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Antibody Clustering in Systemic lupus Erythematosus and their clinical correlates

Allam,N. [1]; Gaber,W. [2]; Helaly M.[3]

1,2 : Department of Rheumatology and Rehabilitation, Faculty of Medicine, Cairo University, Cairo, Egypt
3 : El-Galaa Military Family Hospital, Rheumatology Department, Cairo, Egypt.

Corresponding author: Dr Helaly M. e-mail: dr.marwahelaly@gmail.com

Abstract: The aim of the present work was to study the presence of lupus autoantibodies in clusters and their associations with clinical features and organ damage in Egyptian patients with systemic lupus erythematosus. ***Methods*:** A cross-sectional study included 150 SLE patients. All patients had full systemic examination with routine laboratory and immunological profile testing (ANA, Anti-dsDNA, anti-Sm, anti Ro/SSA, anti La/SSB, anti-RNP, Lupus anticoagulant and ACL (IgG and IgM) antibodies). In addition, items on Systemic Lupus International Collaborating Committee/ American College of Rheumatology damage index (SLICC/ACR damage Index) were calculated. ***Results*:** Patients in cluster-1 (DNA/RNP/Sm) were distinguished by a younger mean age at diagnosis and a statistically significant higher prevalence of pleurisy and nephritis (mainly class IV), a higher renal damage and a higher mean cumulative dose of cyclophosphamide. Patients in cluster-2 (Ro/La) had a statistically significant higher prevalence of sicca manifestations, arthritis, discoid rash, photosensitivity, a higher dermatologic damage. ***Conclusion*:** this study supports the existence of lupus autoantibodies in clusters with distinct clinical features, allowing the prediction of subsequent disease course and organ damage and may help direct the treatment and predict the prognosis of SLE patients.

Keywords: SLE, autoantibodies, clustering.

1. Introduction

Systemic lupus erythematosus is a chronic autoimmune disease that has protean manifestations and follows a relapsing and remitting course. More than 90% of cases of SLE occur in women, frequently starting at childbearing age. It can affect almost any organ system; thus, its presentation and course are highly variable, ranging from indolent to fulminate. The disease course is characterized by exacerbations and remissions, with the development of new organ manifestations or progression of existing manifestations [1].

Over one hundred sixteen autoantibodies were described in SLE patients. These include autoantibodies that target nuclear antigens, cytoplasmic antigens, cell membrane antigens, phospholipid-associated antigens, blood cells, endothelial cells, and nervous system antigens, plasma proteins, matrix proteins, and miscellaneous antigens [2]

Even though the presence of antibodies in SLE has been known for more than 60 years, still nowadays a great effort is being made to understand the pathogenic, diagnostic and prognostic meaning of such antibodies. Earlier studies have shown that individual autoantibodies have some association with the clinical features of patients with systemic lupus erythematosus [3].The correlation of specific autoantibodies with certain clinical findings may help direct the treatment and predict the prognosis of SLE patients [4].

Autoantibody clustering can help us to differentiate between various subsets of SLE, allowing the prediction of subsequent disease course and organ damage [5].It is now thought that SLE can be divided into more homogeneous subsets with pathogenic, therapeutic, or prognostic significance [6].

Thus the aim of the study was to examine the presence of lupus autoantibodies in clusters and their associations with

clinical features and organ damage in Egyptian patients with systemic lupus erythematosus.

2. Patients and Methods

It is a cross-sectional study done on one hundred and fifty Egyptian patients (141 female and 9 male) diagnosed as having SLE from the inpatient and outpatient clinics of El-Galaa Military Family Hospital will be included in this study. The study was approved by the local ethics committee and the principles of the Declaration of Helsinki were observed and informed consents were obtained from all subjects.

All patients had a definite diagnosis of SLE and fulfilled at least 4 of the 11 ACR criteria for classification and diagnosis of SLE [7].The exclusion criterion was positive antibodies for hepatitis C virus. Systemic work up was done and included: full clinical examination, routine laboratory investigations i.e. complete blood count (CBC), acute phase reactants, liver and kidney function tests, 24 hours urinary proteins, urine analysis and the immune profile including ANA, Anti-dsDNA, anti-Sm ,anti Ro/SSA, anti La/SSB ,anti-RNP, Lupus anticoagulant and ACL (IgG and IgM) antibodies, and Systemic Lupus International Collaborating Committee/ American College of Rheumatology damage index (SLICC/ACR damage Index) were calculated for each patient [8].

Statistical analysis

K-means cluster analysis (nonhierarchical clustering or Quick Cluster; SPSS version 10 software; SPSS, Chicago, IL) was used to identify groups of SLE patients with similar autoantibody patterns. Briefly, this first involves defining a disease metric with which to quantify the degree of similarity between autoantibody patterns in 2 patients. We used Euclidian distance (the square root of the sums of squared differences between patients with respect to each autoantibody). The initial centers for the clusters are chosen in a first pass of data, and patients are assigned to the closest center. Next, the cluster centers are recalculated based on the patients in the cluster and the patients are reassigned. This iterative process continues until the clusters' means do not shift more than a given cutoff value or until the iteration limit is reached [9].The conventional chi-square test was used to compare categorical variables. *P* values less than 0.05 were considered significant. All statistical analyses were performed using SPSS for Windows version 7.0.

3. Results

This study was conducted on 150 Egyptian patients diagnosed as SLE; one hundred forty one (94%) patients of them were females while 9 (6%) patients were males with a female to male ratio of (15.6:1).The age ranged between 15 - 61 years with a mean of 31.7 ± 10.6 years and disease duration ranged between 1 - 23 years with a mean of 8 ± 4.7 years. All patients were taking Hydroxychloroquine (HCQ) at a mean dose of 310±26.5 mg/day, 27.3% had Azathioprine at a mean dose of 105±33 mg/day, 23.3% had Mycophenolatemofitil (MMF) at a mean dose of 1260±245 mg/day, 30% had Methotraxate (MTX) at a mean dose of 14±5.3 mg/week and 24% had cyclosporine A at a mean dose of 93±42 mg/day. The mean cumulative dose of glucocorticoids taken by patients was 29.25±6.52 gm and for the Cyclophosphamide it was 2.6±2.3 gm.

The clinical and laboratory features of all patients are represented in (Table 1, 2). The most prevalent lupus manifestations were arthralgia (94%), constitutional manifestation and renal manifestations (82% for each). Organic brain syndrome was the least frequent manifestation among the studied patients (0.7%).

Table (1): Clinical manifestations of the SLE patients:

	No. (150)	%
Arthralgia	141	94
Constitutional manifestation	123	82
Nephritis	123	82
Alopecia	105	70
Arthritis	97	64.7
Cognitive dysfunction	94	62.7
Photosensitivity	89	59.3
Leucopenia	77	51.3
Raynauds phenomenon	72	48
Malar rash	65	43.3
Pleurisy	58	38.7
Lupus headache	55	36.7
Thrombocytopenia	54	36
Sicca manifestations	42	28
Livedo reticularis	39	26
Pericarditis	37	24.7
Oral ulcers	25	16.7
DVT	18	12
Hemolytic anemia	11	7.3
Seizures	11	7.3
AVN	10	6.7
Pulmonary hypertension	9	6
Peripheral neuropathy	8	5.3
Discoid rash	6	4
Myocarditis	5	3.3
Psychosis	2	1.3
Organic brain syndrome	1	0.7

DVT: deep venous thrombosis, AVN: avascular necrosis

Table (2): Immunological manifestations of the SLE patients:

	No. (150)	%
Anti ds-DNA	84	56
Lupus anticoagulant	57	38
aCL-IgM	54	36
aCL-IgG	57	38
Anti-Sm	53	35.3
Anti-Ro	42	28
Anti-RNP	33	22
Anti-La	12	8

Patients were then analyzed using the K-means cluster procedure, based on these 7 autoantibodies. The resulting autoantibody profiles revealed that 96.7% of the cases segregated into one of two distinct clusters defined by autoantibodies against DNA/RNP/Sm or Ro/La auto-antigens.

Ninety seven patients (64.7%) were assigned to cluster-1 (DNA/RNP/Sm) and 48 patients (32%) patients were assigned to cluster-2 (Ro/La). 5 patients (3.3%) did not fit into any of the clusters. The frequency of the specific autoantibodies in each cluster is shown in (Table 3).

Table (3): Frequency of specific autoantibodies in both clusters:

Autoantibody	Cluster-1 DNA/RNP/Sm n=97 (%)	Cluster-2 Ro/La n=48 (%)	*P-value*
ds-DNA	77 (79.4)	7 (14.6)	< 0.001**
Sm	43 (44.3)	10 (20.8)	0.006*
RNP	29 (29.9)	4 (8.3)	0.003*
Ro	12 (12.3)	30 (62.5)	< 0.001**
La	0	12 (25)	< 0.001**
LAC	40 (39)	12 (25)	0.08
aCL	32 (33)	11 (23)	0.07

*Values are significantly different. **Values are strongly significantly different .

Comparing the demographic and clinical features between both clusters (table 4); it was found that patients in cluster-1 (DNA/RNP/Sm) were distinguished by a younger mean age at diagnosis and a statistically significant higher prevalence of pleurisy and nephritis. Patients in cluster-2 (Ro/La) had a statistically significant higher prevalence of sicca manifestations, arthritis, discoid rash and photosensitivity.

Table (4): Demographic and clinical features in both clusters

Clinical feature	Cluster-1 (DNA/RNP/Sm) n=97	Cluster-2 (Ro/La) n=48	*P-value*
Age at diagnosis	21.9 ± 12	27.1 ± 23.3	< 0.001**
Disease duration	9 ± 2.2	6 ± 5.1	0.1
Photosensitivity	48 (49.5%)	41 (85.4%)	0.04*
Discoid rash	1 (1%)	5 (10.4%)	0.01*
Malar rash	35 (36%)	30 (62.5%)	0.07
Sicca manifestations	17 (17.5%)	25 (52.1%)	<0.001**
Arthritis	52 (53.6%)	45 (93.7%)	0.03*
pericarditis	30 (30.9%)	7 (14.6%)	0.09
Pleurisy	49 (50.5%)	9 (18.7%)	0.01*
Leucopenia (<4000/mm^3)	57 (58.7%)	20 (41.6%)	0.3
Thrombocytopenia (<150x10^3/mm^3)	38 (39.2%)	16 (33.3%)	0.6
Hemolytic anemia	10 (10.3%)	1 (2%)	0.2
Proteinuria (>0.5 g/day)	72 (74.2%)	16 (33.3%)	0.01*
Urinary cellular casts	76 (78.4%)	11 (22.9%)	< 0.001**
Neuro-psychiactric	9 (9.3%)	2 (4.5%)	0.5

*Values are significantly different. **Values are strongly significantly different.

The two clusters were then compared for the severity of damage using the lupus damage index (SLICC/ACR damage index). Cluster-1(DNA/RNP/Sm) patients had a higher renal damage while patients of cluster-2 (Ro/La) showed a higher dermatologic damage (table-5). Comparing patients of cluster-1 (DNA/RNP/Sm) and cluster-2 (Ro/La) regarding mean dose of immunosuppressive drugs there was no statistically significant difference.

Table (5): Comparison of organ damage according to autoantibody cluster:

Variable	Cluster-1 (DNA/RNP/Sm) n=97 %	Cluster-2 (Ro/La) n=48 %	*P* value
Musculoskeletal damage	25 (25.8)	16 (33.3)	0.6
Renal damage	19 (19.5)	1(2.1)	0.003*
Neuropsychiatric damage	18 (18.5)	5 (10.4)	0.3
Cardiovascular damage	8 (8.2)	4 (8.3)	1
Gastrointestinal damage	6 (6.2)	2 (4.2)	1
Diabetic mellitus	6 (6.2)	3 (6.3)	1.0
Ocular damage	4 (4.1)	0	0.3
Peripheral vascular damage	4 (4.1)	0	0.3
Pulmonary damage	2 (2.1)	1(2.1)	1
Dermatological damage	1 (1.03)	4 (8.3)	0.04*
SLICC/ACR damage Index(mean±SD)	5.3 ± 1.6	2.4 ± 2.2	0.4

SLICC: Systemic Lupus International Collaborating Committee/ American College of Rheumatology damage index (SLICC/ACR damage Index)
*Values are significantly different. **Values are strongly significantly different.

4. Discussion

The extreme heterogeneity of SLE has led some investigators to propose that SLE represents a syndrome rather than a single disease [10]. The diversity in lupus has been attributed to genetic, environmental and socio-demographic factors [11]. Ethnicity and environmental factors such as occupation, pollutants, socio-economic status, and behavior can affect the course and outcome of SLE [12].

It is now thought that SLE could be divided into more homogeneous subsets with pathogenic, therapeutic, or prognostic significance. However, the characterization of the different patterns of SLE expression is complex, especially when small series of patients or cohorts from different ethnicities are analyzed [13].Benign clinical subsets of SLE had been identified and characterized by infrequent severe disease and mucocutaneous manifestations, while other subsets suffered from severe renal disease with heavy proteinuria and renal failure, which conform with what mentioned by [14&15].

Numerous attempts were held to link the presence of particular antibodies detectable in the serum of patients with lupus to clinical features of the disease [16]. Still nowadays a great effort is being made to understand the pathogenetic, diagnostic, and prognostic meaning of such autoantibodies [17].

In the present study, we used the K-mean cluster analysis procedure; (a statistical method that is able to cluster large number of variables into certain patterns), to identify groups of SLE patients with similar autoantibody patterns. The cluster analysis had been used previously to define different patient groups in SLE [3,6,10,13,17].However; the cluster analysis does not provide an explanation of why these clusters exist, and techniques for determining the reliability and validity of clusters have not yet been developed [18]

Population differences of autoantibodies and clinical manifestations among different ethnicities may lead to different clustering or association results [19]. Few studies have examined the relationship between ethnicity and immunologic factors in terms of autoantibody profiles. However the available data provide evidence that ethnicity differences in autoantibody clusters do exist and might be responsible for the divergent clinical presentations in various ethnic groups [6].

Patients in the first cluster had a lower mean age at diagnosis; this is consistent with results found by *Rovenský and colleagues* who systematically reviewed clinical manifestations of SLE in the elderly, they found

that late onset SLE patients manifested higher rate of positive findings for anti-Ro and anti-La antibodies [20].

Cluster-1 (DNA/RNP/Sm) patients showed higher prevalence of serositis (pleurisy) than cluster-2 (Ro/La) patients. This is consistent with the results found by Ching and his colleagues who studied 129 SLE patients for autoantibodies clustering; the Sm/RNP cluster was associated with a higher prevalence of serositis in comparison to the Ro/La cluster and no other clinical characteristics were associated with either cluster [3].

Cluster-1 (DNA/RNP/Sm) patients also showed higher prevalence of renal manifestations than cluster-2 (Ro/La) patients, as anti-Sm and anti-RNP antibodies seem to exert opposite effects on the severity of renal involvement. According to some authors, anti-RNP antibodies are associated with milder renal involvement [21]. Others; like Bastian and his colleagues; found an association between lupus nephritis and anti-RNP antibodies [22].In a recent retrospective case-control study of 127 patients with biopsy-proven lupus nephritis a higher frequency of anti-dsDNA, anti-RNP and anti-Sm antibodies were found in lupus nephritis patients compared with the control group [23].

Patients in cluster-2 (Ro/La) showed a higher prevalence of mucocutaneous manifestations; *Paz and colleagues* reported a direct relationship between anti-Ro antibodies and cutaneous photosensitivity in SLE patients [24]. This was also confirmed in a study by *Leng and colleagues* who found an association between photosensitivity and anti-La antibodies but not anti-Ro antibodies [25].

Cluster-2(Ro/La) patients had less severe lupus and required lower cumulative dose of immunosuppressive therapy during their illness. They represented the SLE subset with the more favorable prognosis. A possible explanation is the well-documented association between anti-Ro/La and milder disease manifestations, for example, lupus-related rash and photosensitivity and a lower risk of renal involvement and seizures compared with anti-Ro/La negative lupus patients [26 &27].

Autoantibody clustering can help us to differentiate between various subsets of SLE, allowing the prediction of subsequent disease course and organ damage. The correlation of specific autoantibodies with certain clinical findings may help to direct treatment and predict the prognosis of SLE patients. Thus, it would seem that further definition of clusters of antibodies that are reflective of the nature and severity of clinical disease may help guide management of the disease [28].

5. Conclusion

The current work supports the existence of lupus autoantibodies in clusters with distinct clinical features and shows that forming clinical subsets according to autoantibody clusters may be useful in predicting the outcome of the disease and may help in the management strategy and predict the prognosis of SLE patients.

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Nashwa Taher Allam : Professor of Rheumatology and Rehabilitation ,Major degree :M.D. , Rheumatology Department, Faculty of Medicine ,Cairo University, 00202/01000118016; nashwa_t_allam@gmail.com

Wafaa Gaber: Assistant Professor of Rheumatology, Major Degree: M.D. Rheumatology Department, Faculty of Medicine ,Cairo University,00202/01227626502, 00202/23493447;
wafaa.gaber@kasralainy.edu.eg

Marwa Helaly: Rheumatologist, Major degree :MS.C.. , Rheumatology department, El-Galaa military family hospital, 00202/01223839851 ; dr.marwahelaly@gmail.com