Original article

Relationship between autoantibody clustering and clinical subsets in SLE: Cluster and association analyses in Hong Kong Chinese

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ABSTRACT

Objectives

This study aims to identify the existence and relation between autoantibody clusters and clinical subsets in Chinese SLE patients.

Methods

The data of 1928 SLE patients from Hong Kong were analysed. Using cluster analysis, patients were grouped by autoantibodies into clusters. The frequencies of different clinical manifestations were then compared between each cluster. Separate association analyses between individual autoantibodies and clinical manifestations, as well as between clinical manifestations were also performed without any prior clustering.

Results

Three separate autoantibody clusters were identified each with significantly different clinical manifestations. Cluster 1 was characterized by anti-dsDNA and the greatest prevalence of renal disorder but the lowest frequencies of other clinical manifestations. Cluster 2 was represented by the predominance of anti-Sm, anti-RNP and aPL, with greater prevalence of malar rash, oral ulcers, arthritis and serositis. Cluster 3 was characterized by anti-Ro and anti-La with greater prevalence of discoid rash, photosensitivity and haematological involvement. Individual association analysis also revealed similar findings. Patients of Cluster 2 and Cluster 3 were more closely related, whilst Cluster 1 was more distinct, associated with renal disorder only and negatively or not associated with other manifestations.

Conclusions

We conclude that autoantibody clustering and clinical subsets exist in SLE patients of

our locality. These clusters may be viewed as a bipolar spectrum of related autoantibody and clinical manifestations. On one end are patients with over-representation of anti-dsDNA and renal disorder; whilst on the other end are two distinct autoantibody clusters (anti-Sm/anti-RNP/aPL and anti-Ro/anti-La) with overlapping of other clinical manifestations.

Key words:

Lupus Erythematosus, Systemic

Autoantibodies

Cluster Analysis

Multivariate Analysis

Prevalence

Epidemiology

Chinese

Hong Kong

INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with heterogeneous manifestations. The diagnosis of SLE is usually made when patients has developed four or more out of the eleven American College of Rheumatology (ACR) criteria [1], which can range from different organ manifestations to production of various autoantibodies. Individual autoantibodies can be used to reflect or predict disease activity, and some are associated with specific disease manifestations [2, 3].

Subsets of patients with distinct patterns of disease manifestations [4-6] and the clustering of autoantibodies [3, 7] have been previously reported, but seldom have these two phenomena been analyzed together. Identification of patient clusters by autoantibody profile, in addition to each cluster's associated features, may potentially be useful for disease prediction. For example, if tested positive for a certain autoantibody, a patient would likely belong to a certain cluster and thus more prone to develop other specific laboratory or clinical manifestations.

Cluster analysis is a statistical method which partitions cases by grouping them into clusters based on similarities between variables; in this study, different autoantibody production. However, cluster analysis does not provide an explanation as why these clusters exist, and techniques for determining the reliability and validity of clusters have not yet been developed. Therefore separate association analysis between individual autoantibodies and clinical manifestations can also be performed.

In this study, we utilize cluster analysis to identify the existence of autoantibody clustering with specific subsets of clinical manifestations in Chinese SLE patients. The predominant autoantibodies of each cluster were also individually associated with the over-represented clinical manifestations of the same cluster. Furthermore, there were significant associations between the representative manifestations of the same

and certain different clusters, which reiterated the observations in cluster analysis. Our findings therefore suggest that autoantibody clustering and the grouping of clinical manifestations may be inter-related.

MATERIAL AND METHODS

Patient population

All patients were of Chinese ethnicity and diagnosed with SLE, having fulfilled at least four of the 1997 Revised Criteria for the Classification of SLE [1]. The patients were recruited from 5 regional hospitals throughout Hong Kong (Queen Mary Hospital, Queen Elizabeth Hospital, Princess Margaret Hospital, Tuen Mun Hospital and Pamela Youde Nethersole Eastern Hospital) as part of a multi-centre study approved by the Institutional Review Board of the University of Hong Kong and Hospital Authority Hong Kong West Cluster; Research Ethics Committee, Kowloon Central and Kowloon East; Clinical Research Ethics Committee, Kowloon West Cluster; New Territories West Cluster, Clinical and Research Ethics Committee; and Ethics Committee, Hong Kong Easter Cluster. All patients gave informed consent. Clinical data were collected by medical record review between 2007 and 2009 and inputted into a clinical database.

Data collection

This was a cross-sectional retrospective study with all clinical data drawn from the study's database Recorded data from this database included gender, age of onset, clinical manifestations and presence of autoantibodies. Clinical manifestations (malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurological involvement, haematological involvement) and autoantibody (anti-double stranded DNA (dsDNA), anti-Smith (anti-Sm) and anti-phospholipid antibodies (aPL)) were defined according to the revised ACR classification criteria for SLE [1]. Additional autoantibodies associated with SLE were also studied, including:

anti-Ro, anti-La and anti-ribonucleoprotein (anti-RNP). Patients were considered positive for certain disease manifestations as diagnosed by physicians in respective hospitals, and considered positive for autoantibodies if there were any positive results in previous serological tests performed at respective hospitals.

Statistical methods

Cluster analysis was performed using the K-means algorithm to group patients with similar autoantibody profiles together. However, K-means clustering is intended for clustering quantitative variables and the presence of autoantibody production is categorical. Factor analysis was therefore performed first with the factor loading scores used in the K-means algorithm. Further details on factor and cluster analyses can be found in the supplementary data and tables.

The frequencies of different autoantibodies and clinical manifestations between cluster groups were compared using the chi-square test with Yates' correction for overall p-values and the Fisher's exact test to compare between individual clusters. To compensate for the effect of multiple comparisons in the Fisher's exact tests, Bonferroni correction was used and only p-values <0.001 were considered significant. Further association analyses using the chi-square test with Yates' correction were performed and odds ratios (OR) with 95% confidence intervals (CI) were used to quantify the relationship between individual autoantibodies and clinical manifestations, as well as between individual clinical manifestations. The Fisher's exact tests were used to calculate the p-values and, after Bonferroni correction, only p-values <0.001 were considered significant. SAS version 9.1 was used for calculating the Akaike's information criterion (AIC) and Bayesian information (BIC) criterion. SPSS version 11.5 was used for all other analyses.

RESULTS

A total of 1928 Chinese SLE patients from Hong Kong were studied. There were 1771 females and 157 males, making a female:male ratio of 11.3:1. The mean (SD) age of onset was 29.8 (13) years, and the mean (SD) disease duration for all patients was 13.1 (8.6) years.

Data regarding arthritis, serositis and neurological involvement were missing for 256 patients, thus any analyses involving these manifestations were calculated after exclusion of these patients. However since clustering was by autoantibody production only, and not clinical manifestations, this would not have any effect on clustering. All other data was otherwise available for 1928 patients. Baseline characteristics including prevalence of clinical manifestations and autoantibody profile in comparison to previous cohorts of other ethnicities are shown in Table 1.

Autoantibody clusters and their differences in clinical manifestations

Using cluster analysis, the 1928 patients were grouped into three separate clusters of autoantibodies, and each cluster had significantly different subsets of clinical manifestations. The frequencies of individual autoantibodies and clinical manifestations in each respective cluster are shown in Table 2.

Cluster 1 consisted of 1211 patients represented by a higher frequency of anti-dsDNA, which was marginally insignificant when compared to Cluster 2 but significantly different to Cluster 3 (78.1% vs. 72.6% [Cluster 2, $p=0.0194$], 43.9% [Cluster 3, $p<0.0001$]). These patients also had significantly lower prevalence of all other autoantibodies; namely aPL (33.4% vs. 50.6% [Cluster 2, $p<0.0001$], 46.3% [Cluster

3, p<0.0001]), anti-Sm (0% vs. 45.4% [Cluster 2, p<0.0001], 2.7% [Cluster 3, p<0.0001]), anti-Ro (29.5% vs. 58.4% [Cluster 2, p<0.0001], 89.5% [Cluster 3, p<0.0001]), anti-La (0% vs. 5.7% [Cluster 2, p<0.0001], 67.0% [Cluster 3, p<0.0001]) and anti-RNP (0% vs. 84.2% [Cluster 2, p<0.0001], 7.5% [Cluster 3, p<0.0001]). Similarly for clinical manifestations, there was significantly greater prevalence of renal disorder (60.3% vs. 48.9% [Cluster 2, p<0.0001], 44.6% [Cluster 3, p<0.0001]), but lowest prevalence of almost all other clinical manifestations. The only exceptions were haematological involvement (53.6% vs. 61.2% [Cluster 2, p=0.0075]), serositis (12.7% vs. 19.4% [Cluster 2, p=0.0028], 10.6% [Cluster 3, p=0.3967]) and neurological involvement (7.9% vs. 7.7% [Cluster 2], 8.3% [Cluster 3], overall p=0.968), which were not significantly different when compared to one or either clusters.

Four-hundred-and-twenty-three patients were assigned to Cluster 2 This cluster had significantly greater prevalence of anti-Sm (45.4% vs. 0% [Cluster 1, p<0.0001], 2.7% [Cluster 3, p<0.0001]) and anti-RNP (84.2% vs. 0% [Cluster 1, p<0.0001], 7.5% [Cluster 3, p<0.0001]) when compared to bother clusters, and greater prevalence of aPL when compared to Cluster 1 (50.6% vs. 33.4%, p<0.0001). Cluster 2 had the highest prevalence of malar rash (67.1% vs. 47.0% [Cluster 1, p<0.0001]) and arthritis (72.0% vs. 53.5% [Cluster 1, p<0.0001]) but these clinical manifestations were only significantly different when compared with Cluster 1, but not with Cluster 3 (all p-values >0.001). Oral ulcers (23.4% vs. 7.9% [Cluster 1, p=0.0122], 21.1% [Cluster 3, p=0.5243]) and serositis (19.4% vs. 12.7% [Cluster 1, p=0.0028], 10.6% [Cluster 3, p=0.0032]) were also most prevalent in this group, but both did not reach statistical significance when compared with other clusters individually.

The remaining 294 patients were assigned to Cluster 3, which was characterized by the significant over-representation of anti-Ro (89.5% vs. 29.5% [Cluster 1, p<0.0001], 58.4% [Cluster 2, p<0.0001]) and anti-La (67.0% vs. 0% [Cluster 1, p<0.0001], 5.7% [Cluster 2, p<0.0001). The frequencies of photosensitivity (32.7% vs. 20.3% [Cluster 1, p<0.0001], arthritis (68.1% vs. 53.5% [Cluster 1, p<0.0001] and haematological disorder (62.9% vs. 53.6% [Cluster 1, p=0.004] were all significantly greater than Cluster 1 but not significantly different with Cluster 2 (all p-values >0.001). Discoid rash was also most prevalent in this group (11.9% vs. 7.9% [Cluster 1, p=0.0373], 10.9% [Cluster 2, p=0.071]), but did not reach statistical significance when compared with other clusters individually.

Associations between individual autoantibodies and clinical manifestations

Separate association analysis between individual autoantibodies and clinical manifestations echoed the previous observations from cluster analysis. Predominant autoantibodies showed associations with the over-represented clinical manifestations of the same cluster. The OR and CI are shown in Table 3. For both Table 3 and Table 4, the data has been presented so that the representative autoantibodies/clinical manifestations of each cluster (as identified by cluster analysis) are listed together for easier visualisation; no prior clustering was performed for this analysis. No associations between any autoantibodies and neurological involvement were found (data not shown).

Anti-dsDNA was positively associated with renal disorder (OR=2.37, CI=1.93-2.90) and negatively associated with photosensitivity (OR=0.68, CI=0.54-0.84). Furthermore, renal disorder was not associated with all other autoantibodies from other clusters. This is consistent with the observations of Cluster 1 from previous

cluster analysis. Serositis (OR=2.04, CI=1.42-2.92) and haematological involvement (OR=1.56, CI=1.28-1.91) were exceptions and found to be positively associated with anti-dsDNA.

Anti-Sm, anti-RNP and aPL (predominant autoantibodies of Cluster 2) were individually associated with malar rash, arthritis and serositis (which were all representative clinical manifestations of the same cluster during cluster analysis), despite some associations failing to reach statistical significance after Bonferroni correction. These three autoantibodies also did not have associations with oral ulcers. Furthermore, aPL was associated with photosensitivity (OR=1.47, CI=1.19-1.81) and haematological involvement (OR=1.43, CI=1.19-1.72); and anti-RNP with photosensitivity (OR=1.52, CI=1.19-1.95), which were all over-represented clinical manifestations of Cluster 3.

Likewise, anti-Ro, and anti-La (characteristic autoantibodies of Cluster 3) were also associated with all the representative clinical manifestations of the same cluster, namely: discoid rash, photosensitivity and haematological involvement (although the associations of anti-La with discoid rash and photosensitivity were not significant). Anti-Ro and anti-La also showed significant associations with the predominant clinical manifestations from Cluster 2. Anti-Ro was significantly associated with malar rash (OR=1.74, CI=1.45-2.09) and arthritis (OR=1.96, CI=1.61-2.40).

Associations between individual clinical manifestations

Individual association analysis was performed between different clinical manifestations. The OR and CI for all associations are shown in Table 4. In most cases the associations were concordant with previous patterns. Renal disorder (Cluster

1) was negatively associated with all other manifestations, except with serositis (OR=1.56, CI=1.17-2.08) and haematological involvement (OR=1.01, CI=0.84-1.21). However there were again much intra- and inter-cluster associations with the over-represented clinical manifestations of Cluster 2 and Cluster 3 as identified by cluster analysis. Eight out of the twelve inter-cluster associations between the over-representative clinical manifestations reached statistical significance. Associations with serositis was again an exception (vs. discoid rash [OR=0.93, CI=0.57-1.53] and photosensitivity [OR=0.73, CI=0.51-1.03]). The associations of discoid rash with arthritis (OR=1.06, CI=0.75-1.49) and oral ulcers (OR=1.72, CI=1.21-2.44) were also not significant. Neurological involvement, not previously significantly different between clusters or associated with individual autoantibodies, was individually associated with serositis (OR=2.82, CI=1.88-4.23) and haematological involvement (OR=2.05, CI=1.38-3.04) (data not shown).

DISCUSSION

This is one of the largest observational studies of autoantibody and clinical manifestations of SLE patients reported. Although cluster analysis based on clinical patterns have been previously performed [6], we are first to report of autoantibody clustering in our locality and have employed a different statistical approach. In order to avoid inappropriately clustering binary data [8], we first performed factor analysis and used the factor loading scores in the K-means algorithm. Furthermore, we also performed separate association analysis to identify the relationships between *individual* variables. Clinicians may be especially interested in these associations between individual autoantibodies and various disease manifestations; as well as associations among individual clinical features only, without reference to autoantibody profile. It is also much easier to interpret and explain these individual associations within the context of known pathophysiology and biological processes in comparison to the agglomerated data of cluster analysis.

The demographics of the patients in this study (Table 1) were mostly comparable to previous large Mainland Chinese [9], Caucasian [10] and African-Black [11] cohorts. Our study and that by Feng *et al.* [9] both show a higher prevalence of anti-Ro (45.0% and 34.3% vs. 25%) and renal involvement (55.4% and 55.9% vs. 39%) in Chinese patients when compared to Caucasians [10]. This is consistent with previous reports [12, 13] and it has been suggested that the presence of anti-Ro may contribute to the likelihood of developing renal involvement [14]. However this pattern is not consistent when Chinese patients are compared to African Blacks [11]. African Blacks had a higher frequency of anti-Ro (60.5%) despite a lower prevalence of renal disorder (48.6%). This is an important observation as renal disease is a major cause of morbidity in our SLE patients [15] and further research into the relationship between

anti-Ro and renal involvement in Chinese patients would be of great value. Furthermore, such population differences of autoantibodies and clinical manifestations amongst different ethnicities may lead to different clustering or association results. An example of this can be seen if we compare our results to other studies such as that by To *et al.* [3] which studied autoantibody clustering amongst patients of different ethnicities. Although we observe the same clustering of anti-Sm and anti-RNP, we did not identify their reported clustering of anti-dsDNA with anti-Ro/anti-La or with the aPL. This may be due to a comparatively higher prevalence of anti-dsDNA in patients of our locality in comparison to their mixed patient sample (71.6% vs. 58.2%).

In this study we identify three separate clusters of patients by autoantibody profile, each showing distinct patterns of clinical manifestations (Table 2). Despite supporting evidence from Euclidean distances between cluster centroids, as well as the AIC and BIC criteria, determining the optimum number of clusters remains difficult. However, the presence of these three clusters is consistent with prior reports of various SLE subsets and relates well with known biological processes. The details of each autoantibody cluster and associations with various clinical manifestations are elaborated on as follows.

Cluster 1 consisted of 62.8% of all patients, with the highest prevalence of anti-dsDNA and renal disorder. Patients from this cluster also had significantly lower prevalence of all other autoantibodies and no patients were positive for anti-Sm, anti-La or anti-RNP. Individual association analysis (Table 3) further showed anti-dsDNA to be strongly associated with renal disorder, but generally not with other manifestations. A very consistent pattern can also be observed in the association analysis between different clinical manifestations (Table 4) with renal disorder

negatively associated with almost all other manifestations. Interestingly, serositis was repeatedly an exception to these patterns, and individual association analysis of haematological involvement showed positive findings with anti-dsDNA and insignificant results with renal disorder. Although relationships between haematological involvement with renal disorder [3, 10, 16] and anti-dsDNA [17] have been reported by some, the associations with serositis have not been well documented.

Anti-Sm, anti-RNP and aPL were over-represented by patients of Cluster 2 which consisted of 21.9% of all patients; and anti-Ro and anti-La were predominant in Cluster 3, which consisted of 15.2% of all patients (Table 2). The clustering of anti-Sm with anti-RNP [18, 19] and anti-Ro with anti-La [10, 19] have been reported previously. The coexistence of anti-Sm and anti-RNP has been attributed to the similarity and cross-reactivity between the targets of these two antibodies [20]; whist anti-Ro and anti-La are both induced by common small cytoplasmic ribonucleoproteins [21, 22]. On the contrary, the associations of aPL with other autoantibodies have not been well described. However, there was no significant difference in aPL between Cluster 2 and Cluster 3 (50.6% [Cluster 2] vs. 46.3% [Cluster 3, $p=0.259$]), and only differentiating value is its relative infrequency in Cluster 1 (33.4%, both $p < 0.0001$).

Cluster 2 (anti-Sm/anti-RNP/aPL) had the highest prevalence of malar rash, oral ulcers, arthritis and serositis, and Cluster 3 (anti-Ro/anti-La) had the highest prevalence of discoid rash, photosensitivity and haematological involvement (Table 2). Although many associations remain controversial, these results were consistent with many previous reports. For example, in Cluster 2: the association between anti-RNP and arthritis [23]; between anti-Sm and serositis [24]; and in Cluster 3: between anti-Ro and photosensitivity [25], discoid lupus and haematological involvement [26].

There were many associations observed between Cluster 2 and Cluster 3, both between their over-represented clinical manifestations and autoantibodies (Table 3), as well as between their clinical manifestations (Table 4). There were also consistently negative associations between these autoantibodies with renal disorder. In contrast, anti-dsDNA, the predominant autoantibody of Cluster 1, was strongly associated with renal disorder but seldom with the predominant features of other clusters. These observations make sense on both the autoantibody and clinical manifestation levels. On the autoantibody level, the pathogenic role for anti-dsDNA in renal involvement has been well established [27, 28]; whilst anti-Sm [29, 30], anti-RNP [31-33], and the combination of anti-Ro/anti-La [34] have been described to have little or even protective roles in the development of renal disease. In fact, anti-RNP was originally described in patients with mixed connective tissue disease who lacked renal involvement and to occur only rarely with anti-dsDNA [35, 36]. Whilst on the level of clinical manifestations, observations by physicians have long suggested that subsets of lupus patients exist. A consistent observation is that patients with renal disease seem to be at decreased risk of developing other manifestations [4]. Of particular interest, this described subset of patients has shown to have greater prevalence of haematological involvement [6, 16]. This is again compatible with our results, where haematological involvement was an exception to the consistently negative associations observed with anti-dsDNA and renal disorder (Table 3 and Table 4).

The prevalence of neurological involvement was not significantly different between clusters, and no association with other clinical manifestations were identified. This may be explained by the low prevalence of neurological involvement in patients of our locality (7.9%) compared with other ethnicities (e.g. African Blacks: 17.1%), which is consistent with previous reports [37, 38].

In interpretation of the results of cluster and association analysis as a whole, this study proposes that the clustering of autoantibody and subsets of clinical manifestations may be related. Although further studies are required to explore any potential underlying mechanisms, from these observations we hypothesize that the clustering of autoantibodies may partially account for the observed clinical subsets. Various clusters of autoantibodies may play common or complementary roles in the pathogenesis of single or similar clinical manifestations. An example of this can be seen between anti-Ro and anti-La with photosensitivity (Cluster 3). Both *in vitro* and in *vivo* experiments have demonstrated that ultraviolet radiation helps the binding of anti-Ro and anti-La onto the surface of keratinocytes [39], and it has been suggested that the binding of these two autoantibodies may have common pathogenic roles in this photosensitive inflammatory skin manifestation [40, 41].

Overall, in this study we observe that patients can be clearly separated into three clusters based in autoantibody profile. Although Cluster 2 and Cluster 3 clearly cluster very distinctly by autoantibody production, there is much overlapping of their representative clinical manifestations. These findings also suggest that SLE may be viewed as a disease with a bipolar spectrum of autoantibody and clinical manifestations; with these three clusters of patients viewed on two different ends of disease manifestations. On one end are patients from Cluster 1 (anti-dsDNA), with the most renal disorder but lowest prevalence of other manifestations. On the other end are patients of Cluster 2 (anti-Sm/anti-RNP/aPL) and Cluster 3 (anti-Ro/anti-La). The exceptions of haematological involvement and serositis may suggest that these manifestations overlap between these two extremes. Furthermore, neurological involvement may exist independently without any significant between clusters or particular autoantibody associations.

There are numerous limitations to this study. For example, patients in this study were recruited from regional hospitals and may lead to an over-representation of patients with more severe manifestations. This may explain the larger number of patients in Cluster 1 (n=1211) than in Cluster 2 (n=423) or Cluster 3 (n=294). Secondly, other than anti-Ro, anti-La and anti-RNP, data for other manifestations not included in the ACR criteria [1] or more detailed sub-classifications were unavailable. However, given the pervasive adoption of these criteria and being able to compare with other research, we believe the choice of studied variables were appropriate. Furthermore, as this is only an observational study, we also plan to use a predictive approach on an independent sample in future study in order to validate our clustering results.

In conclusion, these findings may help guide the future study of potential common pathogenic mechanisms within autoantibody clusters and their effect on disease manifestations. It would be interesting to explore if these relationships between clinical subsets and autoantibody clusters may extend to the genetic level, for example by using these clusters in subphenotype analysis in genetic association studies.

KEY MESSAGES

1. Three autoantibody clusters with different subsets of clinical manifestations exist in Chinese SLE patients.

2. SLE can be viewed as a bipolar spectrum of autoantibody and clinical manifestations.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Table 1 – Prevalence of autoantibodies and clinical manifestations in Hong Kong Chinese patients compared to previous cohorts.

	Present study (Hong Kong)	**Mainland Chinese** [8]	**Caucasian** [9]	**African Blacks** [10]
Number of patients	1928	1790	1000	111
Female (%)	91.9	90.2	91	92.5
Mean (SD) age of onset	29.8 (13)	31.0 (12)	29 (13)	35.1
Autoantibodies (%)				
Anti-dsDNA	71.6	41.8	78	66.7
aPL	39.2	-	-	-
Anti-Sm	10.4	27.6	10	44.2
Anti-Ro	45.0	34.3	25	60.5
Anti-La	11.5	14.9	19	28.4
Anti-RNP	19.6	16.2	13	65.5
Clinical manifestations (%)				
Malar rash	53.1	50.2	58	55.0
Discoid rash	9.2	5.8	10	28.8
Photosensitivity	24.6	14.9	45	33.3
Oral ulcer	19.5	13.6	24	21.6
Arthritis#	59.6	63.9	84	62.2
Serositis#	13.8	16.6	36	28.2
Renal disorder	55.4	55.9	39	48.6
Neurological involvement#	7.9	8.9	-	17.1
Haematological involvement	56.5	-	-	60.5

Clinical manifestations where data were available for 1672 patients.

Table 2 – Clustering of 1928 SLE patients into three clusters by cluster analysis based on autoantibody profile.

a)	Cluster 1 (n = 1211)	Cluster 2 (n = 423)	Cluster 3 (n = 294)	Overall p-value	p-value between individual clusters 1 vs. 2	1 vs. 3	2 vs. 3
Female	1007 (91.4%)	391 (92.4%)	273 (92.9%)	0.637	.	.	.
Mean (SD) age of onset	29.2 (13.2)	30.0 (12.0)	31.9 (13.5)	0.008*	0.75	0.006*	0.156
Anti-dsDNA	**946 (78.1%)**	306 (72.6%)	129 (43.9%)‡	<0.0001*	0.0194	<0.0001*	<0.0001*
aPL	405 (33.4%)‡	**214 (50.6%)**	136 (46.3%)	<0.0001*	<0.0001*	<0.0001*	0.259
Anti-Sm	0 (0%)‡	**192 (45.4%)‡**	8 (2.7%)‡	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Anti-Ro	357 (29.5%)‡	247 (58.4%)‡	**263 (89.5%)‡**	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Anti-La	0 (0%)‡	24 (5.7%)‡	**197 (67.0%)‡**	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Anti-RNP	0 (0%)‡	**356 (84.2%)‡**	22 (7.5%)‡	<0.0001*	<0.0001*	<0.0001*	<0.0001*
b)	Cluster 1	Cluster 2	Cluster 3	Overall p-value	1 vs. 2	1 vs. 3	2 vs. 3
Malar rash	569 (47.0%)‡	**284 (67.1%)**	171 (58.2%)	<0.0001*	<0.0001*	0.0007*	0.0147
Discoid rash	96 (7.9%)	46 (10.9%)	**35 (11.9%)**	0.042*	0.7193	0.0373	0.071
Photosensitivity	246 (20.3%)‡	132 (31.2%)	**96 (32.7%)**	<0.0001*	<0.0001*	<0.0001*	0.6844
Oral ulcer	215 (17.8%)	**99 (23.4%)**	62 (21.1%)	0.031*	0.0122	0.208	0.5243
Arthritis#	571 (53.5%)‡	**252 (72.0%)**	173 (68.1%)	<0.0001*	<0.0001*	<0.0001*	0.3213
Serositis#	136 (12.7%)	**68 (19.4%)**	27 (10.6%)	0.002*	0.0028	0.3967	0.0032
Renal disorder	**730 (60.3%)‡**	207 (48.9%)	131 (44.6%)	<0.0001*	<0.0001*	<0.0001*	0.2548
Neurological involvement#	84 (7.9%)	350 (7.7%)	**21 (8.3%)**	0.968	.	.	.
Haematological involvement	649 (53.6%)	259 (61.2%)	**185 (62.9%)**	0.002*	0.0075	0.004*	0.6959

(a) Comparison of the frequencies of different autoantibodies between each cluster.
(b) Comparison of the frequencies of different clinical manifestations between each cluster.

Results are displayed as the absolute number of patients (proportion of cluster) for given autoantibody or clinical manifestation.
‡ denotes values are significantly different from the other two clusters
* denotes associations reaching statistical significance ($p<0.05$ for overall p-value, and $p<0.001$ for between clusters after Bonferroni correction)
# denotes clinical manifestations where data were available for 1672 patients.
Bold text denotes clusters with the greatest prevalence.

Table 3 – Associations between individual autoantibodies and clinical manifestations.

	Anti-dsDNA[1]	**Anti-Sm[2]**	**Anti-RNP[2]**	**aPL[2]**	**Anti-Ro[3]**	**Anti-La[3]**
Renal disorder[1]	**2.37***	0.79	0.76	0.81	0.75	0.66
	(1.93 - 2.90)	(0.59 - 1.05)	(0.61 - 0.96)	(0.67 - 0.97)	(0.63 - 0.90)	(0.50 - 0.87)
	p<0.0001	p=0.115	p=0.021	p=0.024	p=0.002	p=0.004
Malar rash[2]	1.05	**1.65***	**1.80***	**1.29**	1.74*	1.20
	(0.86 - 1.28)	**(1.22 - 2.24)**	**(1.42 - 2.27)**	**(1.07 - 1.55)**	(1.45 - 2.09)	(0.90 - 1.59)
	p=0.649	**p=0.001**	**p<0.0001**	**p=0.007**	p<0.0001	p=0.224
Arthritis[2]	1.21	**1.57**	**2.16***	**2.01***	1.96*	1.61
	(0.97 - 1.50)	**(1.11 – 2.21)**	**(1.64 - 2.83)**	**(1.62 – 2.49)**	(1.61 – 2.40)	(1.17 – 2.24)
	p=0.086	**p=0.01**	**p<0.0001**	**p<0.0001**	p<0.0001	p=0.004
Serositis[2]	2.04*	**1.63**	**1.69**	**1.16**	1.27	1.10
	(1.42 - 2.92)	**(1.08 - 2.45)**	**(1.22 – 2.33)**	**(0.87 - 1.55)**	(0.96 - 1.68)	(0.72 - 1.68)
	p<0.0001	**p=0.024**	**p=0.002**	**p=0.332**	p=0.101	p=0.655
Oral ulcers[2]	0.80	**1.35**	**1.21**	**0.98**	1.34	1.06
	(0.53 - 1.02)	**(0.95 - 1.91)**	**(0.92 - 1.59)**	**(0.78 - 1.24)**	(1.07 - 1.68)	(0.75 - 1.51)
	p=0.074	**p=0.091**	**p=0.192**	**p=0.906**	p=0.013	p=0.719
Discoid rash[3]	0.82	1.90	1.14	1.57	**1.75***	**1.45**
	(0.59 - 1.14)	(1.24 - 2.90)	(0.78 - 1.66)	(1.15 - 2.14)	**(1.28 - 2.40)**	**(0.93 - 2.23)**
	p=0.255	p=0.004	p=0.489	p=0.005	**p=0.0005**	**p=0.107**
Photosensitivity[3]	0.68*	1.25	1.52*	1.47*	**1.51***	**1.46**
	(0.54 - 0.84)	(0.90 - 1.74)	(1.19 - 1.95)	(1.19 - 1.81)	**(1.23 - 1.86)**	**(1.08 - 1.98)**
	p=0.001	p=0.193	p=0.001	p=0.0003	**p=0.0001**	**p=0.02**
Haematological involvement[3]	1.56*	1.31	1.40	1.43*	**1.54***	**1.64***
	(1.28 - 1.91)	(0.97 - 1.77)	(1.11 - 1.77)	(1.19 - 1.72)	**(1.29 - 1.85)**	**(1.22 - 2.20)**
	p<0.0001	p=0.083	p=0.005	p=0.0002	**p<0.0001**	**p=0.001**

Results are displayed as the odds ratio (95% confidence interval) and p-value for each association.

* denotes associations reaching statistical significance (p<0.001)

[1,2,3] denotes over-represented autoantibodies or clinical manifestations of Cluster 1, Cluster 2 or Cluster 3 from cluster analysis (Table 2)

Bold text denotes associations between the over-represented clinical manifestations of the same cluster from cluster analysis (Table 2)

Table 4 – Associations between individual clinical manifestations.

	Malar rash[2]	**Arthritis[2]**	**Serositis[2]**	**Oral ulcer[2]**	**Discoid rash[3]**	**Photosensitivity[3]**	**Haematological involvement[3]**
Renal disorder[1]	0.71* (0.59 - 0.85) p=0.0002	0.57* (0.46 - 0.69) p<0.0001	1.56 (1.17 - 2.08) p=0.003	0.70 (0.56 - 0.87) p=0.002	0.57* (0.42 – 0.79) p=0.001	0.41* (0.33 - 0.51) p<0.0001	1.01 (0.84 - 1.21) p=0.963
Malar rash[2]	.	**3.04*** **(2.48 - 3.73)** **p<0.0001**	**0.84** **(0.64 - 1.11)** **p=0.227**	**2.78*** **(2.17 - 3.56)** **p<0.0001**	1.81* (1.32 – 2.51) p=0.0003	5.71* (4.42 - 7.36) p<0.0001	1.36* (1.14 - 1.63) p=0.001
Arthritis[2]	.	.	**1.30** **(0.98 - 1.74)** **p=0.083**	**2.53*** **(1.93 - 3.32)** **p<0.0001**	1.06 (0.75 – 1.49) p=0.796	2.29* (1.79 - 2.94) p<0.0001	1.47* (1.21 - 1.79) p=0.0002
Serositis[2]	.	.	.	**1.10** **(0.78 - 1.54)** **p=0.598**	0.93 (0.57 – 1.53) p=0.902	0.73 (0.51 - 1.03) p=0.08	1.77* (1.31 - 2.39) p=0.0001
Oral ulcer[2]	.	.	.	.	1.72 (1.21 – 2.44) p=0.004	2.00* (1.57 - 2.56) p<0.0001	1.96* (1.54 - 2.50) p<0.0001
Discoid rash[3]	.	.	.	.	.	**2.99*** **(2.18 – 4.11)** **p<0.0001**	**1.07** **(0.78 – 1.46)** **p=0.691**
Photosensitivity[3]	.	.	.	.	.	.	**1.06** **(0.86 - 1.31)** **p=0.594**

Results are displayed as the odds ratio (95% confidence interval) and p-value for each association.

* denotes associations reaching statistical significance (p<0.001)

[1,2,3] denotes over-represented autoantibodies or clinical manifestations of Cluster 1, Cluster 2 or Cluster 3 from cluster analysis (Table 2)

Bold text denotes associations between the over-represented clinical manifestations of the same cluster from cluster analysis (Table 2)