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Description of damage in different clusters of patients with antiphospholipid syndrome

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Abstract

Objective: To identify the different clinical phenotypes of antiphospholipid syndrome (APS) by using cluster analysis and describe cumulative damage of disease clusters.
Methods: This retrospective study includes patients with APS (±systemic lupus erythematosus (SLE)). Two-step cluster analysis was applied by considering clinical data. Damage was calculated for all patients by applying damage index for APS (DIAPS).
Results: A total of 237 patients (198 females; median age of 43 years; median follow-up of 9.5 years) were classified into four clusters. Cluster 1 (n = 74) consisted of older patients with arterial-predominant thrombosis, livedo reticularis, and increased cardiovascular risk; cluster 2 (n = 70) of SLE+APS patients with thrombocytopenia and heart valve disease; cluster 3 (n = 59) of patients with venous-predominant thrombosis, less extra-criteria manifestations; and cluster 4 (n = 34) of patients with only pregnancy morbidity with lower frequency of extra-criteria features and cardiovascular risk. Patients with SLE+APS (n = 123) had the highest mean DIAPS. Regarding clusters, 1 and 2 had high cumulative damage. While cumulative survival rates of clusters did not differ, cluster 2 and 3 had lower survival rates at further years. There was no correlation between DIAPS and mortality.
Conclusion: SLE+APS patients with extra-criteria manifestations and older APS patients with arterial thrombosis and increased cardiovascular risk have higher cumulative damage. Effective treatment of SLE disease activity and control of cardiovascular risk may help to reduce cumulative damage in these patients.

Keywords

antiphospholipid syndrome, damage index, cluster analysis, systemic lupus erythematosus, cardiovascular risk

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Introduction

Antiphospholipid syndrome (APS) is an autoimmune disease characterized by vascular thrombosis and/or pregnancy morbidity with the persistent presence of antiphospholipid antibodies (aPL).[1] Beyond vascular thrombosis and pregnancy morbidity, various aPL-related neurologic, cardiac, pulmonary, renal, cutaneous, and hematologic features, referred to as extra-criteria manifestations, have been defined.[2]

Cluster analysis is a statistical technique that aims to identify groups of patients with similar clinical and/or laboratory characteristics. Recently cluster analysis has been used in different APS cohorts to show different disease phenotypes and to compare the frequencies of thrombosis and mortality frequencies between subgroups.[3-5]

Organ damage is defined as "permanent loss of the normal function of an organ system because of a clinical manifestation of the disease."[6] Disease damage has been shown as an essential predictor of survival and a major

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determinant of the quality of life in many studies.[7–10] Identification of the long-term damage in different APS clusters may further improve our understanding of the disease prognosis.

In the Euro-Phospholipid Project where 1000 patients with APS were included, mortality rate was 9.3% with vascular thrombosis ranking first as the most common cause of mortality.[11] In a study by Grika et al.[9] it was shown that APS is a significant cause of morbidity in young individuals as one-third of the patients experienced organ damage during the 10-year follow-up. In patients with systemic lupus erythematosus (SLE), the subgroup with concomitant APS were shown to have a higher frequency of damage and significantly lower survival rates due to thrombotic events.[8,12] A recent study revealed that higher organ damage and presence of APS were associated with increased risk of cardiovascular events and death in patients with SLE.[13]

The Systemic Lupus International Collaborating Clinics (SLICC)/American College of Rheumatology (ACR) damage index (SDI) was developed to assess organ damage in patients with SLE.[14] Although SDI was applied to the patients with APS (±SLE) previously,[9,15] it does not include some thrombotic and extra-criteria manifestations of APS and may underestimate aPL-related damage.[16]

Recently, Amigo et al.[17] developed a damage index for APS (DIAPS) that included 10 organ systems and 37 items and showed content, criterion and construct validity, and strong correlation with EuroQoL, an instrument to measure health-related quality of life (HRQoL),[18] in a cohort of 156 patients with thrombotic APS. In a retrospective study, where researchers analyzed organ damage by DIAPS in 50 patients with primary APS (PAPS) and 50 with SLE+APS it was shown that there was higher baseline damage in PAPS group whilst higher damage accrual in SLE+APS during the 10-year follow-up.[19] In a recent retrospective cross-sectional study that included 84 patients with APS or aPL carriers, DIAPS was independently validated and higher damage was shown in APS (±SLE) and SLE+APS compared to aPL carriers and PAPS, respectively.[20] Another study by Medina et al.[21] showed a negative correlation between damage accrual and HRQoL in a cohort including 67 thrombotic PAPS patients with 15 years of mean follow-up time. A recent analysis by Antiphospholipid Syndrome Alliance for Clinical Trials and International Networking (APS ACTION) of 576 aPL-positives (412 thrombotic and 164 non-thrombotic) with no other systemic autoimmune diseases showed that older age, male gender, hypertension, hyperlipidemia, and obesity were correlated with higher damage in thrombotic PAPS.[22]

Herein, we aimed to identify clinical clusters and describe DIAPS in these clusters in a single center cohort of patients with APS (±SLE).

Methods

Patients

This retrospective study included 237 consecutive APS (±SLE) patients followed up for >1 year in the weekly SLE/APS outpatient clinic of rheumatology unit by a standard protocol between 1982 and 2020. All patients fulfilled Sydney and SLICC classification criteria for APS and SLE, respectively,[1,23] and had complete data regarding demographics, clinical and laboratory characteristics, disease duration, mortality, and damage parameters that were retrieved from the database and revised. Disease duration was defined as the time from the diagnosis of APS to the time of last visit for each patient and as the time from the diagnosis of SLE to the time of last visit for patients who also had SLE. Patients who were not seen in the outpatient clinic within the last 6 months and were non-responsive to telephone calls were searched on the hospital system connected with the national death registration system (NDR). In case of death, cause was extracted from patients' hospital records, NDR, or if unavailable, information obtained from relatives contacted. The study was conducted in accordance with the declaration of Helsinki and was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (approval number: 2021/157). Written informed consent to participate and publish the results was obtained from all patients.

Extra-criteria manifestations retrieved from the database included livedo reticularis, thrombocytopenia, aPL nephropathy and heart valve disease. Diagnosis of livedo reticularis was made by physical examination. Thrombocytopenia was defined as a persistent presence of platelet count of $<100 \times 10^9/mm^3$ and was confirmed by a peripheral blood smear. Regarding aPL nephropathy, only histopathologically confirmed cases were included. Heart valve disease was defined as moderate-severe regurgitation and/or stenosis of mitral and/or aortic valve that were confirmed with echocardiography at our center.[1] Patients with a mean pulmonary artery pressure (mPAP) at resting greater than 20 mmHg detected by right heart catheterization and/or systolic pulmonary artery pressure (sPAP) greater than 35 mmHg detected by echocardiography were considered as having pulmonary hypertension.[24,25]

Cardiovascular risk factors consisting of arterial hypertension, hyperlipidemia, and smoking were obtained from the database and were included in the analysis only if they were present before thrombotic or obstetric events. Arterial hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg on at least 2 occasions[26]; hyperlipidemia as LDL-C ≥160 mg/dL and/or triglyceride ≥175 mg/dL on at least two measurements[27]; and cigarette smoking as being active smoker before thrombotic/obstetric event.

aPL detection and damage assessment

The aPL profile included lupus anticoagulant (LA), anti-cardiolipin (aCL) IgG/IgM and anti-beta-2-glycoprotein I (aβ2GPI) IgG/IgM antibodies and positivity was confirmed at least twice at least 12 weeks apart. The aCL IgG/IgM and aβ2GPI IgG/IgM antibodies were detected by enzyme-linked immunosorbent assay (EUROIMMUN Diagnostics) and positivity threshold was accepted as >40 GPL or MPL units or >99th percentile. LA was measured by aPTT and dilute Russell's viper venom time (dRVVT) assays at the hematology laboratory according to the guidelines of the International Society on Thrombosis and Hemostasis.[28] Before the onset of dRVVT testing, kaolin clotting time was used to measure LA activity by an experienced hematologist. However, all these patients were tested by dRVVT at later years. Triple positive aPL was defined as simultaneous positivity of LA, aCL IgG/IGM, and aβ2GPI IgG/IgM.

The adjusted global antiphospholipid syndrome score (aGAPSS) was calculated as previously defined by adding corresponding points to the risk factors: 3 for hyperlipidemia, 1 for arterial hypertension, 5 for aCL IgG/IgM, 4 for aβ2GPI IgG/IgM, and 4 for LA.[29]

Cumulative damage was calculated by DIAPS at the last visit for all patients and by also SDI for patients with SLE+APS as previously defined.[14,17] Frequencies of each damage item and domain of DIAPS were described for PAPS and SLE+APS groups.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) when normally distributed or as median (range) when not normally distributed and categorical variables were expressed as percentages (%). To identify different clinical phenotypes of the disease, cluster analysis was performed by considering the following variables: age, accompanying SLE, history of thrombosis and pregnancy morbidity, livedo reticularis, thrombocytopenia, aPL nephropathy, heart valve disease, arterial hypertension, hyperlipidemia, smoking, and aGAPSS. Due to the presence of both continuous and categorical variables, we preferred two-step cluster analysis.[30] Two-step cluster analysis is developed from BIRCH algorithm[31] and is suitable for large datasets that contain both categorical and/or continuous variables.[30] First, the objects are assigned to "pre-clusters" to reduce the distance between all possible cases[32] and then the pre-clusters are re-clustered by using hierarchical clustering methods. In the pre-clustering phase, Euclidean distance is used for continuous variables and log-likelihood distance is used for categorical variables. In the second phase, clusters are achieved with the help of a hierarchical clustering algorithm using the log-likelihood based distance measure. To check the quality of the clustering, silhouette measure of cluster cohesion and separation is used. This measure is shown with s (i) and can be calculated as follows: s (i) = [b (i)-a (i)]/max [a (i),b (i)] where a (i) is the average distance of I to the points in its cluster and b (i) is the minimum average distance of i to points in another cluster. Silhouette measure takes values between $-1 \leq 0 \leq 1$ and higher values indicate a better clustering structure. More explicitly, values over 0.5 are considered a sign of reasonable structure and values over 0.7 regarded as an indicator of strong structure.[33]

Since our analysis included categorical variables as well as continuous variables such as age and aGAPSS we used both Euclidean distances and log-likelihood distances. After the clustering phase, differences among clusters were detected either with a one-way ANOVA (followed by a Bonferroni post-hoc test if any differences existed) for continuous variables or with a chi-square test for categorical variables. A two-sided p-value less than 0.05 was considered as statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY).

Results

A total number of 237 patients with APS were included in this analysis. The majority were female (83.5%). The median age, age at diagnosis, and duration of disease for APS were 43 (20–81), 31 (10–67), and 9.5 (1–37.7) years, respectively. Of 237 patients, 114 (48.1%) had PAPS and 123 (51.9%) had SLE+APS. Patients with SLE+APS had a longer duration of APS compared to patients with PAPS (median 10.9 (1–32.8) vs 7.9 [1–37.7], $p = 0.003$).

When patients were grouped according to clinical manifestations, 120 (50.6%) had vascular thrombosis only, 46 (19.4%) had pregnancy morbidity only, and 71 (30%) had both. Of 191 patients who experienced thrombotic events, 77 (40.3%) had arterial thrombosis, 80 (41.8%) had venous thrombosis, 32 (16.8%) had both, and 2 (1%) had small vessel thrombosis. Seventy-eight (40.8%) patients with thrombosis had a history of recurrence. Frequency of any thrombosis, arterial thrombosis, venous thrombosis, and recurrence rate did not differ between PAPS and SLE+APS groups (80.7% vs 80.5%, $p = 0.55$; 45.6% vs 46.3%, $p = 0.51$; 49.1% vs 45.5%, $p = 0.37$ and 45.7% vs 36.4%, $p = 0.24$, respectively).

Cluster analysis resulted with an acceptable level of silhouette measure (0.55) and 237 patients were classified into 4 clusters. Cluster 1 ($n = 74$) consisted predominantly of older patients with arterial vascular thrombosis, livedo reticularis and cardiovascular risk factors. Cluster 2 ($n = 70$) consisted predominantly of patients with concomitant SLE, thrombocytopenia and heart valve disease. Cluster 3 was composed mainly of patients with venous vascular

Table 1. Demographic, clinical and laboratory characteristics of clusters.

Variable	All (n = 237)	Cluster 1 (n = 74)	Cluster 2 (n = 70)	Cluster 3 (n = 59)	Cluster 4 (n = 34)	p
Age (years), median (range)	43 (20–81)	**51 (20–81)**	40 (27–72)	42 (24–69)	40.5 (26–65)	**<0.001**
Duration of APS (years), median (range)	9.5 (1–37.7)	**13.1 (1–37.7)**	10.4 (1–28.7)	8.5 (1–32.8)	7 (1–22.4)	**0.028**
Duration of SLE (years), median (range)	12 (1–35.7)	**15 (1.8–35.7)**	11.2 (1–28.3)	9.5 (1–32.8)	11.9 (1.6–16)	**0.042**
Female, *n* (%)	198 (83.5)	**56 (75.7)**	61 (87.1)	47 (79.7)	**34 (100)**	**<0.05**
SLE, *n* (%)	123 (51.9)	31 (41.9)	**46 (65.7)**	32 (54.2)	14 (41.2)	**<0.05**
Vascular thrombosis, *n* (%)	191 (80.6)	**73 (98.6)**	59 (84.3)	**59 (100)**	0 (0)	**<0.001**
Arterial thrombosis, *n* (%)	109 (46)	**50 (67.6)**	31 (44.3)	28 (47.5)	0 (0)	**<0.001**
Venous thrombosis, *n* (%)	112 (47.3)	36 (48.6)	37 (52.9)	**39 (66.1)**	0 (0)	**<0.001**
Pregnancy morbidity, *n* (%)	117 (49.4)	22 (29.7)	46 (65.7)	15 (25.4)	**34 (100)**	**<0.001**
Livedo reticularis, *n* (%)	38 (16)	**21 (28.4)**	10 (14.3)	5 (8.5)	2 (5.9)	**<0.01**
Thrombocytopenia, *n* (%)	81 (34.2)	4 (5.4)	**65 (92.9)**	4 (6.8)	8 (23.5)	**<0.001**
aPL nephropathy, *n* (%)	12 (5.1)	3 (4.1)	6 (8.6)	3 (5.1)	0 (0)	0.29
Heart valve disease, *n* (%)	92 (38.8)	32 (43.2)	**46 (65.7)**	8 (13.6)	6 (17.6)	**<0.001**
Arterial hypertension, *n* (%)	101 (42.6)	**49 (66.2)**	34 (48.6)	18 (30.5)	0 (0)	**<0.001**
Diabetes mellitus, *n* (%)	6 (2.5)	2 (2.7)	3 (4.3)	0 (0)	1 (2.9)	0.48
Ischemic heart disease, *n* (%)	14 (7.3)	6 (8.2)	5 (8.5)	3 (5.1)	0 (0)	0.87
Hyperlipidemia, *n* (%)	103 (43.5)	**69 (93.2)**	26 (37.1)	0 (0)	8 (23.5)	**<0.001**
Smoking, *n* (%)	58 (24.5)	**31 (41.9)**	7 (10)	17 (28.8)	3 (8.8)	**<0.001**
LA, *n* (%)	156 (65.8)	53 (71.6)	48 (68.6)	35 (59.3)	20 (58.8)	0.36
aCL IgG/IgM, *n* (%)	155 (65.4)	46 (62.2)	46 (65.7)	38 (64.4)	25 (73.5)	0.71
aβ2GPI IgG/IgM, *n* (%)	93 (39.2)	25 (33.8)	33 (47.1)	26 (44.1)	9 (26.5)	0.13
Triple aPL positivity, *n* (%)	45 (19)	12 (16.2)	16 (22.9)	13 (22)	4 (11.8)	0.46
aGAPSS, mean ± SD.	9.2 ± 3.7	10.2 ± 3.4	10.3 ± 3.5	**7.3 ± 3.5**	8.00 ± 3.3	**<0.001**

LA: lupus anticoagulant, aCL: anticardiolipin, aβ2GPI: anti-β2-glycoprotein I, aGAPSS: adjusted global antiphospholipid syndrome score.

Table 2. Comparison of damage index for APS damage domains between PAPS and SLE+APS.

	PAPS, *n* (%)	SLE/APS, *n* (%)	p
Peripheral vascular	43 (37.7)	52 (42.3)	0.28
Pulmonary	24 (21.1)	33 (26.8)	0.18
Cardiovascular	47 (41.2)	60 (48.8)	0.15
Neuropsychiatric	35 (30.7)	36 (29.3)	0.46
Ophthalmologic	1 (0.9)	2 (1.6)	0.52
Renal	5 (4.4)	14 (11.4)	0.07
Musculoskeletal—avascular necrosis	0 (0)	7 (5.7)	**0.009**
Cutaneous—chronic cutaneous ulcers	2 (1.8)	6 (4.9)	0.16
Gastrointestinal	10 (8.8)	6 (4.9)	0.17
Endocrine	3 (2.6)	0 (0)	0.11

thrombosis (n = 59). The frequency of any extra-criteria manifestations (30.5%) and mean aGAPSS was significantly low in this group. Cluster 4 (n = 34) consisted only of patients with pregnancy morbidity (no thrombosis). Extra-criteria features and cardiovascular risk factors were scarce in this cluster. aPL profiles did not differ between clusters. Demographic, clinical and laboratory characteristics of clusters are summarized in Table 1

The mean DIAPS of the cohort was 1.90 ± 1.56. SLE+APS group had higher mean DIAPS compared to patients with PAPS (2.10 ± 1.61 vs 1.69 ± 1.47, p = 0.046). Cardiovascular domain was the most frequently involved DIAPS domain followed by peripheral vascular and neuropsychiatric domains. Persistent proteinuria and avascular necrosis were significantly more frequent in SLE+APS compared to PAPS (9.8 vs 2.2%, p = 0.02 and 5.7 vs 0%,

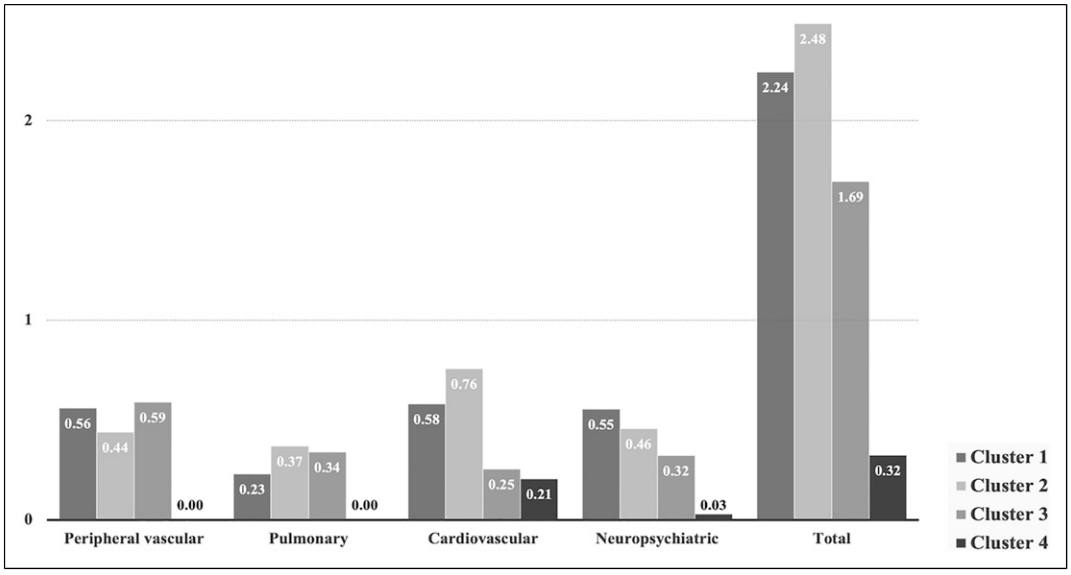

Figure 1. Mean DIAPS values of the clusters. Major domains of the DIAPS were presented as separate bar graphs. DIAPS: damage index for APS

$p = 0.009$, respectively) Table 2 depicts the comparison of DIAPS domains between patients with PAPS and with SLE+APS. DIAPS was positively correlated with disease duration of both APS (r = 0.192, $p = 0.003$) and SLE (r = 0.219, $p = 0.015$). The mean SDI of the SLE+APS patients was 2.12 ± 1.77 and SDI was positively correlated with disease duration of both APS (r = 0.182, $p = 0.044$) and SLE (r = 0.257, $p = 0.004$) in this group. Duration of disease for both APS and SLE were longer in cluster 1 (median 13.1 (1–37.7) and 15 (1.8–35.7) years, respectively) compared to other clusters ($p = 0.042$). While both DIAPS and SDI were positively correlated with disease duration of SLE in cluster 2 (r = 0.327, $p=0.027$ and r = 0.372, $p = 0.011$, respectively) and cluster 3 (r = 0.439, $p = 0.012$ and r = 0.552, $p = 0.001$, respectively), no correlation was shown in cluster 1 (r = −0.278, $p = 0.13$ and r=−0.316, $p = 0.083$, respectively) and cluster 4 (r = 0.454, $p = 0.103$ and r = 0.420, $p = 0.134$, respectively).

Our analysis showed that cluster 2 had the highest cumulative damage (mean DIAPS 2.48 ± 1.67) followed by clusters 1 (2.24 ± 1.44), 3 (1.69 ± 1.27), and 4 (0.32 ± 0.68). Frequencies of patients with damage (DIAPS ≥1) were similar in clusters 1, 2 and 3 whereas cluster 4 had significantly lower frequency of damage (94.6%, 94.3%, 86.4%, and 23.5%, $p < 0.001$). When patients with SLE+APS were analyzed separately, distribution of patients with SDI ≥1 in the clusters was similar (93.5%, 87%, 87.5%, and 21.4%, $p < 0.001$). Regarding domains, cardiovascular and pulmonary damage were more frequent in cluster 2 whereas peripheral vascular in cluster 3 and neuropsychiatric in cluster 1 (Figure 1).

Twenty-four patients died during a follow-up of 38 years and mortality rate was 1.06 per 100 patient-years. Eight of those patients were included in cluster 3, 8 in cluster 2, 6 in cluster 1, and 2 were in cluster 4. Leading cause of death was thrombotic complications with myocardial infarction ($n = 5$) ranking first followed by pulmonary hypertension secondary to pulmonary embolism (n=4), stroke (n=1) and liver failure secondary to Budd-Chiari syndrome ($n = 1$). Eight patients were lost due to infections. These patients had SLE+APS and were on immunosuppressive treatment including high dose steroids. There were 3 patients who died because of intracranial hemorrhage due to uncontrolled warfarin use. Data regarding the cause of death could not be collected in 3 patients. Kaplan–Meier analysis revealed that cumulative survival rates of clusters did not differ significantly albeit clusters 2 and 3 had a tendency for lower survival rates (91.9%, 88.6%, 86.4%, and 94.1% in clusters 1, 2, 3, and 4, respectively; $p = 0.076$). (Figure 2). No correlation was shown between DIAPS and death (r = 0.123, $p = 0.058$ for all patients; r = 0.088, $p = 0.46$ for cluster 1; r = 0.138, $p = 0.25$ for cluster 2; r = 0.056, $p = 0.67$ for cluster 3; and r = 0.065, $p = 0.71$ for cluster 4). When only patients with SLE+APS were evaluated, neither SDI nor DIAPS correlated with death (r = −0.071, $p = 0.43$ and r = 0.056, $p = 0.54$, respectively).

Discussion

Antiphospholipid syndrome is a heterogeneous disease with various clinical and laboratory manifestations. Regardless of its inability to provide a deep insight, clustering may help to recognize different disease subtypes, develop clinical

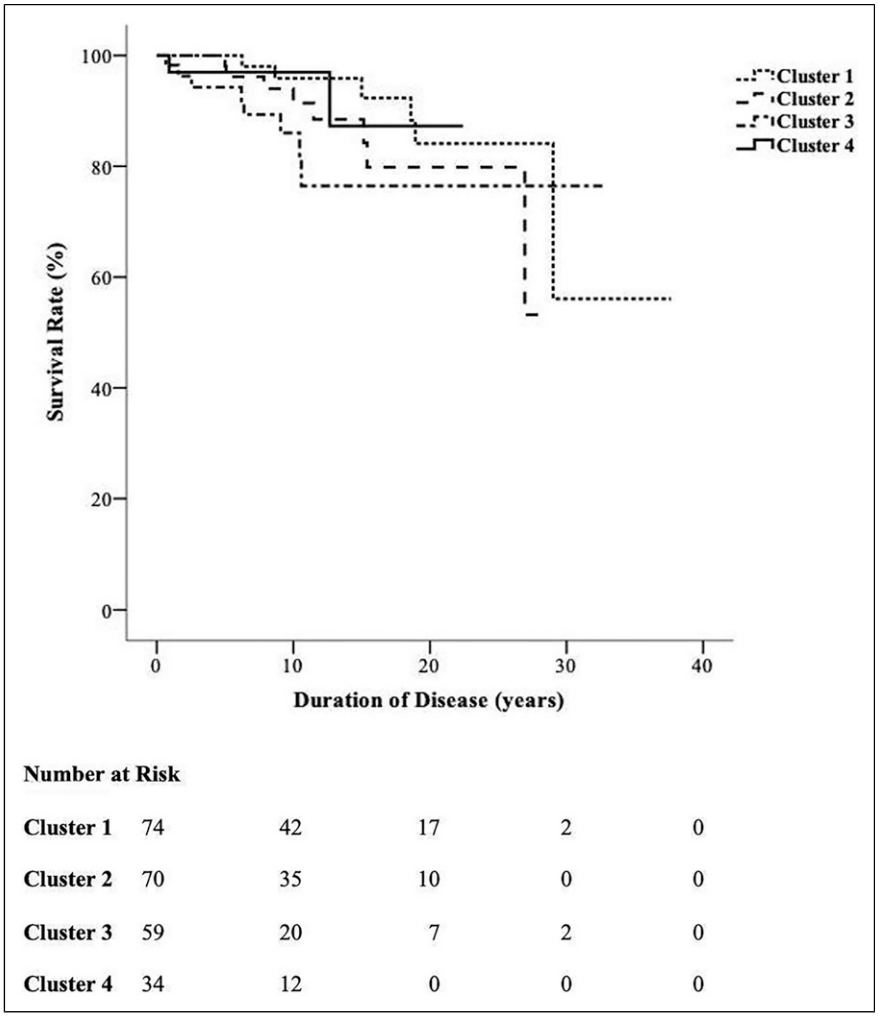

Figure 2. Kaplan–Meier estimates of cumulative survival in clusters. Cumulative survival in cluster 1, 2, 3 and 4 was 91.9%, 88.6%, 86.4%, and 94.1%, respectively (p = 0.076).

approaches accordingly and shape the expectations for the follow-up.

Recently 3 different cluster analyses have been conducted in APS cohorts. Sciascia et al.[3] identified 5 disease clusters according to clinical and laboratory characteristics: thrombotic APS with triple aPL positivity; SLE+APS with anti-dsDNA positivity; obstetric APS; APS with cytopenia (especially thrombocytopenia), and asymptomatic aPL carriers. In another study, a cluster of patients with arterial thrombosis and cardiovascular risk factors, a cluster with SLE+APS and a cluster with venous thrombosis and triple aPL positivity were identified.[4] Frequency of thrombotic events and mortality rate were significantly higher in the first cluster. Cluster analysis of the APS ACTION Registry revealed three different disease phenotypes: female PAPS patients with venous thromboembolism and triple aPL positivity; female SLE/APS patients with venous thromboembolism and extra-criteria manifestations; older male patients with arterial thrombosis and cardiovascular risk factors.[5]

Our two-step cluster analysis revealed 4 clusters. Cluster 1 consisted predominantly of older patients with arterial thrombosis and cardiovascular risk factors, cluster 2 of patients with SLE+APS with extra-criteria manifestations, cluster 3 of patients with venous thrombosis and cluster 4 of females with only obstetric APS. This clustering was based on clinical data and did not include serological characteristics. aGAPSS of patients with arterial thrombosis, namely, cluster 1, was significantly higher compared to aGAPSS of patients with venous thrombosis, namely, cluster 3. As aPL profile was not different between clusters, this difference in comparison reflects the high prevalence of cardiovascular risk factors in cluster 1. Atherosclerotic plaque rupture is the main triggering factor for arterial thrombosis in APS. aPL binding to circulating oxidized—low density lipoprotein (oxLDL) and β2GPI complexes enhances their phagocytosis

by macrophages and accelerates atherosclerosis.[34,35] Conventional cardiovascular risk factors further accelerate the atherogenesis and increase the risk of arterial thrombosis in APS.[36] Andrade et al.[37] have shown that PAPS patients with arterial and/or venous thrombosis and without cardiovascular risk factors do not have premature atherosclerosis. Therefore, clusters 1 and 3 may reflect two different thrombotic APS phenotypes with similar aPL profiles. Corporation of new biomarkers showing endothelial dysfunction and a wider spectrum of non-criteria aPL may be beneficial to differentiate the phenotype with arterial thrombosis in the presence of cardiovascular risk factors and the phenotype with immune-mediated venous thrombosis where cardiovascular risk factors do not seem to play a significant role.

Damage is the most important parameter to determine prognosis and it may be very pronounced in the setting of arterial/venous occlusions where obstruction to the vascular flow impairs the function of the affected organ/tissue as is the case with vascular APS. We found higher cumulative damage by DIAPS in patients with SLE+APS compared to PAPS. In a previous study, while patients with PAPS had higher baseline damage, damage accrual was higher in SLE+APS group during the 10-year follow-up.[19] In a recent retrospective cross-sectional study, patients with SLE+APS also had higher cumulative damage compared to those with PAPS.[20] Longer disease duration, higher frequency of lupus major organ involvement and higher immunosuppressive usage, especially of corticosteroids, may have contributed to higher damage in this group.[7] Compatible with this, in our study, persistent proteinuria as a result of lupus nephritis associated damage and avascular necrosis, mainly a steroid-attributable damage, were the only two damage items that were significantly more frequent in SLE+APS group. SLE+APS patients also had higher disease duration of APS compared to those with PAPS.

In previous studies, most frequently affected DIAPS domains were reported as peripheral vascular[19,22] and neuropsychiatric[20,21] whereas in our cohort, due to high prevalence of heart valve disease, cardiovascular domain ranked the highest (38.8% of the cohort). Diagnosis of heart valve disease was made by transthoracic echocardiography (and transesophageal echocardiography, if necessary) according to Miyakis et al.[1] in our cohort. Different studies have reported heart valve disease prevalence ranging from 14% to 86% in patients with aPL (+) SLE and 30%–82% in patients with PAPS.[38]

Cluster 2 where predominantly SLE patients with thrombocytopenia and heart valve involvement resided, displayed the highest damage score. In our previous cluster work in patients with SLE, we found that patients with aPL positivity and thrombocytopenia had the highest damage rate and worst survival compared to others.[12] Patients in this cluster had significantly a higher frequency of neuropsychiatric manifestations and experienced significantly more thrombotic events. The contribution of thrombocytopenia to damage may be the result of higher cumulative dose of corticosteroids used to treat it or the multisystemic active disease that thrombocytopenia was a part of.

In cluster 1, increased damage was mainly driven by longer disease duration of both APS and SLE, increased thrombotic central nervous system involvement, and heart valve disease. In a recent analysis of APS ACTION registry presented at ACR 2021, older age, male gender, hypertension, hyperlipidemia, and obesity were found to be correlated with higher damage in thrombotic PAPS.[22] In this study hypertension and hyperlipidemia were correlated with damage also in non-thrombotic aPL-positives. Compatible with this study, patients in our cluster 1 where older patients with high damage resided, a higher frequency of hypertension, and hyperlipidemia were found. Despite a high prevalence of peripheral vascular damage in cluster 3, the lower frequency of extra-criteria manifestations lowered overall damage scores in these patients compared to patients in clusters 1 and 2.

Since DIAPS was not developed to evaluate the chronic damage associated with obstetrical impact, cluster 4 consisting only of patients with pregnancy morbidity had significantly lower cumulative damage. Considering the data that suggests obstetric and vascular APS may be different variants of the syndrome, better tools to reflect damage in patients with isolated pregnancy morbidity are needed.

During the follow-up, 24 patients died and cumulative survival rates of clusters did not differ. However, beyond 10 years cluster 3 and 20 years clusters 1 and 2 displayed lower survival rates. Majority of deaths were secondary to thrombosis followed by infections and hemorrhage. Eleven patients with thrombotic events died including four that had pulmonary hypertension secondary to pulmonary embolism and five patients with myocardial infarction. Cervera et al.[11] reported that in a cohort of 1000 patients with APS, mortality rate was 9.3% and 36.5% of total deaths was due to thrombotic events followed by infections (26.9%) and hemorrhages (10.7%). Among these thrombotic events, 14% suffered from pulmonary emboli and 38% from myocardial infarction. In our study, cluster 3 that had a lower survival rate consisted predominantly of patients with venous thrombosis had a higher frequency of pulmonary emboli leading to pulmonary hypertension. Since anticoagulation is the standard treatment of APS and immunosuppressives are widely used especially in patients with concomitant SLE, hemorrhagic and infectious complications are substantially common in the course of the disease. Recently Ajeganova et al.[13] have shown that higher SDI and history of APS were associated with increased risk of cardiovascular events and death in patients with SLE. In our study we found no correlation between mortality and DIAPS in patients with APS. SDI was also not correlated with

death in SLE+APS patients. It is possible that more reliable conclusions regarding the association of damage and mortality could be drawn if data reflecting the change in damage over time was available. Furthermore, taking treatment-related complications such as hemorrhage into consideration may improve the performance of the index in predicting mortality.

Our study has some limitations. Since DIAPS was evaluated cumulatively in a patient population with a variable follow-up time due to the retrospective design, we could not observe the progression of damage over time. Also, data on treatment which may account for damage accrual in patients with APS and SLE was missing. Therefore, a prospective study with a long follow-up period and consecutive damage assessments with the inclusion of cumulative dose of immunosuppressive medication, especially of corticosteroids, would better reflect the correlation of damage with mortality and could have a stronger clinical interpretation. It is also important to consider that SLE patients included in the clusters may affect comparison of damage due to longer disease duration. As mentioned earlier, higher damage in cluster 1 may be associated with longer duration of SLE. However, analyses to show correlation between damage and duration of SLE in each cluster did not show any correlation in clusters 1 and 4.

Despite our efforts to include all published relevant criteria and extra-criteria features of APS, demographic data and cardiovascular risk factors into cluster analysis, it is still possible that adding different variables could increase the performance of the analysis in distinguishing disease phenotypes. Finally, considering the distribution of clinical manifestations in our cohort, despite some significant differences, it is hard to say patients with APS form exclusive clusters as patients with SLE, a disease characterized by many autoantibodies and clinical features. Importantly antiphospholipid antibody profile was not able to make a distinction between the clusters. Whether extending aPL profile to include non-criteria antibodies as well would cause a significant change in clusters is unknown.

In conclusion, the cluster consisting of SLE+APS patients with extra-criteria manifestations had the highest damage and worst survival. Considering underlying SLE as the potential cause, effective treatment of SLE disease activity may help to reduce cumulative damage in this subgroup. Controlling cardiovascular risk may help to lessen organ damage in patients especially with arterial thrombosis and cardiovascular risk factors. As pulmonary emboli causing pulmonary hypertension is the leading cause of death in patients with venous thrombosis, it is important to assess cardiopulmonary function of these patients periodically. DIAPS is the only index developed to evaluate damage in APS. Although it seems to work well, it may still be possible to improve its efficacy by the inclusion of some other parameters like treatment-related complications and possibly new biomarkers causing and showing damage, respectively.

Author Contributions

BA.E. designed the study. Ö.U. and E.G. collected whole data and Ç.Ç. and M.B helped them. E. Ç, Ö.U. and BA.E. carried out data analysis and interpretation. Ö.U. wrote the first draft, BA.E. critically revised it and both shaped the final version. All authors gave intellectual advice, read and approved the final version of the manuscript.

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References

1. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4: 295–306.
2. Sciascia S, Amigo M-C, Roccatello D, et al. Diagnosing antiphospholipid syndrome: 'extra-criteria' manifestations and technical advances. *Nat Rev Rheumatol* 2017; 13: 548–560.
3. Sciascia S, Radin M, Cecchi I, et al. Identifying phenotypes of patients with antiphospholipid antibodies: results from a cluster analysis in a large cohort of patients. *Rheumatology* 2021; 60(3): 1106–1113.
4. Ogata Y, Fujieda Y, Sugawara M, et al. Morbidity and mortality in antiphospholipid syndrome based on cluster analysis: a 10-year longitudinal cohort study. *Rheumatology* 2021; 60(3): 1331–1337.
5. Zuily S, Clerc-Urmès I, Bauman C, et al. Cluster analysis for the identification of clinical phenotypes among antiphospholipid antibody-positive patients from the APS ACTION Registry. *Lupus* 2020: 29(11): 1353–1363.
6. Erkan D, Yazici Y, Sobel R, et al. Primary antiphospholipid syndrome: functional outcome after 10 years. *J Rheumatology* 2000; 27: 2817–2821.
7. Sutton EJ, Davidson JE and Bruce IN. The systemic lupus international collaborating clinics (SLICC) damage index: a systematic literature review. *Semin Arthritis Rheum* 2013; 43: 352–361.

8. Ruiz-Irastorza G, Egurbide M-V, Ugalde J, et al. High impact of antiphospholipid syndrome on irreversible organ damage and survival of patients with systemic lupus erythematosus. *Arch Intern Med* 2004; 164: 77–82.
9. Grika EP, Ziakas PD, Zintzaras E, et al. Morbidity, mortality, and organ damage in patients with antiphospholipid syndrome. *J Rheumatol* 2012; 39: 516–523.
10. Alba P, Gómez-Puerta JA, Goycochea-Robles MV, et al. Organ Damage and Quality of Life in Antiphospholipid Syndrome. *Curr Rheumatol Rep* 2016; 18: 7.
11. Cervera R, Serrano R, Pons-Estel GJ, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis* 2015; 74: 1011–1018.
12. Artim-Esen B, Çene E, Şahinkaya Y, et al. Cluster analysis of autoantibodies in 852 patients with systemic lupus erythematosus from a single center. *J Rheumatol* 2014; 41: 1304–1310.
13. Ajeganova S, Hafström I and Frostegård J. Patients with SLE have higher risk of cardiovascular events and mortality in comparison with controls with the same levels of traditional risk factors and intima-media measures, which is related to accumulated disease damage and antiphospholipid syndrome: a case-control study over 10 years. *Lupus Sci Med* 2021; 8: e000454.
14. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. *Arthritis Rheum* 1996; 39: 363–369.
15. Barbhaiya M, Erkan D, Rodriguez-Almaraz E, et al. Utility of the systemic lupus international collaborating clinics (SLICC)/American College of Rheumatology (ACR) Damage index for antiphospholipid antibody (aPL) positive patients. [abstract]. *Arthritis Rheum* 2011; 63(Suppl 10): 7.
16. Barbhaiya M and Erkan D. The optimal tool for assessment of organ damage in antiphospholipid syndrome. *J Rheumatol* 2013; 40(1): 89.
17. Amigo M-C, Goycochea-Robles MV, Espinosa-Cuervo G, et al. Development and initial validation of a damage index (DIAPS) in patients with thrombotic antiphospholipid syndrome (APS). *Lupus* 2015; 24: 927–934.
18. EuroQol Group. EuroQol–a new facility for the measurement of health-related quality of life. *Health Policy (Amsterdam, Netherlands)* 1990; 16: 199–208.
19. Torricelli AK, Ugolini-Lopes MR, Bonfá E, et al. Antiphospholipid syndrome damage index (DIAPS): distinct long-term kinetic in primary antiphospholipid syndrome and antiphospholipid syndrome related to systemic lupus erythematosus. *Lupus* 2020; 29: 256–262.
20. Radin M, Foddai SG, Cecchi I, et al. Quality of life in patients with antiphospholipid antibodies differs according to antiphospholipid syndrome damage index (DIAPS). *Eur J Intern Med* 2021; 92: 134–136.
21. Medina G, Cimé Aké EA, Vera-Lastra O, et al. Damage index for antiphospholipid syndrome during long term follow-up: correlation between organ damage accrual and quality of life. *Lupus* 2021; 30: 96–102.
22. Balbi G, Ahmadzadeh Y, Tektonidou M, et al. Damage accrual measured by DIAPS in antiphospholipid antibody (aPL)-positive Patients: results from antiphospholipid syndrome alliance for clinical trials and international networking (APS ACTION) clinical database and repository ("registry") [abstract]. *Arthritis Rheumatol* 2021; 73(suppl 10).
23. Petri M, Orbai AM, Alarcón GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheumatism* 2012; 64: 2677–2686.
24. Simonneau G, Montani D, Celermajer DS, et al. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019; 53: 1801913.
25. Rudski LG, Lai WW, Afilalo J, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American society of echocardiography. *J Am Soc Echocardiography* 2010; 23(7): 685–713.
26. Williams B, Mancia G, Spiering W, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart Journal* 2018; 39: 3021–3104.
27. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2019; 73: e285–e350.
28. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. *J Thromb Haemost* 2009; 7: 1737–1740.
29. Sciascia S, Sanna G, Murru V, et al. The global antiphospholipid syndrome score in primary APS. *Rheumatology* 2015; 54: 134–138.
30. Hsu C-C, Chen C-L and Su Y-W. Hierarchical clustering of mixed data based on distance hierarchy. *Inf Sci* 2007; 177: 4474–4492.
31. Zhang T, Ramakrishnan R and Livny M. BIRCH: an efficient data clustering method for very large databases. *SIGMOD* 1996: 25(2): 96.
32. Budayan C, Dikmen I and Birgonul MT. Comparing the performance of traditional cluster analysis, self-organizing maps and fuzzy C-means method for strategic grouping. *Expert Syst Appl* 2009; 36: 11772–11781.
33. Kaufman L and Rousseeuw PJ. *Finding groups in data: an introduction to cluster analysis*. John Wiley & Sons, New York, 2009.
34. Matsuura E. and Lopez L. R.. Are Oxidized LDL/β2-glycoprotein I complexes pathogenic antigens in autoimmune-mediated atherosclerosis? *Clin Developmental Immunol* 2004; 11: 103–111.

35. Hasunuma Y, Matsuura E, Makita Z, et al. Involvement of beta2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. *Clin Exp Immunol* 1997; 107: 569–573.
36. Di Minno MND, Scalera A, Tufano A, et al. The association of adjusted Global AntiphosPholipid Syndrome Score (aGAPSS) with cardiovascular disease in subjects with antiphospholipid antibodies. *Atherosclerosis* 2018; 278: 60–65.
37. Andrade D, Bortolotto L, Bonfá E, et al. Primary antiphospholipid syndrome: absence of premature atherosclerosis in patients without traditional coronary artery disease risk factors. *Lupus* 2016; 25: 472–478.
38. Cervera R, Tektonidou M, Espinosa G, et al. Task force on catastrophic antiphospholipid syndrome (APS) and non-criteria APS manifestations (I): catastrophic APS, APS nephropathy and heart valve lesions. *Lupus* 2011; 20: 165–173.