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

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Conflict of Interest:

Dr. Michelle Petri (Hopkins Lupus Cohort) is supported by a grant from the NIH RO1 AR069572. Dr. Fortin is recipient of a tier 1 Canada Research Chair on Systemic Autoimmune Rheumatic Diseases. The rest of the authors report no conflicts of interest.

Short running head:

aPL Stability Over Time

Abstract:

Objective: 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 a) whether baseline characteristics differ between patients with stable and unstable aPL profiles, and b) predictors of unstable aPL profiles over time.

Methods: Clinically meaningful aPL profile was defined as positive lupus anticoagulant (LA) test and/or anticardiolipin (aCL)/anti-β_2 glycoprotein-I (aβ_2GPI) IgG/M $\geq$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 years), 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 (Odds Ratio [OR] 0.25, 95% Confidence Interval [CI] 0.10-0.64, p=0.004) and isolated LA 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 profile at baseline maintain such at a median follow-up of five 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.

Introduction:

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by thrombosis and pregnancy morbidity in patients with persistently positive antiphospholipid antibodies (aPL). Antiphospholipid antibodies that are used for APS classification include lupus anticoagulant test (LA), anticardiolipin antibodies (aCL), and anti-β_2 glycoprotein-I antibodies (aβ_2GPI)(1).

The assessment of aPL profile 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 LA positivity, high titer aCL/aβ_2GPI, 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 APS patients.

In this analysis of the APS ACTION Registry, our primary objective was to determine whether clinically meaningful aPL profiles (defined as positive LA test and/or aCL/aβ_2GPI IgG/M $\geq$40 U) at baseline remain stable over time in persistently (on two or more occasions at least 12 weeks apart) aPL-positive patients. Our secondary objectives were to determine a) whether demographic, clinical, and laboratory characteristics at baseline differ between patients with stable and unstable aPL profiles over time, and b) predictors of unstable aPL profiles over time.

Methods:

APS-ACTION Registry

The APS-ACTION Registry is a web-based data capture system developed in **R**esearch **E**lectronic **D**ata **Cap**ture-REDCap, that includes patients with persistently positive aPL (positive on two occasions at least 12 weeks apart) with or without other systemic autoimmune disease. Inclusion criteria are positive aPL, based on the Updated Sapporo APS Classification Criteria, tested at least twice within one 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, 796 patients were 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 study, 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), non-criteria 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 LA, aCL IgG/M and aβ_2GPI IgG/M from the Registry were utilized. For the baseline visit, we used the most recent aPL profile (LA, aCL/aβ_2GPI IgG/M). At each annual follow up visit, we used the first available aPL profile that was reported for that time period. High aCL/aβ_2GPI 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 positive LA test and/or aCL/aβ_2GPI IgG/M $\geq$40 U, and a *stable clinically meaningful aPL profile* as a clinically meaningful profile in at least two-thirds of follow-up aPL measurements. We defined aCL/aβ_2GPI IgG/M as "positive" when the reported titer was $\geq$ 40 U. We selected a cutoff positivity of 40 U for aCL/aβ_2GPI given that: a) low aPL titers are generally transient and clinically less significant; and b) limited literature demonstrating that titers above 40 U are more likely to be associated with aPL-related manifestations(1, 3, 4), and therefore are considered more impactful in clinical care of aPL positive patients. *Inconclusive aPL profile* during the follow-up was defined as: a) missing determinant aPL test result(s) (those used to determine the baseline clinically meaningful aPL

profile) with no other positive aPL tests; or b) 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 profile over time. A GLMM framework allowed us to introduce random effects to account for within-subject correlation due to repeated measures of aPL profile across follow-up. T-test (for normally distributed variables), Wilcoxon rank-sum (for non-normally distributed variables), and Fisher's 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 profile (negative LA and aCL/aβ_2GPI 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 (interquartile range [IR]: 39-59) and 349 (74%) were female. The median follow-up was 5.1 years (IR: 4.3-5.8), and the median number of follow-up visits with aPL tests was 2 (IR: 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 one-year follow-up, 216 (72%) at two-year, 177 (72%) at three-year, 135 (73%) at four-year, and 61 (70%) at five-year (Figure 1 and Table 1).

Antiphospholipid Antibody 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 five years. One-hundred-and-fifty-one (32%) patients contributed to the stability analysis with one follow up visit, 99 (21%) with two, 105 (22%) with three, 87 (18%) with four, and 27 (6%) with five. 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 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. Lupus anticoagulant, aCL IgM, aβ_2GPI IgG positivity, and positivity on two or more 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 aβ_2GPI 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 aβ_2GPI 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 aβ_2GPI 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 LA test positivity (17% vs 41%) or isolated aβ_2GPI IgG/M positivity (2% vs 9%). No differences were noted between patients with or without concomitant autoimmune disease at baseline.

Predictors of an Unstable Antiphospholipid Antibody 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% confidence interval (CI) 0.1-0.6, p=0.004) (Table 3). Furthermore: a) patients with isolated LA 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 b) aCL or aβ_2GPI IgG/M $\geq$ 40 U, but not any aCL/aβ_2GPI positivity, was associated with lower odds of unstable aPL profiles over time. In a multivariable logistic model adjusted for age, gender, active smoking, concomitant autoimmune disease, and HCQ use at baseline: a) triple aPL positivity at baseline was associated with lower odds of unstable aPL profiles (OR 0.17, CI 0.1-0.4, p<.0001); and b) isolated LA test positivity and isolated aβ_2GPI positivity at baseline were associated with higher odds of unstable aPL profiles (OR 3.65, CI 1.9-6.8, p<.0001; OR 4.17, CI 1.2-14.1, p=0.02, respectively). When the multivariate model was further adjusted for individual aPL tests: a) baseline LA test positivity (OR 0.26, CI 0.1-0.7, p=0.005); and b) baseline aCL IgG ≥40 U (OR 0.24, CI 0.1-0.7, p=0.006) were associated with lower odds of unstable aPL profiles over time.

Individual Antiphospholipid Antibody Result Stability Over Time:

Table 4 describes the course of aCL and aβ_2GPI IgG/M titers over time based on their assignments to one 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 LA test, 88% of patients with baseline isolated LA 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 LA test and/or aCL/aβ_2GPI IgG/M ≥40 U, remains stable during a median follow up of five years independent of age, active smoking, concomitant systemic autoimmune disease, and HCQ use at baseline. Triple aPL-positivity increases and isolated LA positivity decreases the odds of a stable aPL profile.

In patients with triple aPL positivity (LA, aCL and aβ_2GPI 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 aβ_2GPI of the same isotype), and 40% for isolated aPL test positivity (LA, aCL or aβ_2GPI)(5). Based on a limited number of studies, 70-90% of patients with persistently positive aPL profiles remain positive during follow up ranging from two to ten years(6-8). In contrast, one 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, the different cut-off levels used to define aPL positivity ($\geq$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 five years. Our results are based on explicit and clinically relevant definitions of aPL profile positivity, and prospectively collected clinical and laboratory data.

Interpretation of an aPL test should be done cautiously since not every positive test is clinically important. Triple aPL positivity(10, 11) or LA positivity(12) is known to confer a higher risk for aPL-

related clinical events compared to aCL and aβ_2GPI positivity. Additionally, IgG aCL and aβ_2GPI 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 aβ_2GPI, as defined by the Updated Sapporo APS Classification Criteria, are more likely to be associated with APS. To that point, this study shows that patients who maintain a stable clinically meaningful aPL profile at five years of follow up are more likely to have at baseline LA test positivity, two 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 aPL profile over time had more frequently history of arterial events ($p=0.01$) or transient ischemic attacks ($p=0.04$) at baseline, and were more frequently on 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 (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 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. Firstly, the use of HCQ was considered a potentially contributing factor as a retrospective study has demonstrated that patients with SLE and persistently positive aPL profiles (positive LA and/or an aCL/aβ_2GPI ≥40 U) were less likely to be on HCQ, compared to patients with transiently positive or negative profiles(14); HCQ may also decrease aCL IgG/M levels, and dRVVT (dilute Russell's Viper Venom Time) prolongation(15). Secondly, 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 auto-antibody production; a small study has supported that lupus activity was higher in patients with persistently positive aPL tests (LA and aCL)(17). Therefore, even after adjusting for age, gender, 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.

Lupus anticoagulant test, when persistently positive, is highly associated with obstetric and thrombotic events. Despite guidelines, LA results among laboratories may be discrepant due to lack of standardization and use of different screening tests. In addition, LA results may be unreliable when tested on anticoagulation including direct oral anticoagulants (DOACs) (18, 19). An exercise among four different laboratories demonstrated that discordant or inconclusive LA 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 LA test positivity had significantly higher odds of being associated with an unstable aPL profile; we speculate that this finding was due to relatively high number of anticoagulated patients. For more accurate assessment, future APS-ACTION studies will be completed using Core Laboratory LA test results, which have been performed using methods with minimal interference with anticoagulation.

Our study has several limitations. Firstly, we have missing follow-up data as aPL testing was based on the discretion of the treating physician; however, we plan to re-assess the aPL profiles in future studies using stored blood samples from each patient visit. Secondly, 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 aPL profile remained negative when no data were available. Thirdly, median aCL/aβ_2GPI titers may have been underestimated as: a) for titers reported as "greater than x units" we used the upper limit; and b) we used all available titers irrespective of positivity. Fourthly, 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, APS ACTION Registry is comprised of 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.

Conclusion:

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 five years. These results will help guide future validation studies of stored blood samples through APS ACTION Core Laboratories.

Acknowledgments and affiliations:

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Figure Legends:

Figure 1: ANTIPHOSPHOLIPID ANTIBODY PROFILES OVER TIME (N=482)

*Reasons for inconclusive follow-up aPL profile: a) missing determinant aPL test(s) (those used to determine the baseline clinically meaningful aPL profile) with no other positive aPL tests (n: 28); and b) negative determinant aPL test result(s) with missing other aPL test result(s) (n: 24).

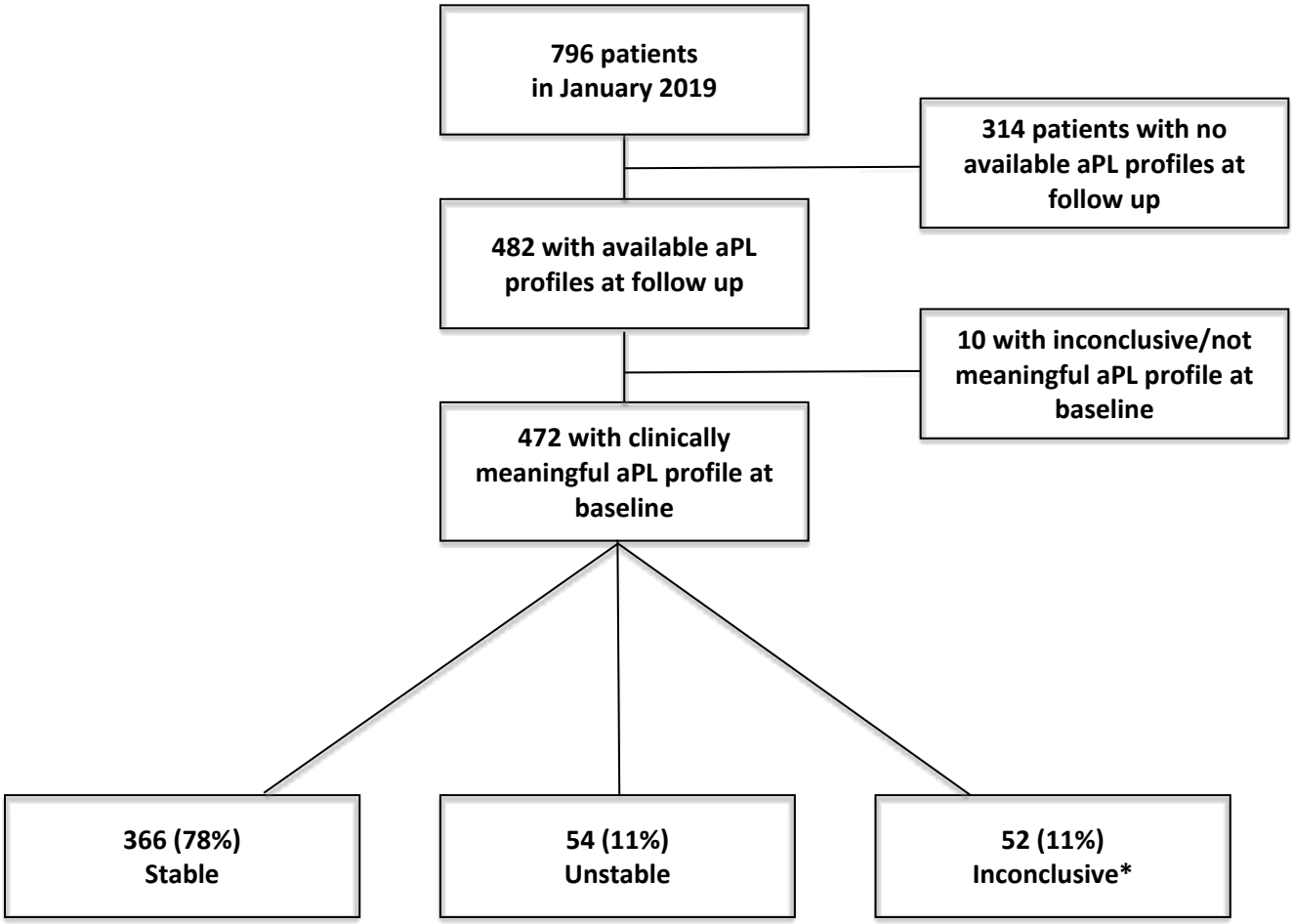

Table 1: ANTIPHOSPHOLIPID ANTIBODY PROFILES OVER TIME (N=482)

	Baseline	0-12M	12-24M	24-36M	36-48M	48-60M
# of Patients with Follow-up	N/A	452	398	357	282	138
# of Patients with aPL Results	482	348	302	245	184	87
Meaningful aPL Profile*	472 (98%)	254 (73%)	216 (72%)	177 (72%)	135 (73%)	61 (70%)
Not Meaningful aPL Profile**	3 (1%)	31 (9%)	29 (10%)	24 (10%)	14 (8%)	7 (8%)
Inconclusive aPL Profile***	7 (1%)	63 (18%)	57 (19%)	44 (18%)	35 (19%)	19 (22%)

#: Number **aPL:** Antiphospholipid Antibody, **M:** Months, **N/A:** Not Applicable

*Positive LA test and/or aCL/aβ_2GPI IgG/M $\geq$40 U; **Negative LA test and aCL/aβ_2GPI IgG/M <40 U;

***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).

Table 2: BASELINE CLINICAL AND LABORATORY CHARACTERISTICS OF PATIENTS (N=420) WITH STABLE OR UNSTABLE CLINICALLY MEANINGFUL ANTIPHOSPHOLIPID ANTIBODY PROFILES AT FOLLOW UP

	Total	Clinically meaningful aPL Profile		p-value
	(n=420)	Stable (n=366)	Unstable (n=54)	
Female	305 (73%)	267 (73%)	38 (70%)	0.74
Age Median (IR)	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 SAIDx	148 (35%)	128 (35%)	20 (37%)	
aPL-Related History				
Vascular Event$ (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*	136	119	17	0.83
Spontaneous Abortions**	13	10	3	0.21
Premature Birth***	37	34	3	0.56
Unexplained Fetal Death****	76	67	9	0.80
aPL Tests				
Lupus Anticoagulant^ (+)	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*
aβ_2GPI IgG ≥40 U	139 (33%)	130 (36%)	9 (17%)	*0.005*
aβ_2GPI 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)				
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*
aβ_2GPI IgG	19 [3, 74]	22 [3, 83]	3 [1, 30]	*<0.001*
aβ_2GPI IgM	9 [2, 33]	10 [2, 39]	4 [1, 20]	*0.04*
aPL Profiles				
Triple aPL Positivity	174 (41%)	167 (46%)	7 (13%)+	*<0.0001*
Double aPL Positivity#	120 (29%)	106 (29%)	13 (26%)	0.75
Isolated LA Test Positivity	84 (20%)	62 (17%)	22 (41%)	*0.0002*
Isolated aCL IgG/M Positivity	29 (7%)	23 (6%)	6 (11%)	0.24
Isolated aβ_2GPI 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

IR: Interquartile Range, **aPL:** Antiphospholipid Antibody**, APS:** Antiphospholipid Syndrome, **SAIDx:** Systemic AutoImmune Diseases, **TIA:** Transient Ischemic Attack, **aCL:** Anticardiolipin Antibody, **aβ_2GPI:** Anti-β_2-Glycoprotein-I Antibody, **U:** Units

*Out of 207 patients with history of pregnancy (with or without morbidity); **Three consecutive unexplained spontaneous abortions before 10th week; ***Premature birth before 34th week due to eclampsia, preeclampsia or placental insufficiency; ****Unexplained fetal death at or beyond 10th week; $^{\#}$Any combination of two positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria; $^{\$}$During an average follow-up of five years, new thrombosis occurred in 30 (7%) of 420 patients (24 with history of baseline thrombosis, and in 6 patients without); 29/30 of these patients had a stable clinically meaningful aPL profile at follow up; $^{\wedge}$Lupus anticoagulant test was reported by each center as positive or negative (screening by dilute Russell's 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 not reported method in 2% of patients); $^{+}$Seven triple aPL-positive patients with unstable aPL profile were mostly on warfarin with fluctuating lupus anticoagulant test status, and relatively low level aPL ELISA at baseline (two patients only had IgM isotype).

Table 3: BASELINE PREDICTORS OF UNSTABLE ANTIPHOSPHOLIPID ANTIBODY PROFILE AT FOLLOW-UP

Univariate (unadjusted)	Odds Ratio (95% CI)	p-value
Baseline aPL Profile		
Triple aPL Positive	0.25 (0.10-0.64)	*0.004*
Double aPL Positive[#]	0.67 (0.31-1.46)	0.32
Isolated LA Test Positivity	3.30 (1.53-7.13)	*0.002*
Isolated aCL IgG/M Positivity^[$]	2.13 (0.71-6.37)	0.18
Isolated aβ_2GPI IgG/M Positivity^[$]	2.31 (0.49-10.75)	0.29
Baseline Individual aPL Tests		
LA 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*
aβ_2GPI IgG ≥ 40 U	0.26 (0.11-0.65)	*0.004*
aβ_2GPI IgM ≥ 40 U	0.28 (0.08-0.93)	*0.04*
Age	1.27 (0.98-1.63)	0.06
Gender (male)	0.70 (0.33-1.48)	0.35
Hydroxychloroquine 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)	**Odds Ratio (95% CI)**	**p-value**
Baseline aPL Profile		
Triple aPL Positive*	0.17 (0.07-0.39)	*<0.0001*
Isolated LA Test Positivity*	3.65 (1.94-6.84)	*<0.0001*
Isolated aβ_2GPI Positivity*	4.17 (1.24-14.1)	*0.02*
Baseline Individual aPL Tests		
LA test Positivity**	0.26 (0.10-0.66)	*0.005*
aCL IgG ≥ 40 U***	0.24 (0.09-0.66)	*0.006*

LA: Lupus Anticoagulant**, aCL:** Anticardiolipin Antibody, **aβ_2GPI:** Anti-β_2-Glycoprotein-I Antibody, **CI:** Confidence Interval, **HCQ:** Hydroxychloroquine, **U:** Units

[#]Any combination of two positive aPL tests based on the laboratory criteria of the Updated Sapporo APS Classification Criteria; ^Any titer above normal range; [$]The small number of patients in isolated aCL and aβ_2GPI aPL profile categories precluded from further analysis of isolated IgG or IgM isotype for these groups; *Adjusted for age, gender, active smoking, concomitant autoimmune disease, and HCQ use at baseline; ** Adjusted for age, gender, active smoking, concomitant autoimmune disease, HCQ use at baseline, clinically meaningful (≥ 40 U) aCL IgG and IgM at baseline, and clinically meaningful (≥ 40 U)

aβ$_2$GPI IgG and IgM at baseline; *** Adjusted for age, gender, active smoking, concomitant autoimmune disease, HCQ use at baseline, LA test result at baseline, clinically meaningful (≥ 40 U) aCL IgM at baseline, and clinically meaningful (≥ 40 U) aβ$_2$GPI IgG and IgM at baseline.

Table 4: INDIVIDUAL ANTIPHOSPHOLIPID ANTIBODY COURSE OVER TIME BASED ON TITERS AT BASELINE

				aPL Titer at Follow-Up (in Units)			
	BL Titer	**# of Pts at BL**	**# of f/u aPL**	**0-19**	**20-39**	**40-79**	**≥80**
aCL IgG	**0-19 U**	195	420	89%	9%	2%	1%
aCL IgM		281	652	91%	5%	4%	1%
aβ_2GPI IgG		159	375	90%	5%	3%	2%
aβ_2GPI IgM		206	477	94%	3%	1%	1%
aCL IgG	**20-39 U**	53	145	41%	23%	24%	12%
aCL IgM		54	140	51%	24%	18%	7%
aβ_2GPI IgG		34	83	36%	30%	19%	14%
aβ_2GPI IgM		31	72	60%	24%	7%	10%
aCL IgG	**40-79 U**	74	199	25%	22%	33%	21%
aCL IgM		49	113	29%	14%	33%	24%
aβ_2GPI IgG		41	90	20%	21%	31%	28%
aβ_2GPI IgM		17	37	51%	14%	19%	16%
aCL IgG	**≥80 U**	111	255	10%	7%	18%	65%
aCL IgM		41	104	6%	4%	29%	62%
aβ_2GPI IgG		68	139	6%	5%	9%	79%
aβ_2GPI IgM		40	90	11%	8%	11%	70%

aPL: Antiphospholipid Antibody, **aCL:** Anticardiolipin Antibody, **aβ_2GPI:** Anti-β_2-Glycoprotein-I Antibody, **BL:** Baseline, **f/u:** Follow-Up, **#:** Number, **Pts:** Patients, **U:** Units