Cluster Analysis of Autoantibodies in 852 Patients with Systemic Lupus Erythematosus from a Single Center

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ABSTRACT. ***Objective.*** Associations between autoantibodies and clinical features have been described in systemic lupus erythematosus (SLE). Herein, we aimed to define autoantibody clusters and their clinical correlations in a large cohort of patients with SLE.

Methods. We analyzed 852 patients with SLE who attended our clinic. Seven autoantibodies were selected for cluster analysis: anti-DNA, anti-Sm, anti-RNP, anticardiolipin (aCL) immunoglobulin (Ig)G or IgM, lupus anticoagulant (LAC), anti-Ro, and anti-La. Two-step clustering and Kaplan-Meier survival analyses were used.

Results. Five clusters were identified. A cluster consisted of patients with only anti-dsDNA antibodies, a cluster of anti-Sm and anti-RNP, a cluster of aCL IgG/M and LAC, and a cluster of anti-Ro and anti-La antibodies. Analysis revealed 1 more cluster that consisted of patients who did not belong to any of the clusters formed by antibodies chosen for cluster analysis. Sm/RNP cluster had significantly higher incidence of pulmonary hypertension and Raynaud phenomenon. DsDNA cluster had the highest incidence of renal involvement. In the aCL/LAC cluster, there were significantly more patients with neuropsychiatric involvement, antiphospholipid syndrome, autoimmune hemolytic anemia, and thrombocytopenia. According to the Systemic Lupus International Collaborating Clinics damage index, the highest frequency of damage was in the aCL/LAC cluster. Comparison of 10 and 20 years survival showed reduced survival in the aCL/LAC cluster.

Conclusion. This study supports the existence of autoantibody clusters with distinct clinical features in SLE and shows that forming clinical subsets according to autoantibody clusters may be useful in predicting the outcome of the disease. Autoantibody clusters in SLE may exhibit differences according to the clinical setting or population. (First Release May 15 2014; J Rheumatol 2014;41:1304–10; doi:10.3899/jrheum.130984)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS | CLUSTER | AUTOANTIBODIES
DISEASE PATTERN | DAMAGE | SURVIVAL

Systemic lupus erythematosus (SLE) is an autoimmune disease with a diversity of antibodies. Some of those antibodies have established associations with disease manifestations and thus diagnostic importance, and play pathogenic roles in various tissues. Because of heterogeneous course and outcome, there have been different approaches to identify subsets of patients with SLE and different disease patterns. Clustering based on autoantibody profile is one of those and it has been the subject of some previous studies[1,2,3,4,5,6,7]. In those studies, it has been shown that autoantibodies tend to occur in clusters and are associated with clinical subsets in SLE. The most remarkable of these associations are anti-dsDNA with renal disorder[8], anti-Ro and anti-La with sicca symptoms[3], anti-RNP with Raynaud phenomenon (RP)[9], and lupus anticoagulant (LAC) and anticardiolipin antibodies (aCL) with thromboembolic events[10].

Herein, we aimed to define autoantibody clusters and their correlations with clinical characteristics and prognosis in a large cohort of patients with SLE from a single center.

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MATERIALS AND METHODS

Patients. Records of 852 patients with SLE admitted to our clinic between January 1980 and May 2010 were studied. Patients fulfilled at least 4 of the American College of Rheumatology (ACR) criteria for SLE and had sufficient clinical and laboratory data with a complete autoantibody profile. All patients registered after 1991 were followed up using a standard protocol in the weekly SLE clinic. The protocol consisted of data on demographic characteristics, SLE classification criteria, mortality, autoantibody profile,

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treatment history, antiphospholipid syndrome (APS; classification criteria[11]), features of nephritis including histopathology when available, and Systemic Lupus International Collaborating Clinics (SLICC) damage index. The SLICC damage index reflected the patients' last visits. Duration of disease was defined as the time from the diagnosis of SLE to the time of the last visit and duration of followup was defined as the time from the first to the last visit of the patient at our SLE outpatient clinic. Patients were classified as lost to followup if they were not seen in the outpatient clinic for more than 6 months, could not be contacted by repeated phone calls, and no information was available on their medical condition.

For this study, demographic characteristics, cumulative clinical features, autoantibody profiles, damage, and mortality data were retrieved from the database.

Autoantibodies. Seven autoantibodies were selected for cluster analysis to identify subsets of patients with SLE and similar autoantibody patterns: anti-dsDNA, anti-Sm, anti-RNP, anticardiolipin IgG or IgM (aCL IgG/M), LAC, anti-Ro, and anti-La. All antibodies were tested at the Immunology Laboratory, Istanbul University, in a routine clinical setting. Immunoblotting was used to detect anti-Sm, RNP, Ro, La, and aCL IgG/M (EUROIMMUN Diagnostics), and anti-dsDNA was detected by using immunofluorescence microscopy with *Crithidia luciliae* (INOVA Diagnostics). Positivity was confirmed at least twice and results reflect the cumulative data for each patient. A positive test result for aCL was defined as IgM > 40 U/ml and/or IgG > 40 U/ml. LAC was measured by kaolin clotting time and/or dilute Russell's viper venom time assays in the hematology laboratory.

Statistical analysis. All statistical analyses were performed using SPSS version 16. Because the number of cases in the cohort was high, 2-step cluster analysis procedure was conducted over the chosen 7 antibodies. Two-step cluster analysis is developed from BIRCH algorithm[12] and is suitable for large datasets that contain both categorical and/or continuous variables[13]. First, the objects are assigned to "preclusters" and then the preclusters are clustered by using hierarchical clustering methods. The goal of preclustering is to reduce the distance between all possible cases[14]. At the preclustering phase, the 2-step algorithm uses Euclidean distance for continuous variables and log-likelihood distance for discrete 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 with:

$$s(i)= [b(i)-a(i)]/\max[a(i),b(i)]$$

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 $-1 \le 0 \le 1$ 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[15].

Because the entire antibody variables were in the binary form, we chose log-likelihood distance for the distance measure in our cohort. Different numbers of clusters were tried. Considering the satisfactory silhouette measure (0.6) and the match of the cluster characteristics with the initial expectations, we chose 5 clusters for final analysis. To determine whether there were any significant differences between the clusters, the 1-way ANOVA was used for continuous variables and the chi-square test was used for categorical variables. P values ≤ 0.05 were considered significant. Posthoc comparisons were made using the Tukey-Kramer test. The probability of survival of the 5 clusters of patients was determined by using the Kaplan-Meier method.

RESULTS

The total number of patients in this cohort was 852. Eighty-seven percent of the patients were female. The mean age at diagnosis was 31 ± 12.5 years (range: 4–72), the mean duration of disease and followup were 115 ± 85.9 months (1-600) and 86.5 ± 78.7 (1–412), respectively. The frequencies of the selected autoantibodies in the cohort were as follows: anti-dsDNA 70.5% (n = 601), anti-Sm 19.1% (n = 163), anti-RNP 15.4% (n = 131), anti-Ro 24.1% (n = 208), anti-La 10.7% (n = 91), LAC 10.8% (n = 92), and aCL IgG or M 29% (n = 247).

Five clusters were identified by cluster analysis as shown in Table 1.

Cluster 1 consisted of patients who did not belong to any of the clusters formed by autoantibodies selected for analysis. Cluster 2 was the anti-dsDNA-only cluster, cluster 3 consisted predominantly of anti-Sm and anti-RNP, cluster 4 of aCL IgG/M and LAC, and cluster 5 of anti-Ro and anti-La autoantibodies. Clusters 3, 4, and 5 also had anti-dsDNA antibodies besides the dominant antibodies, cluster 4 with the highest frequency of occurrence. Basic demographic characteristics of the clusters are shown in Table 2, and clinical and laboratory features are shown in Table 3.

Comparison of clinical and laboratory characteristics in 5 clusters revealed that cluster 2 (dsDNA) had the highest incidence of renal involvement and cluster 5 (Ro/La) had the lowest (44%, 54%, 33%, 44%, and 26% in clusters 1, 2, 3, 4, and 5, respectively; $p < 0.001$). In cluster 4 (aCL/LAC), there were more patients with neuropsychiatric manifestations such as seizures and psychosis, and comparison of difference was statistically significant for psychosis (8% vs 2%, 3%, 3%, 1%; $p = 0.002$ in clusters 1, 2, 3, and 5, respectively). Patients in cluster 4 experienced significantly more arterial (24.6%) and/or venous (18.9%) thrombotic events (vs 3%, 6%, 6%, 8%; $p < 0.001$ for arterial; 2%, 3%, 4%, 8%; $p < 0.001$ for venous thrombotic events in clusters 1, 2, 3, and 5, respectively). Autoimmune hemolytic anemia (AIHA, 17%) and thrombocytopenia (36%) were significantly more frequent in cluster 4 (vs 4%, 9%, 14%, 6%; $p = 0.003$ for AIHA; 24%, 21%, 25%, 18%; $p = 0.001$ for thrombocytopenia in clusters 1, 2, 3, and 5, respectively). There was a higher frequency of RP in cluster 3 (Sm/RNP) compared with other clusters (39% vs 25%, 23%, 21%, 24%; $p = 0.001$ in clusters 1, 2, 4, and 5, respectively, all shown in Table 3).

According to the SLICC Damage Index, the highest occurrence of overall damage was in cluster 4 (aCL/LAC; 63% vs 42%, 43%, 45%, 36% in clusters 1, 2, 3, and 5, respectively; $p < 0.001$). The separate analysis of SLICC damage items showed that damage due to arterial and/or venous thrombotic events was significantly high in cluster 4 (aCL/LAC). These patients had more neuropsychiatric damage due to cerebrovascular accident (16.6% vs 3%, 4%, 4%, 7%; $p < 0.001$ in clusters 1, 2, 3, 5, respectively) and cognitive impairment (8% vs 1%, 3%, 3%, 2%; $p = 0.021$ in

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Table 1. Frequency of autoantibodies in clusters, n (%).

Autoantibody	Cluster 1, n = 83	Cluster 2, n = 271	Cluster 3, n = 170	Cluster 4, n = 175	Cluster 5, n = 153
Anti-dsDNA	0 (0.0)	271 (100)*	91 (53.5)	130 (74.3)	109 (71.2)
Anti-Sm	0 (0.0)	0 (0.0)	159 (93.5)*	3 (1.7)	1 (0.65)
aCL IgG/IgM	0 (0.0)	0 (0.0)	52 (30.5)	162 (92.5)*	33 (21.5)
LAC	0 (0.0)	0 (0.0)	16 (9.4)	65 (36.5)*	11 (7.18)
Anti-RNP	0 (0.0)	0 (0.0)	131 (100.0)*	0 (0.0)	0 (0.0)
Anti-Ro	0 (0.0)	0 (0.0)	53 (31.1)	2 (1.1)	153 (98)*
Anti-La	0 (0.0)	0 (0.0)	20 (11.8)	1 (0.57)	70 (45.7)*

*Significantly different from the 4 other clusters, p < 0.001. Ig: immunoglobulin; LAC: lupus anticoagulant; aCL: anticardiolipin antibody.

Table 2. Comparison of baseline features according to autoantibody cluster.

Characteristic	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	p
Age at diagnosis, yrs, mean ± SD	32.4 ± 11.1	30.5 ± 12.6	31.1 ± 13.0	29.7 ± 12.1	33.4 ± 12.8	0.079
Disease duration, mos, mean ± SD	137.78 ± 103.2*	122.9 ± 85.6	100.7 ± 80.8	120.9 ± 81.5	102.1 ± 83.6	0.001
Duration of followup, mos, mean ± SD	105.3 ± 106.41	89.1 ± 74.5	80.9 ± 79.2	88.7 ± 72.8	76.5 ± 73.2	0.072
Female sex, n (%)	69 (83.1)	229 (84.8)	143 (84.1)	153 (87.4)	145 (94.7)*	0.021
Lost to followup, n (%)	14 (16.8)	30 (11.1)	16 (9.4)	20 (11.4)	13 (8.5)	0.355

*Significantly different from the other 4 clusters.

Table 3. Comparison of clinical and laboratory features according to autoantibody cluster, n (%).

Characteristic	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	p
Malar rash	40 (48.2)	129 (47.8)	93 (54.7)	83 (47.4)	69 (45.1)	0.122
Discoid rash	11 (13.3)	17 (6.3)	14 (8.2)	9 (5.1)	7 (4.6)	0.090
Photosensitivity	51 (61.4)	157 (58.1)	100 (58.8)	99 (56.6)	82 (53.6)	0.788
Oral ulcer	13 (15.7)	40 (14.8)	30 (17.6)	33 (18.9)	13 (8.5)	0.092
Arthritis	52 (62.7)	200 (74.1)	128 (75.3)	118 (67.4)	111 (72.5)	0.148
Pericarditis	7 (8.4)	37 (13.7)	25 (14.7)	30 (17.1)	19 (12.4)	0.406
Pleuritis	9 (10.8)	47 (17.4)	30 (17.6)	37 (21.1)	30 (19.6)	0.354
Renal	37 (44.6)	146 (54.1)	57 (33.5)	78 (44.6)	40 (26.1)*	< 0.001
Leukopenia	33 (39.8)	118 (43.9)	78 (45.9)	72 (41.4)	76 (49.7)	0.513
Lymphopenia	51 (61.4)	172 (63.9)	105 (61.8)	105 (60.3)	93 (60.8)	0.946
AIHA	4 (4.8)	25 (9.3)	24 (14.1)	30 (17.2)*	10 (6.5)	0.003
Thrombocytopenia	20 (24.1)	59 (21.9)	43 (25.3)	64 (36.8)*	28 (18.3)	0.001
Seizure	3 (3.6)	10 (3.7)	4 (2.4)	14 (8.0)	5 (3.3)	0.080
Psychosis	2 (2.4)	8 (3.0)	5 (2.9)	15 (8.6)*	1 (0.7)	0.002
Raynaud phenomenon	21 (25.3)	62 (23.3)	67 (39.6)*	37 (21.5)	39 (25.8)	0.001
Arterial thrombosis	3 (3.6)	16 (5.9)	10 (5.9)	43 (24.6)*	12 (7.8)	< 0.001
Venous thrombosis	2 (2.4)	8 (3.0)	7 (4.1)	33 (18.9)*	13 (8.5)	< 0.001
APS	0 (0)	0 (0)	19 (11.2)	82 (47)	20 (13.1)	< 0.001

*Significantly different from the other 4 clusters. AIHA: autoimmune hemolytic anemia; APS: antiphospholipid syndrome.

clusters 1, 2, 3, 5, respectively). Renal damage was more frequent in clusters 2 (dsDNA; 11%) and 4 (aCL/LAC; 12% vs 6%, 5%, 3%; p = 0.009 in clusters 1, 3, and 5, respectively) with incidence of endstage renal disease highest in cluster 2 (dsDNA; 7%) and reduced glomerular filtration rate in cluster 4 (aCL/LAC; 11% vs 3%, 1%, 4%, 0%, 7%; p = 0.004 for endstage renal disease in clusters 1, 3, 4, and 5, respectively and vs 6%, 9%, 3%, 3%; p = 0.009 in clusters 1, 2, 3, 5, respectively). Peripheral vascular damage was prominent in cluster 4 (aCL/LAC) and though not statistically significant, the proportion of patients with heart valve damage was higher in this group. Pulmonary hypertension was more common in cluster 3 (Sm/RNP) when compared with other clusters (12% vs 9%, 4%, 8%, 7%; p = 0.018 in clusters 1, 2, 4, and 5, respectively, Table 4).

Kaplan-Meier survival analysis comparison of 10 years

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Table 4. Comparison of organ damage according to clusters, n (%).

Characteristic	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	p
Overall damage	35 (42.1)	116 (43.1)	77 (45.2)	110 (63)*	56 (36.6)	< 0.001
Cataract	5 (6.0)	14 (5.2)	7 (4.1)	14 (8.0)	10 (6.5)	0.606
Cognitive impairment	1 (1.2)	8 (3.0)	5 (2.9)	14 (8.0)*	4 (2.6)	0.021
Seizure	0 (0.0)	5 (1.9)	3 (1.8)	9 (5.1)	4 (2.6)	0.089
Cerebrovascular accident	3 (3.6)	11 (4.1)	7 (4.1)	29 (16.6)*	11 (7.2)	< 0.001
Cranial/peripheral neuropathy	4 (4.8)	13 (4.8)	9 (5.3)	5 (2.9)	2 (1.3)	0.291
Reduced GFR	5 (6.0)	24 (8.9)	5 (2.9)	19 (10.9)*	5 (3.3)	0.009
Proteinuria	2 (2.4)	16 (5.9)	7 (4.1)	10 (5.7)	2 (1.3)	0.195
Endstage renal disease	3 (3.6)	19 (7.1)*	2 (1.2)	8 (4.6)	1 (0.7)	0.004
Pulmonary hypertension	8 (9.6)	11 (4.1)	22 (12.9)*	14 (8.0)	11 (7.2)	0.018
Pulmonary fibrosis	0 (0.0)	1 (0.4)*	7 (4.1)	7 (4.0)	3 (2.0)	0.017
Angina/CABG	0 (0.0)	2 (0.7)	3 (1.8)	1 (0.6)	1 (0.7)	0.601
Myocardial infarction	2 (2.4)	5 (1.9)	4 (2.4)	6 (3.4)	4 (2.6)	0.893
Cardiomyopathy	0 (0.0)	3 (1.1)	2 (1.2)	5 (2.9)	1 (0.7)	0.286
Valvular heart disease	4 (4.8)	17 (6.3)	15 (8.8)	21 (12.0)	11 (7.2)	0.180
Peripheral vascular	1 (1.2)	4 (1.5)	9 (5.3)	11 (6.4)*	3 (2.0)	0.019
Gastrointestinal	0 (0.0)	0 (0.0)	1 (0.6)	1 (0.6)	0 (0.0)	0.569
Osteoporotic fracture	1 (1.2)	7 (2.6)	3 (1.8)	5 (2.9)	1 (0.7)	0.581
Avascular necrosis	11 (13.3)	25 (9.3)	12 (7.1)	19 (10.9)	15 (9.8)	0.577
Skin	9 (10.8)*	10 (3.7)	11 (6.5)	5 (2.9)	3 (2.0)	0.011
Premature gonadal failure	2 (2.4)	11 (4.1)	5 (2.9)	17 (9.8)*	2 (1.3)	0.002
Malignancy	1 (1.2)	7 (2.6)	4 (2.4)	8 (4.6)	2 (1.3)	0.355

*Significantly different from the other 4 clusters. GFR: glomerular filtration rate; CABG: coronary artery bypass graft.

(95%, 92.9%, 98.3%, 85.4%, 97.3%) and 20 (95%, 87.3%, 98.3%, 74.7%, 88.3%) in the 5 clusters showed reduced survival in cluster 4 (p = 0.022; Figure 1).

DISCUSSION

SLE has a wide spectrum of clinical presentations that may lead to different clinical courses and outcome. Previous

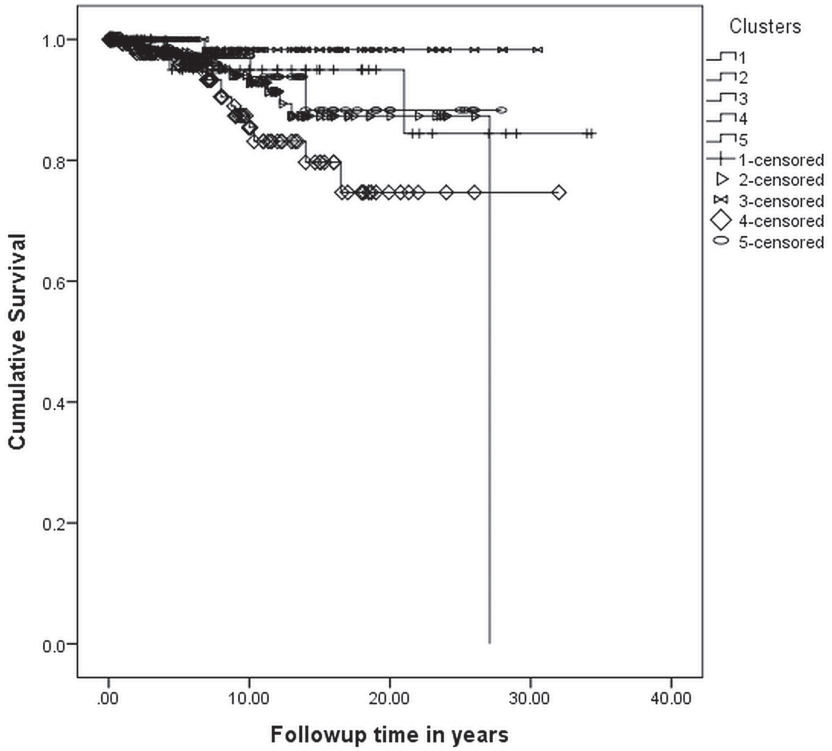

Figure 1. Kaplan-Meier survival curve for clusters.

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reports[1,2,3,4,5,6,7] have shown that autoantibodies, one of the main features of the disease, may aid in recognizing different clinical patterns. Clusters of autoantibodies and their associations with clinical features have been studied in a small number of large cohorts[1,2,6].

In a cohort of 1357 patients, To and Petri reported 3 clusters of autoantibodies with anti-Sm/RNP, anti-dsDNA/Ro/La, and anti-dsDNA/LAC/aCL antibodies and described clinical differences between clusters[6]. The anti-Sm/RNP cluster had the lowest incidence of renal manifestations and anti-dsDNA/Ro/La cluster had the highest. The cluster with anti-dsDNA/LAC/aCL was associated with neurologic and thrombotic manifestations. They found strong associations between the anti-dsDNA/LAC/aCL cluster and damage, such as cerebrovascular events, neuropathy, and venous thrombosis. Hoffman, *et al* identified 5 clusters with anti-Sm/RNP, Ro/La, ribosomal P, histone, and ds-DNA antibodies[3]. In a study from south China, patients with SLE were classified into 3 clusters: anti-Ro/Sm/RNP, anti-Ro, and no anti-extractable nuclear antigen (ENA). In this analysis, there was no difference in the prevalence of anti-dsDNA antibodies and renal disease between the clusters. The anti-Ro/Sm/RNP cluster had a high frequency of cutaneous manifestations[1]. Another study from south China revealed 3 clusters. A cluster consisted of patients with mucocutaneous manifestations and arthritis; a cluster of renal and hematologic findings, as well as pulmonary and gastrointestinal involvement; and a third cluster showed a heterogeneous presentation where a clear distinction from others was not possible[16].

In our study, the analysis revealed 5 different clusters according to the selected autoantibodies. Cluster 1 consisted of patients who lacked selected autoantibodies, but satisfied the ACR SLE classification criteria and were all antinuclear antibody (ANA)-positive. With the exception of cluster 2, which consisted solely of anti-dsDNA antibodies, all 3 other clusters had a considerably higher frequency of anti-dsDNA autoantibodies and a predominant second autoantibody family, i.e., anti-Sm and anti-RNP in cluster 3, aCL IgG/M and LAC in cluster 4, and anti-Ro and anti-La in cluster 5.

Cluster 2, the cluster with only anti-dsDNA positivity in the absence of other autoantibodies, had the highest risk for renal involvement and carried a high risk for renal damage. In previous studies where this association was shown, anti-dsDNA antibodies were accompanied by other autoantibodies[1,6,17]. In a study of patients with pediatric onset SLE, where 3 clusters were described, contrary to our data, the cluster with only anti-dsDNA antibodies was found to be associated with a milder form of the disease and infrequent major organ involvement[17]. This discrepancy might be related to the lower incidence of anti-dsDNA antibodies in this cluster compared with other clusters where anti-dsDNA antibodies accompanied the dominant autoantibody[17].

Anti-Sm and anti-RNP antibodies dominated cluster 3 in our cohort. Anti-Sm antibodies are detected in 5% to 30% of patients with SLE and are nearly always accompanied by anti-RNP antibodies[18,19]. Despite their diagnostic importance, the clinical significance and pathogenic role of anti-Sm antibodies is not clear. In a cohort of 91 patients with SLE, cluster analysis revealed that an absence of antibodies to ENA increased the risk of SLE nephropathy[20]. The authors suggested that anti-Sm/RNP antibodies might have an association with the absence or a relatively benign form of SLE nephropathy. Hoffman, *et al*[3] reported a low prevalence of urine cellular casts in patients with Sm/RNP antibodies and To and Petri[6] have observed that Sm/RNP cluster had the lowest incidence of renal manifestations. Sm/RNP cluster was found to be associated with pulmonary hypertension and Raynaud phenomenon in our cohort. In a recent study, 12 out of 93 (13%) patients with SLE were found to have an elevated systolic pulmonary artery pressure and consistent with our findings, they had significantly higher occurrence of anti-Sm and antiphospholipid antibodies (aPL)[21]. Of note, this cluster had a high positivity for aPL as well. In a previous study, where we investigated the characteristics of patients with SLE and pulmonary hypertension, we found that aPL positivity and RP were significantly higher in this patient group[22]. Hoffman, *et al* have also reported that patients with at least 1 of the antibodies of anti-Sm/RNP pair had a higher risk for RP[3].

In our study, we confirmed the findings of previous studies by showing that the cluster with aPL dominance (cluster 4) was significantly associated with central nervous system manifestations, vascular thrombosis, and overall disease damage[6,23,24,25,26,27,28]. Neuropsychiatric SLE occurs as a result of multiple mechanisms, including neuronal or cerebrovascular injury mediated by aPL or other antibodies[29]. This cluster also displayed a high incidence of thrombocytopenia and AIHA. Thrombocytopenia has been reported frequently with aPL, and there are a considerable number of studies showing a positive correlation between AIHA and aPL and increased risk for thromboembolism in patients with AIHA[30,31,32,33].

Cluster 5 (anti-Ro and anti-La) did not show a distinctive characteristic despite frequent major organ involvement and organ damage. We could not demonstrate any significant associations of this cluster with cutaneous lesions, as was previously reported[34].

A subset of patients bearing none of our selected autoantibodies but with ANA positivity populated cluster 1. Despite the lack of a significant association, these patients had a considerably higher frequency of organ involvement. This finding may question the utility of clustering; however, the autoantibodies that have not been analyzed and are present in this group of patients should also be considered.

Few studies have reported survival rates of different clusters. One from South China showed that the cluster with renal and hematologic manifestations had a higher stand-

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ardized mortality ratio (SMR) compared with other 2 clusters, one of which had a significantly higher prevalence of mucocutaneous findings and the other showed heterogeneous characteristics and no clear distinctions could be made from the cluster with the highest SMR[16]. Comparison of survival between our clusters showed that survival at 20 years was significantly reduced in cluster 4 (aCL IgG/M, LAC). This cluster displayed the highest overall damage rate. The most striking differences were in vascular and neuropsychiatric domains followed by renal. This finding is in concordance with previous studies that have shown that the SLICC damage index is predictive of mortality in patients with SLE[35,36]. Additionally, APS has been shown to be a predictor of mortality in patients with SLE owing to thrombosis and organ damage, with neurologic damage playing the leading role[37,38]. It has also been shown that aPL are predictive of early damage in SLE[39]. To and Petri have reported that the cluster with anti-dsDNA/LAC/aCL was the only cluster associated with damage such as cerebrovascular events, vascular thrombosis, and neuropathy[6].

Although the data originate from a dedicated center, this study has limitations to consider. Our hospital is a reference hospital and the patient cohort includes a disproportionally higher number of severe cases. The analysis is based on the cumulative findings and progression of clinical features and the antibody profile by time cannot be traced. Clustering procedure has been applied over the selected antibodies with a real-life approach, and other possible autoantibody combinations and potentially pathogenic autoantibodies such as anti-C1q or antinucleosome antibodies cannot be tested in all patients over the years. Some differences between the clusters might be due to different pathogenic properties of the heterogeneous subgroups of the same autoantibody tested (e.g., anti-dsDNA antibodies).

We report the application of clustering approach, to our knowledge for the first time, to the largest SLE cohort from Turkey with a significant longterm followup. Our results confirm that autoantibodies may occur in clusters in SLE. We identified subsets of patients with SLE and different autoantibodies and clinical phenotypes displaying differences in organ involvement and damage. We identified a cluster with aPL positivity and decreased survival and another cluster with anti-Sm/RNP associated with RP and pulmonary arterial hypertension. We also found that a subgroup of patients exists who have SLE without selected autoantibodies but with ANA positivity, with significant organ involvement. It is evident that we need better tools for predicting outcome in patients with SLE, but although imperfect, tests for autoantibodies are widely available and a profile of autoantibodies can still be useful for predicting outcomes in the clinic.

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