2023 ACR/EULAR Antiphospholipid Syndrome Classification Criteria

Supplement

Table: **Development and Validation of the 2023 ACR/EULAR Antiphospholipid Syndrome Classification Criteria**

PHASE I: CRITERIA GENERATION

- Part A:
  - Survey with open-ended questions to help identify candidate criteria (54 collaborators) (152 items)
- Part B:
  - Criteria generation and expansion based on the survey results and literature screening (20 steering committee [SC] members) (261 items)

PHASE II: CRITERIA REDUCTION

- Part A:
  - Survey A with Likert scale (low specificity items eliminated) (61 collaborators and selected SC members)
  - Criteria reduction based on survey A results, comprehensive literature reviews, meta-analyses, expert consensus, and the following principles: a) remaining criteria should demonstrate good face, construct, and discriminant validity; and b) items with low sensitivity or specificity, poor reliability, redundancy, or insufficient feasibility should be removed (64 items and 10 domains)
- Part B:
  - Survey B with Likert scale (low specificity items) (19 SC members)
  - Further criteria reduction based on survey B results, comprehensive literature reviews, meta-analyses, expert consensus, and the same principles discussed above (27 items and 6 domains)

PHASE III: CRITERIA DEFINITION, FURTHER REDUCTION, AND WEIGHTING, AND CLASSIFICATION THRESHOLD IDENTIFICATION

- Part A: Clinical Definitions (SC)
  - Establishment of clinical domain subcommittees
  - Comprehensive literature reviews
- Part B: Laboratory Definitions (SC)

- o Establishment of laboratory domain subcommittee
- o Comprehensive literature reviews
- Part C: Refinement of Definitions with the Guidance of Real-world Patient Scenarios
  - o Establishment of entry criteria (SC)
  - o Real-world case collection (derivation cohort, n: 314) (20 selected SC members and collaborators)
  - o Descriptive and statistical analyses of the derivation cohort (SC)
  - o Consensus discussions to finalize clinical and laboratory definitions, further criteria reduction, and hierarchically rank ordering criteria within each domain (SC) (20 items and 8 domains)
- Part D: Multi-Criteria Decision Analysis (MCDA) (n:17)
  - o In-person consensus meeting discussions and candidate criteria weight determination: through a consensus-based MCDA approach
  - o Threshold score identification

PHASE IV: CRITERIA VALIDATION

- Part A: Real-world case collection (two validation cohorts, n: 568) (30 collaborators)
- Part B: Assessment of cases for APS classification (three independent adjudicators)
- Part C: Testing the performance characteristics of the revised Sapporo and new classification criteria in two separate cohorts, using consensus among independent adjudicators as the "gold standard"
- Part D: Sensitivity Analyses

Supplement Section 2: <u>Phase I/II Results</u>

Table: Proposed Domains and Candidate Criteria Following Phase I Item Generation and Phase II Item Reduction

Proposed Domains (n: 6)	Proposed Items (n: 27)
Macrovascular	Superficial Vein Thrombosis, Venous Thromboembolism, Arterial Thrombosis, Transient Ischemic Attack
Microvascular	Livedo Racemosa, Livedoid Vasculopathy, Adrenal Hemorrhage or Plexus Thrombosis, Acute Ischemic Encephalopathy, Cardiac Microvascular Disease, Diffuse Alveolar Hemorrhage, Acute aPL Nephropathy, Chronic aPL Nephropathy
Obstetric	Pre-fetal Death <10 weeks (w) of Gestation, Early Fetal Death Between 10w to <16w of Gestation, Fetal Death Between 16w to 34w of Gestation, Pre-eclampsia with Severe Features <34w of Gestation, Placental Insufficiency with Severe Features <34w of Gestation
Cardiac Valve Disease	Non-infectious Valve Vegetation, Valve Thickening
Hematologic	Mild, Moderate, Severe Thrombocytopenia
Laboratory **aPL testing, coagulation-based functional assays**	Lupus anticoagulant test
aPL testing, solid-phase-based assays	IgG aCL, IgM aCL, IgG aβ_2GPI, IgM aβ_2GPI

aPL: antiphospholipid antibodies; **aCL:** anti-cardiolipin antibody; and **aβ_2GPI:** anti–β_2-glycoprotein I antibody

Supplement Section 3: <u>Phase III Methodology (Additional Information)</u>

During phase III-A and phase III-B, domain subcommittees conducted comprehensive literature reviews and held teleconferences for expert consensus-based decisions. A Steering Committee survey, critical review of recent international guidelines, and consultation with international cardiologists and vascular medicine specialists also assisted in identifying traditional thrombosis risk factors. Proposed clinical and laboratory criteria definitions were finalized and approved by the Steering Committee.

During phase III-C, we: a) established an international derivation cohort using phase III-A/B definitions; b) calculated relative risks of each candidate criterion in association with "highly likely" compared to "equivocal or unlikely" Antiphospholipid Syndrome (APS) cases, as rated by the treating physicians; c) refined the candidate criteria definitions; and d) further reduced and hierarchically organized criteria within domains using expert consensus guided by relative risk calculations ("A" items received lowest rank, and subsequent letters e.g., "B" to "E", received higher rank).

During phase III-C, we conducted an international case collection (derivation cohort), when 20 multidisciplinary international collaborators from Europe, and North and South America provided real-world cases reflecting the broad spectrum of APS-associated clinical manifestations. We designed a standardized case collection form to represent items included in the 2006 revised Sapporo APS Classification Criteria as well as the candidate criteria identified for the new APS classification criteria at the end of Phase II. We collected de-identified patient-specific information on the case collection form in accordance with the definitions as defined in Phase III-A/B. For each case submitted, experts provided an overall score for APS likelihood using a Likert scale of +3 to -3 (+3= highly likely APS, 0=not for or against APS, and -3=very unlikely APS). We evaluated the univariate association between each candidate criterion and likelihood of APS, comparing "highly likely" APS cases (Likert scores of +2 or +3) versus "equivocal or unlikely" APS (Likert scores +1, 0, -1, -2, -3). We calculated risk ratios, 95% confidence intervals and p-values of each candidate criterion in association with "highly likely" APS cases compared to "equivocal or unlikely" cases. The detailed methodology will be published elsewhere (1). Steering committee decisions on rank ordering of domains prior to the multicriteria decision analysis (MCDA) meeting were guided using derivation cohort analyses along with literature and expert consensus, as summarized in Supplement Section 5.

During phase III-D, 15 SC members, one collaborator not involved in phase I/II/III decisions, and one patient representative, participated in a two-day face-to-face moderated consensus meeting employing

MCDA methodology and nominal group technique (NGT) exercise. Participants voted on pairwise scenarios, with each pair including two candidate criteria from two distinct domains. Relative weights were derived using 1000Minds software. Following the meeting, an additive score was assigned to each derivation cohort case based on the weighted criteria from each domain; cases were then rank ordered based on the additive score. Panel members assessed each case for APS classification and selected a minimal threshold for classification, above which cases would be classified as APS. The group iteratively discussed each unique case and resolved disagreements until 100% consensus was achieved. Finally, a provisional threshold for APS classification criteria was established and individual criterion weights were refined and simplified for interpretability.

Reference:

(1) Barbhaiya M, Zuily S, Ahmadzadeh Y, Costenbader K, Naden R, Erkan D. Development of New International Classification Criteria for Antiphospholipid Syndrome: Phase III Case Collection Results [abstract]. Arthritis Rheumatol. 2020; 72 (suppl 10). https://acrabstracts.org/abstract/development-of-new-international-classification-criteria-for-antiphospholipid-syndrome-phase-iii-case-collection-results/. Accessed June 27, 2023.

Supplement Section 4: <u>Phase III-A/B Results (Additional Information)</u>

Table: Description of "Otherwise Unexplained" Included in the Definitions of Classification Criteria Items

Domain	Classification Criteria Items	"Otherwise Unexplained" Requires the Exclusion of "Equally Likely" or "More Likely" Causes Based on Investigator's Judgement that Include but are Not Limited to:
1	Venous Thromboembolism (VTE)	Thoracic outlet syndrome associated with external venous compression and upper extremity deep vein thrombosis, or genetic thrombophilia *(major/minor VTE risk factors required for scoring [Table 2] are not reasons for exclusion).*
2	Arterial Thrombosis (AT)	Thoracic outlet syndrome associated with external arterial compression and upper extremity arterial thrombosis, or atrial fibrillation *(high/moderate cardiovascular disease risk factors required for scoring [Table 2] are not reasons for exclusion).*
3	Livedoid Vasculopathy Lesions (by physical examination)	Genetic thrombophilia (e.g., protein C or S deficiency), systemic autoimmune diseases, or solid organ or hematologic malignancies.
3	Antiphospholipid Antibody Nephropathy (by physical examination and laboratory tests)	Active lupus nephritis, acute renal artery or venous thrombosis, diabetes mellitus, malignant hypertension, reduced renal perfusion or intravascular volume loss, infections, or medications (e.g., non-steroidal anti-inflammatory drugs, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, antimicrobials [aminoglycosides, vancomycin, amphotericin, anti-viral agents], chemotherapeutics, immunosuppressive drugs [e.g., cyclosporine, tacrolimus], or contrast media).
3	Pulmonary Hemorrhage (by clinical symptoms and imaging):	Pulmonary infections, vasculitis, toxic exposures, or congestive heart failure.

3	Livedoid Vasculopathy (by physical examination and pathology)	Protein C or S deficiency, systemic autoimmune diseases, and solid organ or hematologic malignancies.
3	Pulmonary Hemorrhage (by bronchoalveolar lavage [BAL] or pathology)	Antineutrophilic cytoplasmic antibody (ANCA)-associated vasculitis, drug-induced vasculitis, other systemic autoimmune diseases, cryoglobulinemia, bone marrow transplant recipient, anti–glomerular basement membrane disease, or IgA vasculitis (Henoch-Schönlein).
3	Myocardial Disease (by imaging or pathology)	Other systemic autoimmune diseases such as systemic sclerosis or vasculitis.
3	Adrenal Hemorrhage or microthrombosis (by imaging or pathology)	Sepsis and medications (e.g., tyrosine kinase inhibitors)
4	Pre-fetal Death (pre-embryonic or embryonic loss) <10 weeks	Known genetic abnormalities in products of conception, uterine malformations, poorly controlled maternal conditions such as diabetes or thyroid diseases, and parental karyotype abnormalities.
4	Fetal Death (≥10 weeks to <34 weeks)	Fetal anomalies, genetic abnormalities, feto-maternal hemorrhage, umbilical cord accidents, placental abruption, preterm rupture of membranes, congenital infections (e.g., syphilis, toxoplasmosis, or cytomegalovirus), chorioamnionitis, or red cell alloimmunization.
5	Valve Thickening	Systemic lupus erythematosus or rheumatic fever.
5	Valve Vegetation	Systemic lupus erythematosus, cancer, or infective endocarditis
6	Thrombocytopenia	Active systemic lupus erythematosus, pseudo-thrombocytopenia, medications (e.g., myelosuppressive or chemotherapeutic medications, or heparin), radiation treatment, gestational thrombocytopenia, chronic alcohol abuse, liver disease, splenomegaly, severe infections/sepsis, or primary hematologic malignancies.

Supplement Section 5: Phase III-C Results (Additional Information)

Table: Summary* of the Steering Committee (SC) Phase III-C Discussions and Consensus For Further Criteria Reduction and Final Domain Definitions/Levels for Phase III-D Multi Criteria Decision Analysis (MCDA).

Domains	Discussions[1,2,3] - Derivation Cohort Analysis[4] – Consensus for MCDA
Clinical	
1-Macrovascular (Venous Thromboembolism [VTE]) and Arterial Thrombosis [AT]):	**Discussions:** ● Two different domains for VTE and AT are necessary to allow for additive points. ● Based on general population guidelines, cumulative minor VTE and cumulative moderate cardiovascular disease (CVD) risk factors may lead to a stronger risk of thrombosis, comparable to major VTE and high CVD risk factors. ● Based on a Steering Committee (SC) survey of individual VTE and CVD risk factors, 72-89% agreed with proposed risk factors and definitions. ● Based on literature and SC guidance, superficial vein thrombosis (SVT) and transient ischemic attack (TIA) have low specificity for antiphospholipid syndrome (APS); in addition, the definition of TIA is non-specific. **Derivation Cohort Analysis:** ● Any VTE or AT event was significantly associated with "highly likely" APS cases versus "equivocal or unlikely" APS cases; however, no difference in SVT and TIA was observed. ● When stratified by additional thrombosis risk factors, VTE/AT cases *without* any risk factors had a significantly positive association with "highly likely" APS cases, unlike VTE/AT cases *with* risk factors. **Consensus for MCDA:** ● Two-level macrovascular disease domain for VTE and AT (in two different domains) based on minor/major VTE and moderate/high CVD risk profiles; and eliminate SVT and TIA.
2- Microvascular:	**Discussions:** ● The importance of a two-level microvascular domain was agreed upon based on the degree of objective evidence to support particular organ-specific involvement, i.e., clinical suspicion (e.g., by physical examination) *versus* established evidence (e.g., by imaging or biopsy). ● In the absence of skin biopsy, livedoid vasculopathy lesions can be clinically suspected by otherwise unexplained physical examination; in the absence of renal biopsy, aPL-nephropathy can be clinically suspected by otherwise unexplained clinical and laboratory parameters; and in the

	absence of bronchoscopy or lung biopsy, pulmonary hemorrhage can be suspected based on otherwise unexplained clinical symptoms and suggestive imaging findings. ● Given the complexity of adrenal (micro) vascular structure, and the limited number of histopathological studies and imaging techniques, further studies are needed to elucidate the origin of aPL-related thrombosis i.e., plexus or arterioles/venules and capillaries vs adrenal vein/artery. ● Literature review demonstrated lack of data regarding specificity of acute ischemic encephalopathy, as well as difficulties in defining acute ischemic encephalopathy. **Derivation Cohort Analysis:** ● All microvascular candidate criteria had positive association with "highly likely" APS cases, except acute ischemic encephalopathy. ● Evaluation of a two-level definition of microvascular involvement, i.e., suspected versus established, demonstrated a strong and significant association for established microvascular involvement with "highly likely" APS, and the association was positive but not significant for suspected microvascular disease. **Consensus for MCDA:** ● Two-level microvascular disease domain based on "suspected" and "established" involvement; and eliminate acute ischemic encephalopathy.
3-Obstetric:	**Discussions:** ● Based on literature review and SC consensus, pre-fetal death (pre-embryonic or embryonic loss) (<10 weeks of gestation) is not specific for APS. ● Fetal death is: a) relatively common and non-specific (especially early fetal death between 10 weeks 0 days and 15 weeks 6 days of gestation) even when other potential causes excluded; b) more specific for APS when associated with severe pre-eclampsia (PEC) or severe placental insufficiency (PI); and c) often related to obstetric management at the time of the delivery, i.e., whether the baby was delivered before the fetal death could occur, than to the underlying pathophysiology of conditions such as PEC or PI. ● When PEC with severe features and PI with severe features develop simultaneously, the combination is more specific for APS than each feature alone. **Derivation Cohort Analysis:** ● Pre-fetal death (<10 weeks) and early fetal death (10-16 weeks) were not associated with highly likely APS. ● Fetal death (16-34 weeks) and PEC with severe features (<34 weeks) were significantly associated with highly likely APS. ● There was a positive but not significant association for PI with severe features (< 34 weeks) and highly likely APS (lack of statistical significance likely due to low number of cases due to poor documentation in the medical records).

	Consensus for MCDA:
	● Three-level obstetric domain: a) recurrent pre-fetal deaths less than 10 weeks and/or early fetal death(s) between 10 weeks 0 days and 15 weeks 6 days of gestation; b) PEC with severe features or PI with severe features before 34 weeks of gestation; and c) PEC with severe features AND PI with severe features before 34 weeks of gestation.
	    ○ Do not assign additional weight to fetal death (16 weeks 0 days and 34 weeks 0 days) alone (without PEC or PI with severe features), compared to recurrent pre-fetal (<10w) and/or early fetal death alone (10 weeks 0 days and 15 weeks 6 days of gestation).
	    ○ Retain the prevailing definition of "recurrent", i.e., three or more, as used in previous classification criteria given the lack of data against or for the current definition.

4-Cardiac Valve:	**Discussion:** ● Based on literature review, cardiac valve disease, i.e., valvular thickening or vegetation, in primary APS patients is significantly higher than in individuals without APS. ● Valve dysfunction (stenosis or regurgitation) without thickening or vegetation is not specific for APS and may be the consequence of valve damage. **Derivation Cohort Analysis:** ● Cardiac valve disease had a positive and significant association with "highly likely" APS; cardiac vegetation had a significant and strong association with "highly likely" APS, but the association with cardiac valve thickening was weaker without significance. **Consensus for MCDA:** ● Two-level cardiac valve disease domain based on thickening or vegetation.
5-Hematology:	**Discussion:** ● Challenges exist related to converting a continuous variable, i.e., platelet count, to dichotomous variable for classification criteria purposes. ● "Mild" thrombocytopenia, defined as platelet count more than 131×10^9/L but less than the laboratory reference range, decreases the specificity, and the closer the abnormal platelet count to the lower end of the reference range, the more variability is introduced (the lower end of reference range is typically reported as 140×10^9/L or 150×10^9/L based on different laboratories). ● "Severe" thrombocytopenia, which was agreed as platelet count $< 20 \times 10^9$/L, also decreases specificity as it is generally due to other etiologies. ● "Moderate" thrombocytopenia was defined as platelet count between $20\text{-}130 \times 10^9$/L and was considered to be more specific for APS by expert consensus. **Derivation Cohort Analysis:**

	● Thrombocytopenia ($<150 \times 10^9$/L) was significantly associated with highly likely APS; however, there was no association between "severe" thrombocytopenia and "highly likely" APS cases. ● Both "mild" and "moderate" thrombocytopenia had positive and significant association with highly likely APS, but "mild" thrombocytopenia had very low frequency and wide confidence intervals. **Consensus for MCDA:** ● One-level hematology domain-based thrombocytopenia (lowest platelet count 20-130 x 10^9/L).
Laboratory:	
6-Laboratory	**Discussion:** ● Separation of two distinct laboratory domains based on: a) coagulation-based functional assays (lupus anticoagulant [LAC] test); and b) solid phase assays (anticardiolipin antibody [aCL] and anti-β_2-glycoprotein-I [aβ_2GPI] antibodies, is important due to mechanistic differences, and also allows for assessment of single, double and triple positive aPL profiles. ● Prospective studies demonstrate that single (one-time) LAC positivity may predict LAC persistence after 12 weeks; additionally, repeat LAC testing may not be available or feasible due to false positive or negative results after initiation of anticoagulation. ● IgM isotypes for aCL/aβ_2GPI confer lower APS likelihood and specificity than IgG isotypes. ● There is insufficient data supporting the inclusion of the IgA isotype in the classification criteria until there is better understanding of its pathogenic and prognostic significance. ● Assessment of simultaneously positive high level aCL IgG and aβ_2GPI IgG as a separate category captures double aPL (aCL+ aβ_2GPI) positivity. ● Threshold of $\geq$40U has excellent ability to discriminate between low and moderate–high aCL and aβ_2GPI IgG titers using ELISA but not for new automated systems, which require further investigation (see main manuscript Table 1 for further discussion). ● "Negative" laboratory results differ from "not tested"; however, as full aPL testing should be performed for all patients, for classification purposes, these two categories can be combined. ● For other solid phase assay-based aPL tests, e.g., anti-Domain-I antibodies, anti-phosphatidylserine-prothrombin antibodies, besides limited commercial availability, additional research is needed to define their feasibility, clinical correlation, and standardization. **Derivation Cohort Analysis:** ● In "highly likely" (versus equivocal or unlikely) APS cases, a progressively stronger association was demonstrated for persistent and most recent positive LA results as follows: any positive, persistently positive LA, and persistently positive LA with the most recent test positive. No association was demonstrated for single LA positivity. ● Persistently positive aCL IgG or aβ_2GPI IgG was strongly associated with "highly likely" APS, which was further strengthened when the most recent test was positive; no association was

demonstrated for most recent levels <40U but a significant positive association with levels of 40-79U, which was increased further for levels ≥80 U.

- Persistently positive aCL IgM or aβ₂GPI IgM was relatively weakly associated with "highly likely" APS; further evaluation based on the most recent levels demonstrated: a) no association for aCL levels <40U or 40-79U but a weak positive association with levels ≥80 U; and b) no association for aβ₂GPI levels <40U but a weak positive association with levels of 40-79U and ≥80 U.

Consensus for MCDA:

- Two-level LAC positivity and four-level aCL/aβ₂GPI-positivity after separating LAC and aCL/aβ₂GPI into two different domains.
- Include single LAC positivity, for the reasons discussed above, despite the negative results of the derivation cohort analysis; however, consensus was to be cautious in weighting cases with single LAC alone.
- Define "moderate" level aCL/aβ₂GPI IgG/M positivity as 40-79 U, and "high" level as ≥80 U based on:
   ○ Derivation cohort analysis demonstrating no significant association for aCL/aβ₂GPI levels of <40 U with highly likely APS cases.
   ○ Derivation cohort analysis demonstrating pronounced sequential relative risk increase above aCL/aβ₂GPI IgG ≥40, supported by our literature review demonstrating the increased association with aCL/aβ₂GPI levels of ≥40 U and aPL-related events.
   ○ Consensus among the SC members that aCL/aβ₂GPI levels: a) above the laboratory cut-off but <40 U do not provide enough confidence for APS classification for research purposes, even in the setting of an acceptable clinical event; and b) above ≥80 U provide unquestionable confidence for APS diagnosis or classification in the setting of an acceptable clinical event.
   ○ Derivation cohort analysis demonstrating a) the upper limit of aCL/aβ₂GPI normal range varied between 5U and 40U based on the reference ranges of the derivation cohort; b) median titers of aCL/aβ₂GPI IgG in the "highly likely" cases were 96/90U, compared to 35/46U in the "equivocal or unlikely" cases (for IgM, 50/48 U versus 30/55 U).
- Include aCL IgM and/or aβ₂GPI IgM as a separate category, independent of moderate or high positivity, given the nominal relative risk increase above 40U.
- Include simultaneous high level aCL IgG and aβ₂GPI IgG positivity as a separate category

*A summary of the discussions and consensus decisions based on phase I/II (1), phase III-A/B literature reviews (2-3), phase III-C derivation cohort analysis (4), and the steering committee consensus are reported here.

References:

(1) Barbhaiya M, Zuily S, Ahmadzadeh Y, Amigo MC, Avcin T, Bertolaccini ML et al. Development of a New International Antiphospholipid Syndrome Classification Criteria Phase I/II Report: Generation and Reduction of Candidate Criteria. Arthritis Care Res (Hoboken) 2021;73:1490-1501.

(2) Barbhaiya M, Zuily S, Amigo MC, Avcin T, Bertolaccini MB, et al. Development of a New International Antiphospholipid Syndrome Classification Criteria Phase III-A Report: Clinical Domain Definitions (pending manuscript submission).

(3) Barbhaiya M, Zuily S, Bertolaccini MB, Willis R, Devreese KMJ, et al. Development of a New International Antiphospholipid Syndrome Classification Criteria Phase III-B Report: Laboratory Domain Definitions (pending manuscript submission).

(4) Barbhaiya M, Zuily S, Ahmadzadeh Y, Costenbader K, Naden R, Erkan D. Development of New International Classification Criteria for Antiphospholipid Syndrome: Phase III Case Collection Results [abstract]. Arthritis Rheumatol. 2020; 72 (suppl 10). https://acrabstracts.org/abstract/development-of-new-international-classification-criteria-for-antiphospholipid-syndrome-phase-iii-case-collection-results/. Accessed March 1, 2022 (pending manuscript submission).

Supplement Section 6: <u>Phase IV Methodology (Additional Information)</u>

Sensitivity Analyses:

- We evaluated the performance of the new Antiphospholipid Syndrome (APS) classification criteria after: a) exclusion of cases that did not meet the entry criteria; and b) inclusion of cases considered unclassifiable due to missing data.

Additional Analyses:

- Given that two different validation cohorts were sequentially assessed by the same expert adjudicators, we anticipated a potential evolution in the thinking of expert adjudicators while rating cases, particularly after the planned moderated discussion of discordant cases of the first validation cohort. Thus, we assessed inter-rater agreement (using kappa coefficients) among expert adjudicators before the moderated discussions for both validation cohorts.
- We calculated Youden's index, a global index that incorporates both sensitivity and specificity, for the revised Sapporo and new APS classification criteria in each validation cohort.
- We compared the clinical and laboratory characteristics of false negative cases with true positive cases to evaluate reasons for cases not achieving APS classification.

Supplement Section 7: <u>Phase IV Results (Additional Information)</u>

Sensitivity Analyses:

- In the 229 (41%) patients not meeting entry criteria, mutually exclusive reasons for not meeting the entry criteria were: no clinical criterion (85 [37%]); no laboratory criterion (92/229 [40%]); no clinical and laboratory criteria (13 [6%]); more than three years between the clinical and laboratory criteria (15 [7%]); and low level aCL/aβ_2GPI positivity, i.e. above normal laboratory range but less than 40U (24 [11%]) with a negative LAC test. All cases not meeting the 'entry criteria' (n: 122 in cohort 1, n: 107 in cohort 2) were classified as 'no APS' by adjudicators. After excluding cases not meeting the entry criteria, performance characteristics were similar (cohort 1 specificity 0.98 [95% CI 0.95-1.00] and sensitivity 0.83 [95% CI 0.75-0.90]; and cohort 2 specificity 0.96 [95% CI 0.91-1.00] and sensitivity 0.84 [95% CI 0.77-0.91]).

- In the second sensitivity analysis, we examined "unclassifiable" cases excluded due to a non-scoreable macrovascular domain with missing data about provoking risk factors. In these cases, it was unknown whether the case fulfilled the entry criteria as no other clinical criteria were present. Based on SC consensus, if these cases were assigned to the lowest possible non-zero macrovascular domain level (i.e. score of one point), as opposed to the higher domain level to avoid potential overestimation of aPL positivity's role in the thrombotic event, the operating characteristics were not significantly different (cohort 1 specificity 0.99 [95% CI 0.98-1.00] and sensitivity 0.79 [95% CI 0.71-0.87]; and cohort 2 specificity 0.99 [95% CI 0.98-1.00] and sensitivity 0.78 [95% CI 0.70-0.85]).

Additional Analyses:

- Inter-rater agreement among experts was excellent and improved slightly between the two validation cohorts (Cohort 1: Kappa coefficient=0.73, 95%CI [0.68-0.78]; Cohort 2: Kappa coefficient= 0.84; 95%CI [0.80-0.89]), as anticipated.

- Youden's index was similar for the new and revised Sapporo criteria (with overlapping confidence intervals) in cohort 1 (New APS classification criteria: 0.82 [0.73-0.88]; Revised Sapporo criteria: 0.91 [0.84-0.94]) and cohort 2 (New APS classification criteria: 0.83 [0.74-0.88]; Revised Sapporo criteria: 0.85 [0.78-0.90]).

- Comparison of false negative cases to true positives demonstrated that the majority (83%) of false negatives were one of the three scenarios: a) moderate or high titer IgM aCL/aβ_2GPI alone with clinical criteria met (n:8 in cohort 1, n:6 in cohort 2); b) VTE or AT alone in patients with high

VTE or CVD risk profiles, with laboratory criteria met (n:2, n:3); and c) pre-fetal deaths or fetal death alone with laboratory criteria met (n:5, n:5) (Tables A and B below).

Table A: Clinical and Laboratory Characteristics of False Negative *versus* True Positive Cases (Validation Cohort 1) *

	False Negative n: 17		True Positive n: 81
	Clinical Criteria Met & Laboratory Criteria Not Met (n: 8)	Clinical Criteria Not Met & Laboratory Criteria Met (n: 9)	Clinical & Laboratory Criteria Met
Clinical Domains			
Macrovascular (Venous Thromboembolism [VTE]			
• VTE with a High-risk VTE Profile	0	0	2 (3%)
• VTE without a High-risk VTE Profile	3	0	44 (54%)
Macrovascular (Arterial Thrombosis [AT]			
• AT with a High-risk CVD Profile	0	2	6 (7%)
• AT without a High-risk CVD Profile	4	0	28 (35%)
Microvascular			
• Suspected	0	1**	6 (7%)
• Established	0	0	11 (14%)
Obstetric			
• ≥3 Pre-fetal and/or Early Fetal Deaths, or ≥ 1 FD 16-34w only without pre-eclampsia (PEC) or placental insufficiency (PI)	0	5	7 (9%)
• PEC or PI (+/- FD)	1	0	12 (15%)
• PEC and PI (+/- FD)	0	0	2 (3%)
Cardiac Valve			
• Thickening only	0	0	4 (5%)
• Vegetation (+/- thickening)	0	0	3 (4%)
Hematologic			
- Thrombocytopenia	1	1	21 (26%)

Laboratory Domains			
Lupus Anticoagulant Positive			
• Positive (single – one time)	0	0	10 (12%)
• Positive (persistent)	0	9	64 (79%)
aCL/aβ₂GPI IgG/M Positive			
• aCL and/or aβ₂GPI IgM Moderate or High Level Alone	8	1	4 (5%)
• aCL and/or aß₂GPI IgG Moderate Level with/without IgM	0	4	12 (15%)
• aCL <u>or</u> aβ₂GPI IgG High Level with/without IgM	0	2	17 (21%)
• aCL <u>and</u> aβ₂GPI IgG High Level with/without IgM	0	0	21 (26%)

aCL: anti-cardiolipin antibody; **aβ₂GPI:** anti–β₂-glycoprotein I antibody; **CVD**: cardiovascular disease

*Rows not mutually exclusive; **Livedoid vasculopathy lesions by physical examination.

Table B: Clinical and Laboratory Characteristics of False Negative *versus* True Positive Cases (Validation Cohort 2) *

	False Negative n: 18**		True Positive n: 95
	Clinical Criteria Met & Laboratory Criteria Not Met (n: 6)	Clinical Criteria Not Met & Laboratory Criteria Met (n: 10)	Clinical & Laboratory Criteria Met
Clinical Domains			
Macrovascular (Venous Thromboembolism [VTE])			
• VTE with a High-risk VTE Profile	0	4	2 (2%)
• VTE without a High-risk VTE Profile	2	0	50 (53%)
Macrovascular (Arterial Thrombosis [AT])			
• AT with a High-risk CVD Profile	0	1	7 (7%)
• AT without a High-risk CVD Profile	1	0	34 (36%)
Microvascular			
• Suspected	0	1***	10 (11%)
• Established	0	0	19 (20%)
Obstetric			
• ≥3 Pre-fetal and/or Early Fetal Deaths, or ≥ 1 Fetal Death (FD) 16-34w only without pre-eclampsia (PEC) or placental insufficiency (PI)	1	5	9 (10%)
• PEC or PI (+/- FD)	2	0	16 (17%)
• PEC and PI (+/- FD)	0	0	0
Cardiac Valve			
• Thickening only	0	0	4 (4%)
• Vegetation (+/- thickening)	0	0	9 (10%)
Hematologic			
• Thrombocytopenia	1	0	26 (27%)

Laboratory Domains			
Lupus Anticoagulant Positive			
• Positive (single – one time)	0	0	4 (4%)
• Positive (persistent)	0	10	87 (92%)
aCL/aβ₂GPI IgG/M Positive			
• aCL and/or aβ₂GPI IgM Moderate or High Level Alone	6	1	2 (2%)
• aCL and/or aß₂GPI IgG Moderate Level with/without IgM	0	0	10 (11%)
• aCL or aβ₂GPI IgG High Level with/without IgM	0	3	19 (20%)
• aCL and aβ₂GPI IgG High Level with/without IgM	0	4	22 (23%)

aCL: anti-cardiolipin antibody; **aβ₂GPI:** anti–β₂-glycoprotein I antibody; **CVD**: cardiovascular disease

* Rows not mutually exclusive; ** Two cases did not meet either clinical or laboratory criteria threshold;

***Livedo racemosa by physical examination and aPL-nephropathy suspicion by laboratory tests

Supplement Section 8: <u>References for International Guidelines Used to Formulate Venous Thromboembolism (VTE) and Cardiovascular Disease (CVD) Risk Factors</u>

Major VTE Risk Factors
- **Active malignancy** (1-3)
- **Hospital admission** (4-6)
- **Major trauma** (6-8)
- **Surgery** (2, 5, 6)

Minor VTE risk factors (two or more of the following at the time of the event (5)):
- **Active systemic autoimmune disease or active inflammatory bowel disease** (2, 5-7)
- **Acute/active severe infection** (6, 7)
- **Central venous catheter** (9)
- **Hormone replacement therapy, estrogen containing oral contraceptives, or ongoing *in vitro* fertilization treatment** (4-7)
- **Long distance travel** (1, 6, 7, 10)
- **Obesity** (3, 5-7)
- **Pregnancy or postpartum period** (2, 5, 7)
- **Prolonged immobilization** (2, 5)
- **Surgery** (2, 5, 6)

High CVD Risk Factors:
- **Arterial hypertension** (11-13)
- **Chronic kidney disease** (11, 13, 14)
- **Diabetes mellitus** (11, 13-15)
- **Hyperlipidemia** (11, 13, 16)

Moderate CVD risk factors: (three or more of the following at the time of the event (15))
- **Arterial Hypertension** (17, 18)
- **Current Tobacco smoking** (14, 19)
- **Diabetes mellitus** (11, 13, 15)
- **Hyperlipidemia** (11, 13, 14)
- **Obesity** (11, 12, 20, 21)

*1.	Schünemann et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: prophylaxis for hospitalized and non-hospitalized medical patients. Blood Adv. 2018;2:3198-225.*

*2.	Kearon et al. Categorization of patients as having provoked or unprovoked venous thromboembolism: guidance from the SSC of ISTH. J Thromb Haemost. 2016;14:1480-3.*

*3.	Mazzolai et al. Diagnosis and management of acute deep vein thrombosis: a joint consensus document from the European Society of Cardiology working groups of aorta and peripheral vascular diseases and pulmonary circulation and right ventricular function. Eur Heart J. 2018;39:4208-18.*

*4.	Kearon et al. Antithrombotic Therapy for VTE Disease: CHEST Guideline and Expert Panel Report. Chest. 2016;149:315-52.*

*5.	Ortel et al. American Society of Hematology 2020 guidelines for management of venous thromboembolism: treatment of deep vein thrombosis and pulmonary embolism. Blood Adv. 2020;4:4693-738.*

*6.	Kakkos et al. Editor's Choice - European Society for Vascular Surgery (ESVS) 2021 Clinical Practice Guidelines on the Management of Venous Thrombosis. Eur J Vasc Endovasc Surg. 2021;61:9-82.*

*7.	Konstantinides et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS): The Task Force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). Eur Respir J. 2019;54.*

*8.	Kearon et al. Antithrombotic therapy for venous thromboembolic disease: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008;133(6 Suppl):454s-545s.*

*9.	Gould et al. Prevention of VTE in nonorthopedic surgical patients: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e227S-e77S.*

*10.	Byard RW. Deep venous thrombosis, pulmonary embolism and long-distance flights. Forensic Science, Medicine and Pathology. 2019;15:122-4.*

*11.	Piepoli et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J. 2016;37:2315-81.*

12.	Williams et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018;39:3021-104.

13.	Mach et al. 2019 ESC/EAS Guidelines for the management of dyslipidemias: lipid modification to reduce cardiovascular risk. Eur Heart J. 2020;41:111-88.

14.	Arnett et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;140:e596-e646.

15.	Cosentino et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eur Heart J. 2020;41:255-323.

16.	Grundy et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;139:e1046-e81.

17.	Mansia et al. 2007 ESH-ESC Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Blood Press. 2007;16:135-232.

18.	Whelton et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Soc Hypertens. 2018;12:579.e1-.e73.

19.	Khan et al. The risk and burden of smoking related heart disease mortality among young people in the United States. Tob Induc Dis. 2015;13:16.

20.	Jensen et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. J Am Coll Cardiol. 2014;63(25 Pt B):2985-3023.

21.	Waist circumference and waist–hip ratio: report of a WHO expert consultation, Geneva, 8–11. December 2008.