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ORIGINAL RESEARCH

Identifying high-risk profile in primary antiphospholipid syndrome through cluster analysis: French multicentric cohort study

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ABSTRACT

Introduction Antiphospholipid syndrome (APS) is an autoimmune disease characterised by thrombosis (arterial, venous or small vessel) or obstetrical events and persistent antiphospholipid antibodies (aPL), according to the Sydney classification criteria. Many studies have performed cluster analyses among patients with primary APS and associated autoimmune disease, but none has focused solely on primary APS. We aimed to perform a cluster analysis among patients with primary APS and asymptomatic aPL carriers without any autoimmune disease, to assess prognostic value.

Methods In this multicentre French cohort study, we included all patients with persistent APS antibodies (Sydney criteria) measured between January 2012 and January 2019. We excluded all patients with systemic lupus erythematosus or other systemic autoimmune diseases. We performed hierarchical cluster analysis on the factor analysis of mixed data coordinates results with baseline patient characteristics to generate clusters.

Results We identified four clusters: cluster 1, comprising 'asymptomatic aPL carriers', with low risk of events during follow-up; cluster 2, the 'male thrombotic phenotype', with older patients and more venous thromboembolic events; cluster 3, the 'female obstetrical phenotype', with obstetrical and thrombotic events; and cluster 4, 'high-risk APS', which included younger patients with more frequent triple positivity, antinuclear antibodies, non-criteria manifestations and arterial events. Regarding survival analyses, asymptomatic aPL carriers relapsed less frequently than the others, but no other differences in terms of relapse rates or deaths were found between clusters.

Conclusions We identified four clusters among patients with primary APS, one of which was 'high-risk APS'. Clustering-based treatment strategies should be explored in future prospective studies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Better risk stratification is a significant issue in antiphospholipid syndrome (APS). Clusters analysis have already been performed among APS patients, but none aimed to focus on primary APS patients.

WHAT THIS STUDY ADDS

⇒ We performed a cluster analysis in primary APS and identified four clusters, one of which was 'high-risk APS'.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Clustering-based treatment strategies should be explored in future prospective studies.

INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disease characterised by thrombosis (arterial, venous or small vessel) or obstetrical events and persistent antiphospholipid antibodies (aPL), according to the Sydney classification criteria.[1] APS can be primary or associated with other autoimmune diseases, particularly systemic lupus erythematosus (SLE) and patients with infectious diseases and several other inflammatory conditions can develop positive aPL.[2] Patients with primary APS seem to develop other autoimmune diseases infrequently during their clinical course.[3] Some clinical and biological elements are known to have a prognostic impact in primary APS,[4–6] but better risk stratification remains a significant issue in these patients. Many studies have performed cluster analyses among patients with primary APS and associated autoimmune disease, but none has focused on patients with primary APS.[7–9] The aim of our study was to perform a cluster analysis among patients with primary

APS and asymptomatic aPL carriers without any other autoimmune disease, to describe their characteristics, and to assess new APS events and death rates, in order to explore prognostic value.

METHODS

Data collection and population

In this French multicentre retrospective cohort study, we included all patients with persistent aPL (according to Sydney criteria), measured between January 2012 and January 2019, from Saint Antoine and Tenon University Hospitals (Paris), Tours University Hospital and Brest University Hospital. Patients with SLE or other systemic autoimmune diseases were excluded. Clinical, laboratory and treatment data were collected retrospectively from the medical records during the first in-hospital contact, and were considered as baseline variables. Non-criteria manifestations included: livedo reticularis; immune thrombocytopenia and/or autoimmune haemolytic anaemia; APS nephropathy; Libman Sachs endocarditis and neurological disorders, including multiple sclerosis-like disease and seizure.

Lupus anticoagulant assay and antiphospholipid IgM/IgG determination

Lupus anticoagulant testing was performed according to International Society on Thrombosis and Haemostasis (ISTH) guidelines[1] with STA-PTTA (Diagnostica Stago) and confirm dilute Russell's viper venom time (dRVVT) using a kit containing LA-1 screening reagent and LA-2 confirmation reagant (Ref OQGP). All assays were performed on STA R Max3 instrument (Diagnostica Stago, France, 92 600 Asnières-sur-Seine) at 37°C.

Quantitative values of anti-beta-2 glycoprotein 1 (β2GP1) antibodies IgM/IgG and anticardiolipin antibodies IgM/IgG were measured using a standard ELISA with BioPlex 2200 APLs Multiplex kit (Bio-Rad, France, 92 430 Marnes-La-Coquette).

Lupus anticoagulant testing by dRVVT may be influenced by anticoagulant treatments (risk of false positive). In patients with vitamin-K antagonist treatment, testing was performed according to the international normalised ratio (INR) patient's value. In patient with INR below 1.5, normal test was performed. In patients with INR between 1.5 and 3.5, the test was performed on M+T plasma mixture, and if the INR was over 3.5, test was not performed. In patient with unfractionated heparin or low-molecular-weight heparin, the dRVVT was not performed if thrombin time was over 150 s and anti-Xa activity over 1 IU/mL. Among patients with direct oral anticoagulant, dRVVT was performed after pretreatment with DOAC-Remove (Endotell AG, Suisse, 4123 Allschwil), activated carbon.

Anti-β2GP1 antibodies (IgM/IgG) were considered medium-high titre positive if above the 99th percentile and high titre positive if higher than 80 UGPL for IgG and 80 UMPL for IgM. Anticardiolipin medium-high titre was considered positive if higher than 40 UGPL for IgG and 40 UMPL for IgM and high titre positive if higher than 80 UGPL for IgG and 80 UMPL for IgM. Persistent aPL were defined according to Sydney criteria as persistent positivity of APS laboratory criteria detected on two or more occasions at least 12 weeks apart. Triple positivity was defined as the presence of three aPL with ELISA of the same isotype either G or M.

Statistical analysis

Data are expressed as medians with IQRs and numbers with frequencies. Qualitative and quantitative variables were compared using Fisher's test and the Kruskal-Wallis test, respectively. In order to generate clusters, we first used baseline patient characteristics (shown in online supplemental table S1 in supporting information) to perform a factor analysis of mixed data (FAMD) on the individuals. Missing data were handled in the FAMD analysis by multiple imputation, using the R package 'missMDA', V.1.18. Next, we performed a hierarchical cluster analysis on the FAMD coordinates results using Euclidean distance and the Ward agglomerative method. The optimal number of clusters was found using multiple clustering validity indices, shown in online supplemental figure S1 in supporting information. APS event during the follow-up period was defined as the occurrence of any new venous or arterial thrombosis or obstetrical adverse event during the follow-up. Follow-up duration was defined as the time between the date of diagnosis or the date of the first confirmed APS laboratory criteria positivity, and the date of the outcome incidence or the date of the last medical contact available within ten years from the date of diagnosis. Cumulative incidence curves of death and APS event were generated using Kaplan-Meier among the total population and each cluster population, and were compared using the log-rank test. Two-sided testing was used, with $p<0.05$ considered statistically significant. All analyses were performed using R software, V.3.6.0 for Mac (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We included 253 patients with persistent aPL in this study, most of whom were women (n=174, 68.8%); the median age was 52 years (IQR 38–65 years). Among them, 125 (49.4%) had thrombosis (venous or arterial), 56 (24.2%) had obstetrical adverse events and 107 (42.3%) were aPL carriers without any classifying clinical criteria, according to the Sydney classification. Triple positivity was found in 47 patients (19.3%), and 71 patients (28.2%) presented non-criteria manifestations. Further details are provided in table 1.

Based on clinical and laboratory data listed in online supplemental table S1, a hierarchical cluster analysis was performed on the FAMD coordinates results to obtain the dendrogram shown in figure 1. The optimal number of clusters was four, according to various methods shown

Table 1 Patient characteristics, according to clusters

	All patients N=253	Cluster 1 N=101	Cluster 2 N=67	P value*	Cluster 3 N=58	P value†	Cluster 4 N=27	P value*‡
Female sex, n (%)	174 (68.8)	63 (62.4)	31 (46.3)	0.06	58 (100.0)	<0.001	22 (81.5)	0.10
Age (years, median, IQR)	52.0 (38.0–65.0)	54.0 (41.0–62.0)	58.0 (43.5– 70.0)	0.16	48.0 (36.0– 62.0)	0.36	41.0 (33.5–54.5)	0.047
Arterial hypertension, n (%)	81 (40.9)	27 (35.1)	23 (46.0)	0.30	22 (44.0)	0.41	9 (42.9)	0.69
Dyslipidaemia, n (%)	49 (25.1)	16 (1.3)	15 (30.6)	0.34	12 (24.0)	0.90	6 (28.6)	0.69
Tobacco, n (%)	35 (26.5)	15 (27.3)	10 (22.7)	0.78	3 (23.1)	1.00	7 (35.0)	0.72
Diabetes mellitus, n (%)	22 (15.5)	8 (13.3)	6 (13.0)	1.00	6 (40.0)	0.045	2 (9.5)	0.94
Overweight, n (%)	52 (31.5)	14 (22.2)	12 (28.6)	0.61	16 (39.0)	0.10	10 (52.6)	0.02
IgG anti-cardiolipin (IU, median, IQR)	13.0 (4.0–43.0)	9.7 (3.0–33.9)	8.6 (4.0–50.0)	0.23	12.0 (5.0–27.2)	0.41	51.5 (22.9– 200.3)	<0.001
IgG anti-cardiolipin medium-high titre positive, n (%)	64 (28.7)	19 (20.9)	21 (32.8)	0.14	10 (22.7)	0.98	14 (58.3)	<0.01
IgG anti-cardiolipin high titre positive, n (%)	30 (14.0)	7 (7.9)	9 (15.3)	0.25	6 (14.0)	0.43	8 (33.3)	<0.01
IgM anti-cardiolipin (IU, median, IQR)	8.5 (2.0–36.4)	7.0 (2.0–30.2)	14.50 (2.6– 46.5)	0.28	7.0 (2.2–20.0)	0.76	45.0 (7.5–82.0)	<0.01
IgM anti-cardiolipin medium-high titre positive, n (%)	51 (24.1)	16 (18.0)	16 (27.6)	0.24	7 (16.7)	1.00	12 (52.2)	<0.01
IgM anti-cardiolipin high titre positive, n (%)	21 (9.9)	6 (6.7)	5 (8.6)	0.92	3 (7.1)	1.00	7 (30.4)	<0.01
IgG anti-β2GP1 (IU, median, IQR)	3.4 (1.0–25.0)	2.2 (1.0–10.0)	3.0 (1.0–19.2)	0.44	6.5 (1.0–24.5)	0.42	68.5 (11.4– 200.3)	<0.001
IgG anti-β2GP1 medium-high titre positive, n (%)	70 (31.4)	21 (23.6)	15 (24.6)	1.00	18 (39.1)	0.09	16 (59.3)	0.001
IgG anti-β2GP1 high titre positive, n (%)	32 (15.0)	8 (9.0)	6 (10.2)	1.00	6 (14.3)	0.54	12 (50.0)	<0.001
IgM anti-β2GP1 (IU, median, IQR)	3.00 (1.00– 24.00)	4.0 (1.0–20.3)	2.5 (1.0–28.7)	0.91	2.0 (1.0–7.9)	0.07	29.0 (2.0–77.4)	0.001
IgM anti-β2GP1 medium-high titre positive, n (%)	66 (29.7)	24 (27.0)	18 (29.5)	0.88	8 (17.4)	0.31	16 (61.5)	<0.01
IgM anti-β2GP1 high titre positive, n (%)	13 (6.1)	3 (3.4)	2 (3.4)	1.00	2 (4.8)	1.00	6 (26.1)	<0.01
LA, n (%)	102 (65.0)	38 (62.3)	19 (50.0)	0.32	26 (76.5)	0.24	19 (79.2)	0.22
Triple positivity, n (%)	47 (19.3)	10 (10.2)	7 (11.1)	1.00	7 (12.5)	0.87	23 (85.2)	<0.001
Antinuclear antibodies, n (%)	58 (30.7)	15 (19.0)	5 (12.8)	0.56	18 (39.1)	0.03	20 (80.0)	<0.001
Anti-dsDNA antibodies, n (%)	16 (12.4)	6 (10.3)	2 (6.9)	0.90	0 (0.0)	0.382	8 (32.0)	0.04
aPL carrier, n (%)	107 (42.3)	100 (99.0)	0 (0.0)	<0.001	7 (12.1)	<0.001	0 (0.0)	<0.001
Thrombotic phenotype, n (%)	125 (49.4)	1 (1.0)	67 (100.0)	<0.001	31 (53.4)	<0.001	26 (96.3)	<0.001
Arterial thrombosis, n (%)	53 (22.1)	1 (1.0)	19 (28.4)	<0.001	19 (38.8)	<0.001	15 (55.6)	<0.001
Stroke, n (%)	33 (15.8)	1 (1.1)	17 (27.9)	<0.001	6 (15.4)	<0.01	9 (40.9)	<0.001
Transient ischaemic attack, n (%)	3 (1.4)	0 (0.0)	1 (1.6)	0.86	1 (2.6)	0.68	1 (4.5)	0.46
Myocardial infarction, n (%)	12 (5.7)	0 (0.0)	4 (6.6)	0.06	4 (10.3)	0.01	4 (18.2)	0.001

Continued

Table 1 Continued

	All patients N=253	Cluster 1 N=101	Cluster 2 N=67	P value*	Cluster 3 N=58	P value†	Cluster 4 N=27	P value*‡
Pulmonary embolism, n (%)	32 (13.6)	1 (1.0)	22 (34.4)	<0.001	5 (10.0)	0.03	4 (16.0)	<0.01
Deep vein thrombosis, n (%)	60 (25.1)	0 (0.0)	33 (50.0)	<0.001	14 (28.0)	<0.001	13 (48.1)	<0.001
Obstetrical phenotype, n (%)	56 (24.2)	0 (0.0)	0 (0.0)	NA	47 (94.0)	<0.001	9 (33.3)	<0.001
No of obstetrical adverse events, n (%)				NA		<0.001		<0.001
0	174 (75.7)	87 (100.0)	66 (100.0)		3 (6.0)		18 (66.7)	
1	33 (14.3)	0 (0.0)	0 (0.0)		29 (58.0)		4 (14.8)	
2	4 (1.7)	0 (0.0)	0 (0.0)		3 (6.0)		1 (3.7)	
≥3	19 (8.3)	0 (0.0)	0 (0.0)		15 (30.0)		4 (14.8)	
One or more unexplained deaths at or beyond the 10th week of gestation, n (%)	11 (5.3)	0 (0.0)	0 (0.0)	NA	8 (25.8)	<0.001	3 (11.1)	0.01
Three or more unexplained consecutive spontaneous miscarriage before the 10th week, n (%)	15 (7.1)	0 (0.0)	0 (0.0)	NA	13 (39.4)	<0.001	2 (7.4)	0.09
Premature births before the 34th week, n (%)	7 (3.3)	0 (0.0)	0 (0.0)	NA	5 (15.2)	0.001	2 (7.4)	0.09
Preeclampsia, HELLP syndrome or placental abruption, n (%)	16 (7.6)	1 (1.2)	0 (0.0)	1.000	10 (29.4)	<0.001	5 (18.5)	<0.01
Non-criteria manifestations, n (%)	71 (28.2)	31 (30.7)	10 (15.2)	0.04	12 (20.7)	0.24	18 (66.7)	0.001
Autoimmune cytopenia, n (%)	26 (10.4)	13 (12.9)	0 (0.0)	<0.01	4 (7.0)	0.38	9 (33.3)	0.03
Neurological manifestations, n (%)	22 (8.8)	9 (9.0)	5 (7.6)	0.97	4 (7.0)	0.90	4 (14.8)	0.60
APS nephropathy, n (%)	4 (1.6)	2 (2.0)	0 (0.0)	0.67	0 (0.0)	0.74	2 (7.4)	0.42
Libman Sachs endocarditis, n (%)	3 (1.2)	0 (0.0)	0 (0.0)	NA	1 (1.8)	0.78	2 (7.4)	0.06
Livedo reticularis, n (%)	7 (2.8)	4 (4.0)	0 (0.0)	0.26	3 (5.3)	1.00	0 (0.0)	0.66
Thrombotic microangiopathy, n (%)	8 (3.2)	4 (4.0)	2 (3.0)	1.00	0 (0.0)	0.32	2 (7.4)	0.82
Catastrophic APS, n (%)	2 (0.8)	0 (0.0)	0 (0.0)	NA	0 (0.0)	NA	2 (7.4)	0.07
Anticoagulant treatment, n (%)	115 (48.5)	8 (8.2)	50 (80.6)	<0.001	34 (64.2)	<0.001	23 (95.8)	<0.001
Antiplatelet therapy, n (%)	80 (33.9)	15 (15.5)	26 (40.0)	0.001	27 (51.9)	<0.001	12 (54.5)	<0.001
Hydroxychloroquine, n (%)	32 (13.3)	8 (8.2)	4 (6.1)	0.84	10 (19.2)	0.09	10 (40.0)	<0.001
Steroids, n (%)	33 (14.2)	14 (14.7)	4 (6.1)	0.14	7 (14.3)	1.000	8 (34.8)	0.06
Death, n (%)	21 (8.3)	3 (3.0)	12 (17.9)	< 0.01	3 (5.2)	0.79	3 (11.1)	0.21
Relapse, n (%)	44 (17.4)	0 (0.0)	16 (23.9)	<0.001	15 (25.9)	<0.001	13 (48.1)	<0.001
Follow-up duration (months, median, IQR)	38.4 (11.5– 106.3)	120.0 (120.0– 120.0)	24.0 (5.0–84.8)	0.16	36.0 (11.4– 48.0)	0.15	48.0 (24.0– 115.0)	0.21

Continued

Table 1 Continued

	All patients N=253	Cluster 1 N=101	Cluster 2 N=67	P value*	Cluster 3 N=58	P value†	Cluster 4 N=27	P value*‡

Qualitative variables and quantitative variables were compared between clusters using Fisher and Kruskal-Wallis tests, respectively.
*Comparison between cluster 1 and cluster 2.
†Comparison between cluster 1 and cluster 3.
‡Comparison between cluster 1 and cluster 4.
aPL, antiphospholipid; APS, antiphospholipid syndrome; dsDNA, double-stranded DNA; HELLP, haemolysis, elevated liver enzymes and low platelet count; LA, lupus anticoagulant; β2GP1, beta-2 glycoprotein 1.

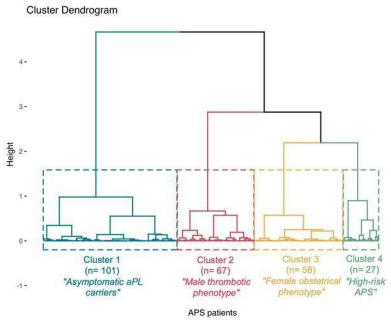

Figure 1 Dendrogram. Hierarchical clustering on principal components analysis of patients with antiphospholipid syndrome (APS) showed four clusters. aPL, antiphospholipid antibodies.

in online supplemental figure S1: cluster 1 'asymptomatic aPL carriers' (n=101, 39.9%) cluster 2 'male thrombotic phenotype' (n=67, 26.5%), cluster 3: 'female obstetrical phenotype' (n=58, 22.9%) and cluster 4 'high-risk APS' (n=27, 50,9%). Figure 2 shows the factorial map of individuals, according to the four clusters obtained.

Cluster analysis results

Cluster 1: 'asymptomatic aPL carriers'

The first cluster was composed of 101 patients, who were mainly women (n=63, 62.4%); the median age was 54 years (IQR 41–62 years). Almost all patients were asymptomatic aPL carriers (n=100, 99.0%; p<0.001 compared with the other clusters) as they did not have any Sydney classification clinical criteria. One patient (1.0%) had a stroke and one (1.0%) had a pulmonary embolism. Triple positivity was found in 10 patients (10.2%), and non-criteria manifestations were found in 31 patients (30.7%), mainly autoimmune cytopenia (n=13, 12.9%). Only 3 patients (3.0%) died, and no patient (0.0%) had any APS event during follow-up.

Cluster 2: 'male thrombotic phenotype'

Cluster 2 was the cluster with the largest number of men (n=36, 53.7%), and had the oldest median age: 58.0 years (IQR 43.5–70.0 years). All patients had a thrombotic phenotype (n=67, 100%, 19 patients (28.4%) with arterial thrombosis and 49 patients (74.2%) with venous thrombosis) and no obstetrical events (n=0.0, 0.0%).

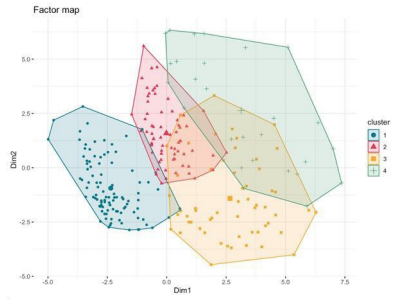

Figure 2 Factor map showing the individuals used to generate the dendrogram. Colours indicate individuals, according to their cluster.

Cluster 2 had more pulmonary embolism than cluster 1, 3 and 4 (pulmonary embolism : n=22, 34.4% vs n=1, 1.0%, n=5, 10% and n=4, 16.0% (p<0.001, p=0.03 and p<0.01, respectively), more deep vein thrombosis than cluster 1 and 3 but not 4: n=33, 50.0% vs n=0, 0.0%, n=14, 28% and n=13, 48.1% (p<0.001, p=0.03 and p=1), respectively). They have more arterial thrombosis (n=19, 28.4%) compared with cluster 1 (n=1 (1.0%), p<0.001) but they had less arterial thrombosis than the cluster 4 (n=15 (55.6%), p=0.003). Non-criteria manifestations were less frequent in this cluster than in the cluster 1 and 4 (n=10, 15.2% vs n=31, 30.7% and n=18, 66.7%, p=0.04 and p<0.001, respectively), but it had the highest number of deaths (n=12, 17.9%), and 16 patients (23.9%) presented an APS event. More details are available in table 1 and online supplemental table S6.

Cluster 3: 'female obstetrical phenotype'

The third cluster was composed exclusively of women (n=58, 100%), with a median age of 48 years (IQR 36–62 years). Among them, 47 (94.0%) had an obstetrical phenotype, with obstetrical adverse events, mainly composed of recurrent unexplained consecutive spontaneous miscarriage before the 10th week (n=13, 39.4%) and unexplained deaths at or beyond the 10th week of gestation (n=8, 25.8%). Almost half of this cluster had a thrombotic phenotype (n=31, 53.4%), with 19 patients (38.8%) presenting an arterial thrombosis, 14 (28.0%) presenting a deep vein thrombosis, and five (10.0%) presenting a pulmonary embolism.

Cluster 4: 'high-risk APS'

The fourth cluster was the youngest, composed of 22 patients (81.5%) with a median age of 41.0 years (IQR 33.5–54.5 years), and mainly comprised female patients (n=22, 81.5%). Triple positivity was highly represented in this cluster (n=23, 85.2%) compared with clusters 1, 2 and 3 (n=10, 10.2%; p<0.001, n=7, 11.1%, p<0.001 and n=7, 12.5%, p<0.001), as were antinuclear antibodies (n=20, 80.0%; p<0.001 vs n=15, 19.0, p<0.001, n=5, 12.8%, p<0.001 and n=18, 39.1%, p=0.01, respectively) and anti-double-stranded deoxyribonucleic acid (anti-dsDNA) antibodies (n=9, 32.0%; p<0.001 vs n=6, 10.3%, p=0.04, n=2, 6.9%, p=0.04 and n=0, 0.0%, p=0.03, respectively). Patients in this cluster also had significantly higher levels of all aPLs and triple positivity (85.2% vs 10.2%, 11.1% and 12.5%, vs clusters 1, 2 and 3 respectively) (table 1 and online supplemental table S6). Almost all patients had a thrombotic phenotype (n=26, 96.3%): 15 patients (55.6%) had arterial thrombosis, mainly stroke and myocardial infarction (n=9, 40.9% and n=4, 18.2%, respectively); and there were a high number of venous thromboembolism events, with 13 patients (48.1%) with deep vein thrombosis and 4 patients (16%) with pulmonary embolism. Cluster four most frequently had non-criteria manifestations (n=18, 66.7%; p<0.001 vs every other cluster) and was the only cluster with catastrophic APS (n=2, 7.4%). This cluster was characterised by the highest APS event rate (n=13, 48.1%; p<0.001 vs cluster 1, p=0.03 vs cluster 2, p=0.07 vs cluster 3), and patients received anticoagulants more frequently than clusters 1 and 3 (n=23, 95.8% vs n=8, 8.2%, p<0.001 and n=34, 64.2%, p<0.01).

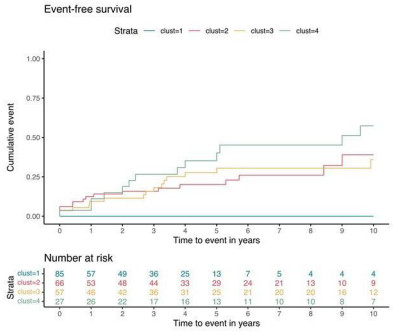

Figure 3 Relapse cumulative incidence curves, according to clusters.

APS event incidence and overall survival

APS event cumulative incidence curves, according to the clusters, are shown in figure 3. Patients from cluster 1 'asymptomatic aPL carriers' did not present any event during follow-up (5-year event incidence rate 0.0%, 95% CI 0.0% to 0.0%), with: 5-year event incidence rate 20.1% (95% CI 9.1% to 29.8%; log-rank test p<0.001) vs 27.6% (95% CI 13.4% to 39.5%; log-rank test p<0.001) and 35.2% (95% CI 13.6% to 51.4%; log-rank test p<0.001), respectively. No significant difference was found between the event incidences of other clusters.

In the cluster 2, 5 patients had an arterial thrombosis, 11 patients had venous thrombosis and no patients experienced an obstetrical event. In the cluster 3, three patients had an arterial thrombosis, eight patients had venous thrombosis and four patients had obstetrical event. Finally, four patients from the cluster 4 had an arterial event whereas seven had a venous thrombosis event and two patients had an obstetrical event. Figure 4 shows the Kaplan-Meier curves of APS event according to the subtype of event in the total population.

Overall survival was not significantly different between the various clusters (shown in online supplemental table S3 and figure S2). More details about individuals' characteristics of patients who experienced an APS event or

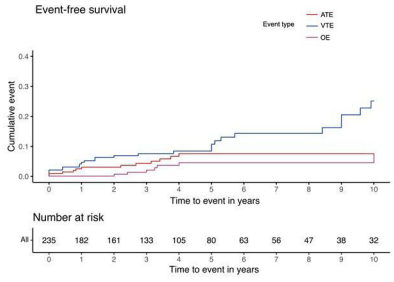

Figure 4 Events incidence according to the type of event. ATE, arterial thrombosis event; OE, obstetrical event; VTE, venous thrombosis event.

death, including causes of death, are available in online supplemental table S4 and S5.

DISCUSSION

In our study, we identified four clusters from a cohort of patients with persistent APS antibodies without any other associated systemic autoimmune disease. The first cluster comprised 'asymptomatic aPL carriers' with a low risk of events during follow-up. The second cluster included 'male thrombotic phenotype', with older patients and more venous thromboembolic events than the other clusters. The third cluster included the 'female obstetrical phenotype', composed exclusively of females with obstetrical adverse events and thrombotic events. The fourth cluster—'high-risk APS'—included younger patients with more frequent triple positivity and arterial events; these patients also had more frequent antinuclear antibodies, anti-dsDNA antibodies and non-criteria manifestations. Regarding survival analyses, asymptomatic aPL carriers had less new APS events than the others, but no other differences in terms of APS events rates or deaths were found between clusters.

Because clustering depends on the variables and profile of the patients included in the analysis,[10] we decided to exclude patients with associated autoimmune diseases that have a known profile, to focus on the exploratory analysis of patients with primary APS or patients with isolated persistent aPL. Compared with primary APS, secondary APS patients have more frequently systemic manifestations such as arthralgia and more severe renal disease with immune complex disease.[11 12] Moreover, recent evidences seem to show important genetical differences between primary and secondary Aps regarding alterations in interferon signature and genes involved in atherosclerotic and inflammatory signalling.[13]

To our knowledge, our study is the first to perform clustering analyses on this restricted population, although a few studies have already investigated clustering analyses among patients with APS with and without associated autoimmune diseases. This study makes it possible to distinguish highly convergent and consistent profiles, from asymptomatic carriers with few non-criteria features and no thrombotic features or other autoimmune laboratory features, through more restricted thrombotic and obstetrical profiles, to mixed APS with triple positivity, associated autoimmune laboratory features and greater use of immunomodulatory drugs.

All previous cluster analyses included all patients with APS, with primary and associated APS without asymptomatic carriers. Thus, Sciascia *et al* performed a cluster analyses on 486 patients with APS (primary and associated APS), and found 5 clusters: one cluster with thrombotic phenotype and triple positivity; one cluster with SLE; one cluster composed of women with pregnancy morbidity; one cluster with asymptomatic aPL carriers; and a 'bridging' cluster between pure primary APS and defined SLE associated with antinuclear antibodies and cytopenia.[8] Another cluster analysis by Zuily *et al* on 497 patients with APS found three different clusters: a cluster with thrombotic events and triple positive patients; another cluster with patients with SLE with non-criteria manifestations; and a final cluster comprising older men with arterial thrombosis and cardiovascular risk factors.[9] Finally, a recent cluster analysis performed on 168 Japanese patients found 3 clusters, in line with the study by Ogata *et al*: one cluster with venous thrombosis and triple positivity; one cluster with arterial thrombosis and cardiovascular risk factors; and one cluster with secondary APS.[7]

Our clusters are concordant with those found by Sciascia *et al*. We found approximately the same clusters in terms of 'male thrombotic phenotype', 'female obstetrical phenotype' and 'asymptomatic aPL carriers'. However, our fourth clusters differ: we did not obtain a mixed SLE/APS cluster, as we excluded patients with SLE, allowing us to detail more precisely a 'high-risk APS' cluster, composed of younger patients with arterial thrombosis, more frequent triple positivity, antinuclear antibodies and non-criteria manifestations, and more prevalent catastrophic APS. Indeed, recent studies showed that antinuclear antibody positivity in primary APS was associated with more prevalent non-criteria manifestations and triple positivity and might be associated with increased relapse rates versus antinuclear antibody-negative patients with APS.[5 14] Cluster 4 also shows a significant proportion of patients with positive dsDNA compared with the others. Though all patients included in our study did not present SLE as we focused on primary APS, we cannot exclude the possibility for those patients to develop SLE after our actual follow-up period. However, we decided to keep these patients so as not to bias the clustering at baseline. In addition to the triple-positive status that is already well known to be associated with worse outcomes in patients with APS,[15–17] the presence of non-criteria manifestation might be associated with an increased risk of relapse.[4 18] Those studies support the hypothesis of a high-risk cluster characterised by a particular clinical and biological profile. Nevertheless, although bivariate comparisons found a significantly higher risk of new APS events in cluster 4 'high-risk APS', survival analyses accounting for time did not find a significant difference. However, our study was probably underpowered to show any difference as this cluster only comprised 27 patients.

Another limit of our study was that we were unable to analyse the course of each cluster over time. The only follow-up information available included new APS event and death rate, and we were unable to determine whether some patients changed cluster over time. In addition, because of the study's retrospective nature, some clinical and biological data may have been underestimated, such as non-criteria manifestations or the provoked or unprovoked nature of venous thrombosis events. We could not clarify the role of traditional cardiovascular risk factors and their treatment on arterial events and how significant the contribution of atherosclerosis was in these

events compared with the thrombotic burden of the aPL, mainly in older men. However, we present individual characteristics of all patients who experienced an APS event in online supplemental table S6, including cardiovascular risk factors and concomitant anticoagulant or antiplatelet drugs at the event occurrence.

Moreover, results obtained from cluster analysis do not always allow a perfect delimitation of the different groups, as we can see in our cluster 1, 'asymptomatic aPL carriers,' which still includes a few symptomatic patients (two subjects with criteria features of APS). Though cluster analyses should be taken with caution and remain exploratory, as there is little consensus on how the number of clusters should be determined, and results may vary depending on the variables used and the study population.[19] For this reason, we used several methods (shown in online supplemental figure S1) to determine the number of clusters that were consistent. Thus, comparing our clusters with several published cluster analyses in the literature on different populations facilitates better understanding of the external validity of these clusters.

CONCLUSIONS

In conclusion, we identified four clusters among patients with primary APS and persistent APS carriers, corresponding to well-defined entities—'asymptomatic aPL carriers', 'male thrombotic phenotype' and 'female obstetrical phenotype'—and a new cluster entitled 'high-risk APS', characterised by triple positivity, non-criteria manifestations and the presence of antinuclear antibodies. Whereas the determinants ensuring that asymptomatic aPL remains asymptomatic need to be better elucidated, a clustering-based treatment strategy should be explored in future prospective studies.

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