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Cluster analysis for the identification of clinical phenotypes among antiphospholipid antibody-positive patients from the APS ACTION Registry

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Abstract

Objective: This study aimed to use cluster analysis (CA) to identify different clinical phenotypes among antiphospholipid antibodies (aPL)-positive patients.

Methods: The Alliance for Clinical Trials and International Networking (APS ACTION) Registry includes persistently positive aPL of any isotype based on the Sydney antiphospholipid syndrome (APS) classification criteria. We performed CA on the baseline characteristics collected retrospectively at the time of the registry entry of the first 500 patients included in the registry. A total of 30 clinical data points were included in the primary CA to cover the broad spectrum of aPL-positive patients.

Results: A total of 497 patients from international centres were analysed, resulting in three main exclusive clusters: (a) female patients with no other autoimmune diseases but with venous thromboembolism (VTE) and triple-aPL positivity; (b) female patients with systemic lupus erythematosus, VTE, aPL nephropathy, thrombocytopaenia, haemolytic anaemia and a positive lupus anticoagulant test; and (c) older men with arterial thrombosis, heart valve disease, livedo, skin ulcers, neurological manifestations and cardiovascular disease (CVD) risk factors.

Conclusions: Based on our hierarchical cluster analysis, we identified different clinical phenotypes of aPL-positive patients discriminated by aPL profile, lupus or CVD risk factors. Our results, while supporting the heterogeneity of aPL-positive patients, also provide a foundation to understand disease mechanisms, create new approaches for APS classification and ultimately develop new management approaches.

Keywords

Antiphospholipid syndrome, APS ACTION, cardiovascular risk factors, systemic lupus erythematosus, triple positivity

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Introduction

Persistent antiphospholipid antibodies (aPL) are recognized risk factors for thrombosis or obstetric morbidity, leading to a diagnosis of antiphospholipid syndrome (APS). Furthermore, aPL are associated with several non-thrombotic manifestations also known as non-criteria manifestations (e.g. thrombocytopaenia, autoimmune haemolytic anaemia, livedo, aPL-related nephropathy, heart valve disease and neurological manifestations).[1] APS can either be associated with another autoimmune disease (mainly systemic lupus erythematosus (SLE)) or be referred to as primary APS when no other concomitant autoimmune disease exists. Thus, clinical presentations of aPL-positive patients represent a wide spectrum, including asymptomatic carriers of aPL, arterial/venous/microvascular thrombosis, obstetric morbidity, non-thrombotic manifestations and the most severe form of the disease, catastrophic APS.[2]

The Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION) was created to design and conduct large-scale, multi-centre studies and clinical trials in persistently aPL-positive patients.[3] The APS ACTION Clinical Database and Repository ('Registry') was created to study the natural course of persistently aPL-positive patients with or without concomitant autoimmune disorders over at least 10 years. The registry allows large-scale cross-sectional and prospective analyses to be performed, which will eventually help us to understand the clinical characteristics of APS patients better.

Cluster analysis (CA) is a data-driven method that can group patients in a way that patients in the same group (cluster) are more like each other than those in other groups. Several studies have used CA to identify phenotypes in chronic diseases such as Parkinson's disease, asthma, inflammatory bowel disease and SLE.[4] This method helps unravel the complex aetiologies of medical conditions which may have pathogenic and therapeutic implications. For example, a therapeutic response in IgG4-related disease was predicted using cluster analysis.[5] In aPL-positive patients, CA has not been used to identify different clinical phenotypes.

Therefore, to improve our understanding of APS disease characteristics and facilitate potential targeted therapies, our primary objective was to use CA to identify different clinical phenotypes among aPL-positive patients. The secondary objective was to identify homogeneous groups of aPL-related clinical manifestations and cardiovascular disease (CVD) risk factors occurring in similar patients.

Methods

The checklist of items that should be included in reports of observational studies is available in Supplemental Table S1 (STROBE Statement).

APS ACTION Registry

An international web-based application – the REDCap (Research Electronic Data Capture)[6] – captures data on patient demographics, aPL-related clinical and laboratory characteristics and medication. The inclusion criteria are age between 18 and 60 years and persistent (at least 12 weeks apart) aPL-positivity within 12 months prior to screening. Positivity is defined as anti-cardiolipin antibodies (aCL) IgG/M/A (>40 GPL/MPL/APL, medium-to-high titre and/or >99th percentile), anti-β_2-glycoprotein-I (aβ_2GPI) IgG/M/A (>40 units, medium-to-high titre) and a positive lupus anticoagulant (LA) test based on International Society on Thrombosis and Haemostasis and other current guidelines.[7–9] Patients are followed every 12 ± 3 months with clinical data and blood collection.

Study cohort and data points

The primary CA was performed on the first 500 persistently aPL-positive patients with or without other systemic autoimmune diseases included in the APS ACTION registry. The goal was to identify variables that could discriminate groups of patients. We used 30 baseline (collected retrospectively at the time of the registry entry) demographic and clinical data points representative of the whole clinical spectrum of aPL-positive patients to generate clusters (Table 1). The choice of these data points was based on all available variables related to aPL in the APS ACTION registry. In a subgroup analysis, we limited our CA to female patients with a history of pregnancy. We only used 15 baseline demographic and clinical data points (Table 1).

For the secondary CA, clinical criteria for definite APS according to the Sydney criteria (arterial thrombosis, venous thromboembolism (VTE), small vessel thrombosis, more than three recurrent early fetal losses, late fetal death, premature birth due to pre-eclampsia/eclampsia), non-criteria manifestations (aPL-related nephropathy, livedo, superficial vein thrombosis, heart valve disease, haemolytic anaemia, thrombocytopaenia, transient ischaemic attack, chorea, cognitive impairment), as well as CVD risk factors (hypertension, hyperlipidaemia, diabetes, smoking, obesity) were analysed. The goal was to identify groups of clinical characteristics instead of groups of patients.

Statistical analysis

The characteristics of the sample are described as percentages for categorical variables and means, standard deviations, medians, quartiles and min/max values for continuous variables. Pearson's chi-square (or Fisher's exact test when assumption of expected frequency was violated) and Student's t-test were applied to compare qualitative variables and quantitative variables, respectively.

To identify clinical phenotypes, the CA method we used was the hierarchical ascending classification method based on Ward's criterion considered as the most relevant. From a statistical point of view, the objective of Ward's method is to find at each stage those two clusters whose fusion gives the minimum increase in the total within-group error sum of squares. This method optimizes the variance criterion.[10]

Regarding the robustness of the primary CA analysis, the Cubic Clustering Criterion and the SPRSQ (semi-partial R^2) were used to identify the optimal number of patient clusters (κ coefficient). The results of this analysis were validated by the bootstrap method (1000 iterations).[11] To identify differences between clusters, analysis of variance and the chi-square test of independence were used. Tests were adjusted for all pairwise comparisons within a row using the Bonferroni correction to identify predominant and discriminant variables. The variable with the highest percentage, which is significantly more common compared with one other cluster only, is defined as the predominant variable, and to all other clusters as the discriminant variable. Alpha risk was fixed at 5% for all analysis. These statistical analyses were done with IBM SPSS Statistics for Windows v22.0 (IBM Corp., Armonk, NY) and SAS v9.4 (SAS Institute, Cary, NC).

Results

After excluding three patients with missing data, 497 persistently aPL-positive patients from international centres were analysed (384 (77%) female, $M_{age} = 44.5 \pm 12.9$ years, $n = 324$ primary aPL/APS and $n = 173$ aPL/APS associated with other systemic autoimmune diseases).

Table 1. Variables used for cluster analysis.

	Variables
Demographics	Sex (male/female)
	Race (white/non-white)
Clinical criteria for definite antiphospholipid syndrome	Arterial thrombosis (yes/no)*
	Venous thromboembolism (yes/no)*
	Biopsy-proven microvascular thrombosis (pulmonary, skin, kidney and 'other') (yes/no)*
	Fetal death after 10th week of gestation (yes/no)*
	Premature birth due to preeclampsia, eclampsia, or placental insufficiency before 34th week of gestation (yes/no)*
	Three or more consecutive pre-embryonic or embryonic losses before 10th week of gestation (yes/no)*
Non-criteria manifestations	Superficial vein thrombosis (yes/no)
	Transient ischaemic attack (yes/no)
	Livedo reticularis/racemosa (past or current/never)
	Persistent thrombocytopenia defined as platelets $<100{,}000 \times 10^9$ tested twice at least 12 weeks apart (past or current/never)
	Autoimmune haemolytic anaemia (past or current/never)
	Echocardiography-proven heart valve disease (yes/no or unknown)
	Biopsy-proven aPL-related nephropathy (yes/no or unknown)
	Neuropsychiatric test-proven cognitive impairment (abnormal/normal or unknown)
	Chorea (yes/no)
	Seizure (yes/no)
	Skin ulcer (yes/no)
	Brain white-matter abnormalities (yes/no or unknown)
Cardiovascular risk factors	Body mass index >30 kg/m^2 (yes/no)*
	Hypertension requiring treatment (yes/no)*
	Diabetes mellitus requiring treatment (yes/no)*
	Hyperlipidaemia requiring treatment (yes/no)*
	Smoking (past or current/never)*
aPL profile	Positive LA test (yes/no)*
	Positive aCL IgG/IgM/IgA (yes/no)*
	Positive aβ_2GPI IgG/IgM/IgA (yes/no)*
Associated autoimmune diseases	SLE based on the American College of Rheumatology Classification Criteria (yes/no)*
	Other autoimmune disease, e.g. lupus-like disease, rheumatoid arthritis, Sjögren's syndrome, systemic sclerosis, idiopathic inflammatory myopathy or vasculitis (yes/no)

*Variables used for the cluster analysis restricted to female aPL-positive patients with a history of pregnancy.
aβ_2GPI: anti-β_2-glycoprotein I antibodies; aCL: anticardiolipin antibodies; aPL: antiphospholipid antibodies; LA: lupus anticoagulant; SLE: systemic lupus erythematosus.

Primary cluster analysis: clinical phenotypes of patients within the entire cohort

Table 2 demonstrates the demographic, clinical and laboratory characteristics of the patients, clustered in three main groups following a dendrogram analysis (Figure 1). The number of clusters was validated through the visual inspection of the dendrogram and confirmed by computation of the κ coefficient, which indicated a robust classification ($\kappa = 0.716$; 95% confidence interval (CI) 0.567–0.863). Discriminant variables in the three clusters were: (a) female patients with no other autoimmune diseases but with VTE and triple-aPL positivity (cluster 1); (b) female patients with SLE, VTE, non-criteria manifestations (aPL-nephropathy, thrombocytopaenia and haemolytic anaemia), positive LA test and positive SLE serology (cluster 2); and (c) older men with arterial thrombosis, heart valve disease, livedo, skin ulcer, neurological manifestations and CVD risk factors (cluster 3). Discriminant variables were triple-aPL positivity (cluster 1), SLE (cluster 2) and sex, older age, arterial thrombosis, heart valve disease, neurological manifestations and CVD risk factors (except diabetes mellitus; cluster 3).

Primary cluster analysis subgroup analysis: clinical phenotypes of female patients with pregnancy history

Table 3 demonstrates the demographic, clinical and laboratory characteristics of 290 female patients with pregnancy history clustered in four main groups: (a)

Table 2. Identification of three distinct clusters of patients among those included in the APS ACTION Registry.

Variables, n (%)	Cluster 1 ($N = 179$)	Cluster 2 ($N = 180$)	Cluster 3 ($N = 138$)
Demographics			
Age (years), $M \pm SD$	41.9 ± 11.6	42.3 ± 12.5	**51.0 ± 12.4**[a,b]
Female	**145 (81.0)**[c]	**145 (80.6)**[c]	92 (66.7)
Male	34 (19)	35 (19.4)	**46 (33.3)**[a,b]
Past medical history			
Clinical criteria*			
Arterial thrombosis	28 (15.6)	51 (28.3)[a]	**95 (68.8)**[a,b]
Venous thromboembolism	**84 (46.9)**[c]	**85 (47.2)**[c]	45 (32.6)
Small-vessel thrombosis	9 (5.0)	11 (6.1)	10 (7.2)
Pregnancy morbidity**	73/97 (75.3)	67/103 (65.0)	42/66 (63.6)
Non-criteria manifestations			
Heart valve disease	9 (5.0)	6 (3.3)	**23 (16.7)**[a,b]
Livedo	15 (8.4)	26 (14.4)	**30 (21.7)**[a]
Skin ulcer	6 (3.4)	11 (6.1)	**14 (10.1)**[a]
Neurological manifestations	22 (12.3)	26 (14.4)	**58 (42.0)**[a,b]
aPL nephropathy	2 (1.1)	**10 (5.6)**[c]	0 (0)
Thrombocytopaenia	22 (12.3)	**45 (25.0)**[a]	22 (15.9)
Autoimmune diseases			
None or unknown	**145 (81.0)**[b]	99 (55.0)	102 (73.9)
SLE	25 (14.0)	**74 (41.1)**[a,c]	26 (18.8)
Other	9 (5.0)	7 (3.9)	10 (7.2)
Cardiovascular risk factors			
Hypertension	14 (7.8)	33 (18.3)[a]	**99 (71.7)**[a,b]
Diabetes	4 (2.2)	5 (2.8)	**12 (8.7)**[a]
Hyperlipidaemia	12 (6.7)	31 (17.2)[a]	**65 (47.1)**[a,b]
Obesity	31 (17.3)	49 (27.2)	**60 (43.5)**[a,b]
Smoking	44 (24.6)	61 (33.9)	**74 (53.6)**[a,b]
Laboratory parameters			
aPL			
LA	129 (72.1)	**152 (84.4)**[a]	105 (76.1)
aCL	**166 (92.7)**[b,c]	63 (35.0)	115 (83.3)[b]
Anti-β_2-GPI antibodies	**138 (77.1)**[b,c]	25 (13.9)	73 (52.9)[b]
Triple-aPL positivity	**99 (55.3)**[b,c]	13 (7.2)	56 (40.6)[b]
Other laboratory parameters			
Haemolytic anaemia	2 (1.1)	**18 (10.0)**[a]	6 (4.3)
ANA	104 (58.4)	**117 (65.7)**[c]	72 (52.2)
Anti-dsDNA	43 (24.0)	**61 (33.9)**[c]	23 (16.7)
Low C3	20 (29.9)	**39 (49.4)**[a]	18 (48.6)
Low C4	24 (35.8)	36 (45.6)	15 (40.5)

The variable with the highest percentage, which is significantly more common compared with one other cluster only, is defined as the predominant variable (shown in bold), and to two other clusters as the discriminant variable (shown in bold and underlined). NB: For each variable, when both discriminant and predominant variables are present, only the discriminant variable is shown in bold to facilitate the reading.

[a,b,c]Significantly ($p < 0.05$) more prevalent than clusters 1, 2 and 3, respectively.

*Several clinical manifestations can occur in the same patient.

**Among 266 aPL-positive female patients who have been pregnant.

SD: standard deviation; aCL: anticardiolipin antibodies; aPL: antiphospholipid antibodies; LA: lupus anticoagulant; SLE: systemic lupus erythematosus; APS ACTION: Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking.

older female patients with arterial thrombosis, CVD risk factors, statin treatment (cluster 1); (b) female patients with pregnancy morbidity only (cluster 2); (c) asymptomatic aPL-positive female patients with aCL/aβ_2GPI treated with aspirin (cluster 3); and (d) female patients with VTE, obesity, SLE, positive LA test and warfarin treatment (cluster 4). Discriminant variables were fetal death (cluster 2), asymptomatic aPL (particularly aβ_2GPI positivity; cluster 3) and SLE, VTE and obesity (cluster 4).

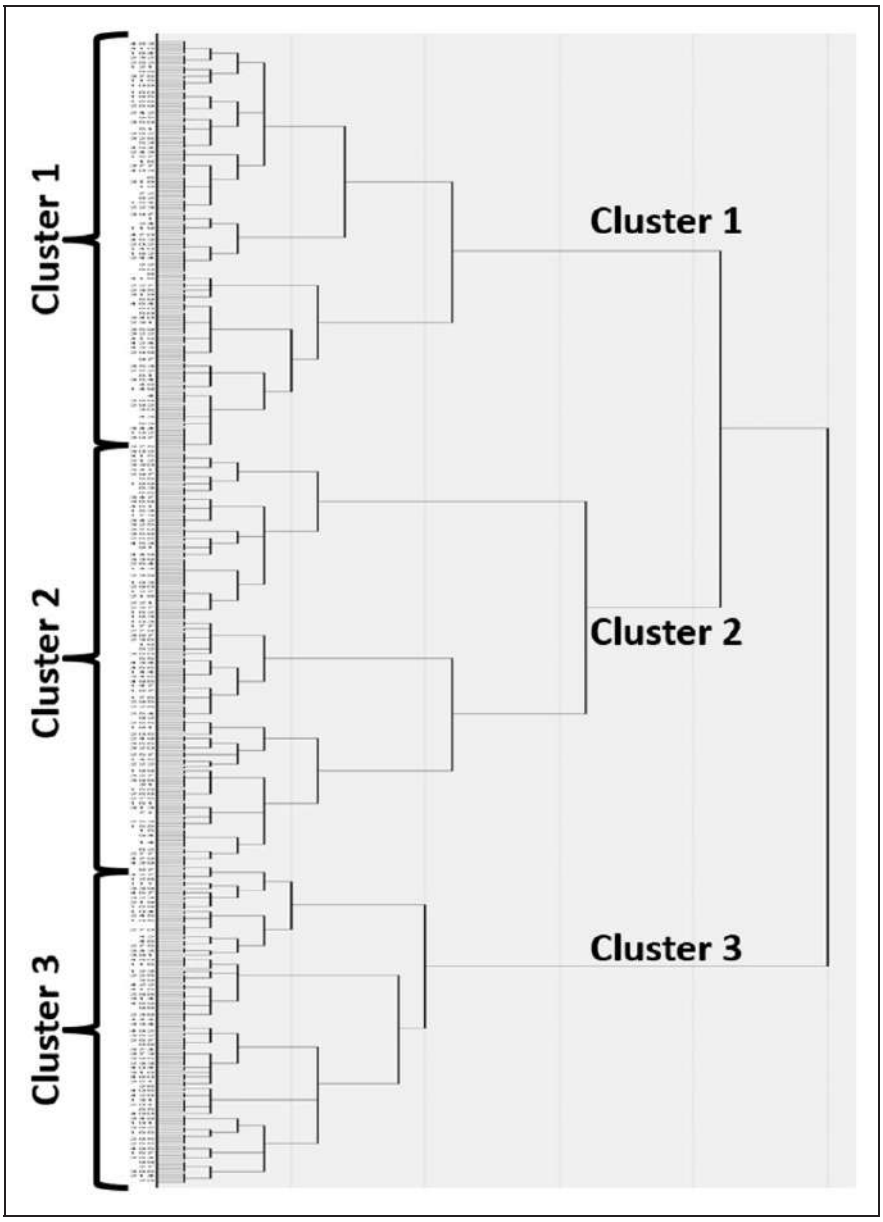

Figure 1. Dendrogram. Using Ward's minimum-variance hierarchical clustering method, 497 subjects were clustered to a single final group. At each generation of clusters, samples were merged into larger clusters to minimize the within-cluster sum of squares or maximize between-cluster sum of squares. With successive clustering, three balanced groups became obvious.

Secondary cluster analysis: clusters of clinical characteristics occurring together

Three main clusters with different combinations of manifestations were identified (Figure 2): (a) obstetric morbidity, non-criteria manifestations and diabetes (cluster A); (b) arterial thrombosis with CVD risk factors (hypertension, hyperlipidaemia and smoking; cluster B); and (c) VTE and obesity (cluster C). When excluding patients with any associated autoimmune disease (mainly SLE), results from 279 patients remained unchanged (Figure 3).

Discussion

According to our hierarchical primary and secondary CA, we confirmed the heterogeneity of clinical phenotypes of aPL-positive patients including aPL-positive females with a history of pregnancy. Factors resulting in this heterogeneity were mainly aPL profile, SLE diagnosis and CVD risk factors. Furthermore, we identified specific clusters in asymptomatic aPL-positive patients and women with obstetric APS only and found that non-criteria manifestations do not share the same cluster of clinical APS criteria.

Table 3. Identification of four distinct clusters of patients with a history of pregnancy among the APS ACTION Registry.

Variables, *n* (%)	Cluster 1 (*N*=85)		Cluster 2 (*N*=69)		Cluster 3 (*N*=92)		Cluster 4 (*N*=44)	
Demographics								
Age (years), $M \pm SD$	**47.95**	**±9.63**[b]	38.94	±11.67	**44.86**	**±11.74**[b]	42.94	±11.30
White	52	(65.8)	35	(54.7)	53	(66.3)	22	(50.0)
Asian	3	(3.8)	**11**	**(17.2)**[a]	8	(10.0)	4	(9.1)
Latin American	22	(27.8)	16	(25.0)	12	(15.0)	12	(27.3)
Black	1	(1.3)	2	(3.1)	5	(6.3)	5	(11.4)
Past medical history								
Clinical criteria*								
Arterial thrombosis	**40**	**(47.1)**[b,d]	12	(17.4)	31	(33.7)	8	(18.2)
Venous thromboembolism	37	(43.5)[c]	27	(39.1)[c]	16	(17.4)	**34**	**(77.3)**[a,b,c]
Small-vessel thrombosis	8	(9.4)	1	(1.4)	4	(4.3)	2	(4.5)
≥3 fetal losses	7	(8.2)	5	(7.2)	8	(8.7)	3	(6.8)
Fetal death >10th week	30	(35.3)[c]	**58**	**(84.1)**[a,c,d]	3	(3.3)	11	(25.0)[c]
Premature birth**	12	(14.1)	**21**	**(30.4)**[d]	**18**	**(19.6)**[d]	1	(2.3)
Classification								
Asymptomatic aPL-carriers	11	(12.9)	3	(4.3)	**33**	**(35.9)**[a,b,d]	5	(11.4)
Obstetric APS	7	(8.2)	**29**	**(42.0)**[a,c,d]	15	(16.3)	3	(6.8)
Thrombotic and obstetric APS	**30**	**(35.3)**[c]	**25**	**(36.2)**[c]	13	(14.1)	12	(27.3)
Thrombotic APS	**37**	**(43.5)**[b]	12	(17.4)	31	(33.7)	**24**	**(54.5)**[b]
Other autoimmune disease								
SLE	21	(24.7)	11	(15.9)	20	(21.7)	**25**	**(56.8)**[a,b,c]
Lupus-like disease	7	(8.2)	4	(5.8)	15	(16.3)	0	(0.0)
Cardiovascular risk factors								
Hypertension	**42**	**(49.4)**[b,c]	12	(17.4)	17	(18.5)	13	(29.5)
Diabetes	4	(4.7)	3	(4.3)	5	(5.4)	2	(4.5)
Hyperlipidaemia	**29**	**(34.1)**[b,c]	6	(8.7)	13	(14.1)	6	(13.6)
Obesity	21	(24.7)	10	(14.5)	21	(22.8)	**25**	**(56.8)**[a,b,c]
Smoking	**14**	**(16.5)**[b]	2	(2.9)	**16**	**(17.4)**[b]	5	(11.4)
Treatments								
Aspirin	32	(38.1)	32	(46.4)[d]	**57**	**(62.0)**[a,d]	9	(20.5)
Warfarin	56	(65.9)[c]	31	(44.9)	35	(38.0)	**33**	**(75.0)**[b,c]
LMWH	7	(8.2)	4	(5.8)	7	(7.6)	2	(4.5)
Statins	**29**	**(34.1)**[b,d]	5	(7.2)	16	(17.4)	5	(11.4)
Hydroxychloroquine	35	(41.7)	23	(33.3)	37	(40.2)	23	(52.3)
Laboratory parameters								
aPL								
LA	**75**	**(88.2)**[c]	**55**	**(79.7)**[c]	53	(57.6)	**39**	**(88.6)**[c]
aCL	**63**	**(74.1)**[d]	**47**	**(68.1)**[d]	**78**	**(84.8)**[d]	7	(15.9)
Anti-β_2-GPI antibodies	37	(43.5)[d]	28	(40.6)[d]	**59**	**(64.1)**[a,b,d]	1	(2.3)
Other parameters								
Anti-Ro	6	(7.1)	6	(8.7)	11	(12.0)	10	(22.7)
Anti-La	1	(1.2)	2	(2.9)	2	(2.2)	4	(9.1)

The variable with the highest percentage, which is significantly more common compared with one other cluster only, is defined as the predominant variable (shown in bold), and to three other clusters as the discriminant variable (shown in bold and underlined). NB: For each variable, when both discriminant and predominant variables are present, only the discriminant variable is shown in bold to facilitate the reading.

[a,b,c,d]Significantly ($p < 0.05$) more prevalent than clusters 1, 2, 3 and 4, respectively.

*Several clinical manifestations can occur in the same patient.

**Due to preeclampsia, eclampsia or placental insufficiency.

LMWH: low molecular weight heparin; APS ACTION: Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking; aCL: anticardiolipin antibodies; aPL: antiphospholipid antibodies; LA: lupus anticoagulant; SLE: systemic lupus erythematosus.

aPL profile, especially triple-aPL positivity, is considered as the most clinically significant laboratory profile that exposes patients to a higher risk for developing aPL-related clinical events.[12] Furthermore, the additive impact of CVD risk factors on the development of thrombosis in aPL-positive patients[13] is well accepted. A similar effect of CVD risk factors (mainly smoking, hypertriglyceridaemia and obesity) on obstetric outcomes are also

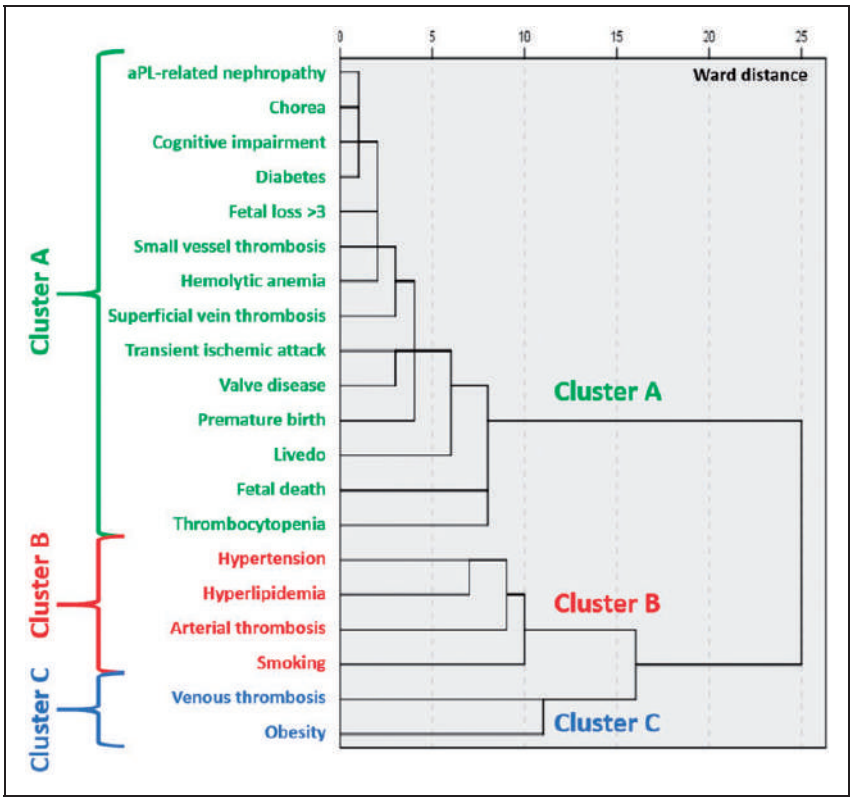

Figure 2. Cluster analysis of antiphospholipid antibody (aPL)-related clinical manifestations and cardiovascular risk factors. Using Ward's minimum-variance hierarchical clustering method ($n = 500$), three main clusters of manifestations were identified (arterial thrombosis and cardiovascular risk factors; venous thromboembolism and obesity; non-criteria manifestations, diabetes and obstetric morbidity).

identified in women with a history of pregnancy.[14] In fact, CVD risk factors are now incorporated into thrombosis prediction models.[15,16] Lastly, overlapping manifestations exist between SLE and APS. While aPL modify the clinical presentation of SLE patients,[17–19] conversely, SLE could also modify the clinical presentation of aPL-positive patients.[20] Thus, as supported by our findings, the identification of triple-aPL positivity, CVD risk factors and SLE in aPL-positive patients is critical for a precise clinical phenotyping allowing a better risk stratification in aPL-positive patients.[21]

Since 2010, new data have confirmed the significant association between some of the non-criteria manifestations and aPL, especially in SLE patients.[19] Indeed, current classification criteria are suboptimal due to several factors, the most relevant being the lack of representation of many heterogeneous manifestations of aPL. In parallel with an international collaborative effort to develop new APS classification criteria,[22] our finding of the significant associations between non-criteria and classical criteria manifestations reinforces the need to take into account these manifestations in the global clinical assessment of aPL-positive patients.

From a pathogenic point of view, several non-criteria manifestations share the same underlying pathogenic process[23]: vascular wall involvement with proliferation and endothelium impairment has been demonstrated in the kidneys of APS patients with aPL-related nephropathy (thrombotic microangiopathy, intimal hyperplasia), in the brain of patients with cognitive decline, in the lungs of patients with pulmonary arterial hypertension (plexiform lesion), in the placentas of women with placental-mediated complications (decidual vasculopathy) and in the vessels of patients with arterial stenosis (coronary and renal artery). This aPL-related vasculopathy is not completely understood. However, there were indications of the AKT/mTORC pathway activation by aPL in cultured endothelial cells in vitro, leading to aPL-related nephropathy lesions,[24] although the activation of this pathway in other organs is still to be demonstrated. We found that regardless of any underlying autoimmune diseases, all non-criteria manifestations were gathered in one cluster, suggesting that patients with these manifestations could share a common phenotype, supporting the hypothesis of a common underlying pathologic mechanism. Together with previous data,[25] our results

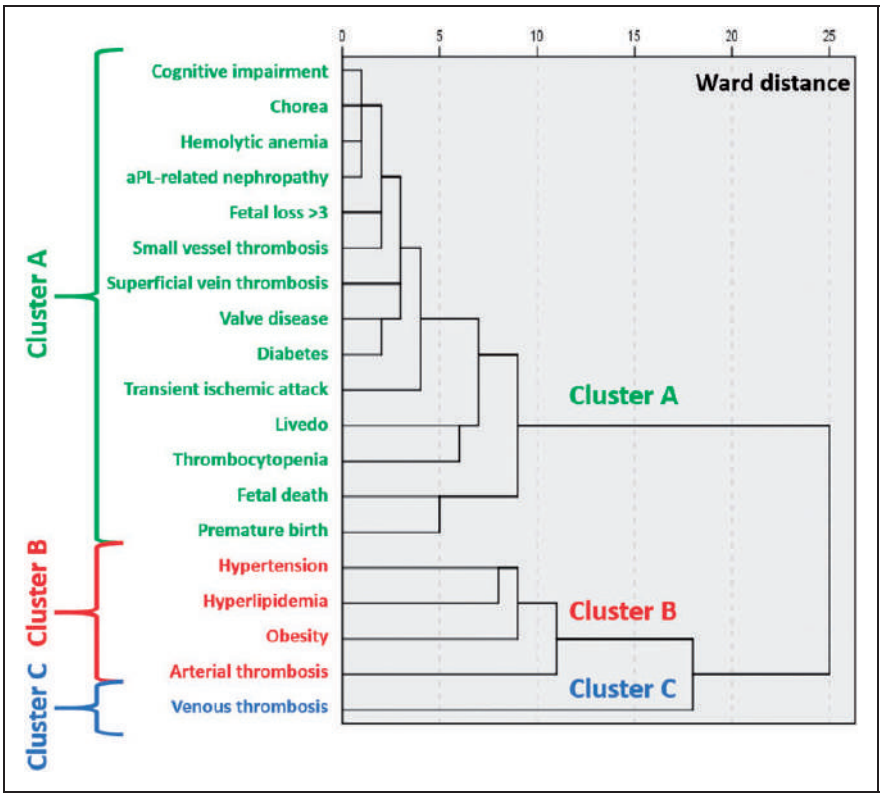

Figure 3. Cluster analysis of clinical manifestations and cardiovascular risk factors in aPL-positive patients with no autoimmune disease. Using Ward's minimum-variance hierarchical clustering method (n=279), three main clusters of manifestations were identified (arterial thrombosis and cardiovascular risk factors; venous thromboembolism only; non-criteria manifestations, diabetes and obstetric morbidity).

contribute to the understanding of the heterogeneity of clinical phenotypes of APS patients.

The limitations of this study include a potential lack of generalizability to other patient populations. However, the APS ACTION Registry represents the largest ongoing prospective collaborative clinical database and repository, gathering a large number of aPL positive patients followed regularly. In fact, confounding factors may impact the results. CA is an exploratory analysis that is used to identify subsets of cases if the grouping is not previously known. Therefore, it does not make any distinction between dependent and independent variables. The CA can identify groups of patients that present with similar symptoms/manifestations and simultaneously maximize the difference between the groups. Thus, even if potential confounding factors are not addressed in a classical fashion (e.g. multivariate analysis), the identification of a clinical heterogeneity between aPL-positive patients can be considered as the major confounding factor that could help understand different outcomes.[26] Another issue is that time is not analysed in this CA. Indeed, this analysis is based on a cross-sectional analysis of data recorded at baseline, and it cannot be excluded that different disease durations and treatments may influence the results. Several risk factors could have started after the aPL events took place, and therefore apparent differences in attributed aPL events could be due to differences in duration of exposure and to heterogeneity of treatment. However, this will be analysed using data collected during the prospective follow-up of the cohort.

In conclusion, our results confirm the heterogeneity of aPL-positive patients and provide a foundation to identify different disease mechanisms, create new approaches for APS classification and ultimately develop new tailored management tactics. Furthermore, our results open new research avenues such as monitoring the long-term follow-up of patients based on their initial clusters, or conducting randomized controlled studies based on different clusters segregations.

Authorship details

Substantial contribution to concept and design: S.Z. Analysis and/or interpretation of data: S.Z., I.C-U., D.W. and D.E. Critical writing or revising the intellectual content: S.Z., I.C.-U., C.B., D.A., S.S., V.P., M.G.T., A.U., M.G., H.M.B., M. A.A.Z., P.F., L.J., M.E., H.C., D.W.B., G.R.d.J., C.N., M.

P., E.R., R.C., J.S.K., T.A., R.W., M.L.B., J.V., D.W. and D.E. Final approval of the version to be published: S. Z., I.C.-U., C.B., D.A., S.S., V.P., M.G.T., A.U., M.G., H. M.B., M.A.A.Z., P.F., L.J., M.E., H.C., D.W.B., G.R.d.J., C.N., M.P., E.R., R.C., J.S.K., T.A., R.W., M.L.B., J.V., D.W. and D.E.

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Supplemental material

Supplemental material for this article is available online.

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