anticoagulation use at time of testing, and missing data.

In conclusion, using a large, multicenter, international database of patients with persistently positive aPL profiles, we demonstrated that the majority, approximately 80%, of clinically meaningful aPL profiles remain stable over time at a median follow-up of 5 years. These results will help guide future validation studies of stored blood samples through APS ACTION Core Laboratories.

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