Autoantibodies in Pediatric Systemic Lupus Erythematosus: Ethnic Grouping, Cluster Analysis, and Clinical Correlations

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ABSTRACT. Objective. (1) To evaluate the spectrum of serum autoantibodies in pediatric-onset systemic lupus erythematosus (pSLE) with a focus on ethnic differences; (2) using cluster analysis, to identify patients with similar autoantibody patterns and to determine their clinical associations.

Methods. A single-center cohort study of all patients with newly diagnosed pSLE seen over an 8-year period was performed. Ethnicity, clinical, and serological data were prospectively collected from 156/169 patients (92%). The frequencies of 10 selected autoantibodies among ethnic groups were compared. Cluster analysis identified groups of patients with similar autoantibody profiles. Associations of these groups with clinical and laboratory features of pSLE were examined.

Results. Among our 5 ethnic groups, there were differences only in the prevalence of anti-U1RNP and anti-Sm antibodies, which occurred more frequently in non-Caucasian patients (p < 0.0001, p < 0.01, respectively). Cluster analysis revealed 3 autoantibody clusters. Cluster 1 consisted of anti-dsDNA antibodies. Cluster 2 consisted of anti-dsDNA, antichromatin, antiribosomal P, anti-U1RNP, anti-Sm, anti-Ro and anti-La autoantibody. Cluster 3 consisted of anti-dsDNA, anti-RNP, and anti-Sm autoantibody. The highest proportion of Caucasians was in cluster 1 (p < 0.05), which was characterized by a mild disease with infrequent major organ involvement compared to cluster 2, which had the highest frequency of nephritis, renal failure, serositis, and hemolytic anemia, or cluster 3, which was characterized by frequent neuropsychiatric disease and nephritis.

Conclusion. We observed ethnic differences in autoantibody profiles in pSLE. Autoantibodies tended to cluster together and these clusters were associated with different clinical courses. (First Release Jan 15 2009; J Rheumatol 2009;36:416–21; doi:10.3899/jrheum.080588)

Key Indexing Terms: PEDIATRICS SYSTEMIC LUPUS ERYTHEMATOSUS AUTOANTIBODIES ETHNIC GROUPS CLUSTER ANALYSIS DISEASE ASSOCIATIONS

The serological hallmark of systemic lupus erythematosus (SLE) is the presence of autoantibodies directed against multiple nuclear and cytoplasmic antigens and phospholipid components of cell membranes. Some of these autoantibodies are useful for classification purposes and are part of the American College of Rheumatology classification criteria for SLE, such as the antinuclear antibodies (ANA), anti-Sm, anti-dsDNA antibodies, and antiphospholipid antibodies. Frequencies of autoantibodies in SLE are reported to differ among ethnic groups, and studies examining the relationship among ethnicity and autoantibody patterns in SLE reported a high prevalence of anti-Sm and anti-U1RNP antibodies in African American patients. In addition, autoantibodies have been associated with specific clinical features of adult-onset SLE (aSLE), such as anti-dsDNA with lupus nephritis, anti-SSA(Ro) and anti-SSB(La) antibodies with sicca symptoms, anti-U1RNP antibodies with Raynaud’s phenomenon, and antiphospholipid antibodies with thrombotic events. Studies in aSLE have reported a tendency of these autoantibodies to occur in pairs or even in clusters, which has led to description of new clinical associations. To date, there have been few studies of autoantibody associations with clinical disease in pediatric-onset SLE (pSLE), and none examining autoantibody clustering.

Our aim was to evaluate the spectrum of serum autoantibodies in pSLE with a focus on differences among ethnic groups. The second aim was to identify groups of pSLE patients with similar antibody patterns using cluster analy-
sis, and to examine the associations of the identified autoantibody clusters with clinical features of pSLE including development of major organ manifestations.

MATERIALS AND METHODS

Study design. A single-center cohort study of all new patients diagnosed with pSLE was performed. All patients were diagnosed and followed in the Rheumatology Division at The Hospital for Sick Children in Toronto, a hospital providing tertiary healthcare for pediatric patients.

Patients. All new patients diagnosed with SLE in the time period September 1998 to May 2006 at the pediatric SLE clinic were eligible for this study if age at diagnosis was < 18 years. All patients met the revised American College of Rheumatology (ACR) criteria for classification of SLE1. Each patient had a standardized clinical assessment and standardized laboratory investigations on each clinic visit. Clinical assessment consisted of a general physical and musculoskeletal examination recorded on specifically designed forms. The following laboratory investigations were done on each visit: complete blood count with differential, liver and kidney function tests, complement and immunoglobulin levels, antiphospholipid antibodies, ANA, and extractable nuclear antibodies (ENA). For the purposes of this study, the following data, present within the first year of SLE diagnosis, were extracted: malar rash, photosensitivity, oral and/or nasal ulcers, arthritis, serositis, lupus nephritis, renal failure, any symptoms of neuropsychiatric disease related to SLE20, Coombs’-positive hemolytic anemia, lymphopenia, thrombocytopenia, and Raynaud’s phenomenon. Renal failure was defined as an increase in serum creatinine ≥ 75% above baseline on at least 2 consecutive occasions. Raynaud’s phenomenon was defined as blanching of fingers and/or toes upon exposure to cold or stress. ACR definitions were used for classification of the other variables21. Patients were categorized into ethnic subsets based on self-designated ethnic origins.

Autoantibodies. At the time of diagnosis, each patient was evaluated for presence of anticardiolipin antibodies (aCL) by ELISA (Varelisa Cardiolipin IgG Kit; Phadia, Freiburg, Germany), lupus anticoagulant (LAC), and anti-dsDNA antibodies at the clinical laboratory at the Hospital for Sick Children. Anti-dsDNA antibodies were determined by ELISA (IgG/IgM ELISA, Kallestad; Bio-Rad Laboratories, Hercules, CA, USA) and by fluorescence microscopy assay using the protozoan Crithidia lucilae (NovaLite dsDNA Crithidia; Inova Diagnostics, San Diego, CA, USA). A patient was considered positive for anti-dsDNA antibodies if the results of either or both tests were positive. In addition, a serum sample from each patient obtained within 12 months after diagnosis was analyzed in a single laboratory (Advanced Diagnostics Laboratory, University of California) using an addressable laser bead immunoassay (ALBIA) for detection of autoantibodies directed to the following autoantigens: chromatin, ribosomal P, U1RNP, topoisomerase I, Jo-1 (histidyl RNA synthetase), Sm, SSA(Ro), and SSB(La) (QuantaPlex ENA 9 kit; Inova Diagnostics),22. This technology was found to be reliable and accurate with a high level of agreement (> 90%) with conventional techniques.23

The study was approved by the Research Ethics Board at The Hospital for Sick Children, Toronto (REB No. 1000004037).

Statistical analysis. Chi-square and Fisher’s exact test were used to evaluate the significance of differences in single autoantibody frequencies among the ethnic groups.

Cluster analysis was used for identification of groups of patients with similar autoantibody profiles. The objective of cluster analysis is to partition a set of observations into mutually exclusive groupings in order to best represent distinct sets of observations within the sample. Agglomerative hierarchical analysis initially utilized the SAS Cluster procedure to generate and profile the clusters. The SAS outputs gave the clustering history a set of observations into mutually exclusive groupings in order to best represent the significance of differences in single autoantibody frequencies among the ethnic groups.

RESULTS

Patient characteristics. The patient cohort consisted of all 169 patients with newly diagnosed SLE (age at diagnosis < 18 yrs) seen between September 1998 and May 2006 at the pediatric SLE clinic at The Hospital for Sick Children. The study cohort consisted of 156 of the 169 newly diagnosed patients. Thirteen patients were excluded because of missing laboratory data (incomplete testing of autoantibodies). Of these 156 patients, 130 (83.4%) were female. Mean age (± standard deviation) at diagnosis was 12.6 ± 3.3 years. Forty-five patients (28.9%) were Caucasian, 40 (25.6%) Asian, 30 (19.2%) Black, 25 (16.0%) South Asian (Indian), and 16 (10.3%) were of other ethnic origin (mixed ethnicities, Hispanic/Latino, Native Canadians). Symptoms of SLE present at diagnosis or within the first year of disease are summarized in Table 1.

Ethnicity and autoantibody patterns. The distribution of autoantibodies among the 5 ethnic groups was examined first. The only differences found among the ethnic groups were in the frequency of anti-U1RNP and anti-Sm antibodies (p < 0.0001 and p = 0.032, respectively) but not in the frequency of the other autoantibodies tested (Table 2). As expected, anti-Jo1 antibodies were not detected in any
patient and were excluded from further analysis. When patients were grouped into large groupings of Caucasians (45 patients) and non-Caucasians (111 patients), there was a statistically significant difference for frequencies of both anti-U1RNP and anti-Sm antibodies, which were each present in only 24.4% of Caucasian patients as compared to 62.2% of non-Caucasian patients for anti-U1RNP (p < 0.0001) and 49.5% for anti-Sm antibodies (p = 0.004).

Clustering of autoantibodies and associations of clusters with disease manifestations. Cluster analysis revealed that the best fit was for 3 major autoantibody clusters. There was a significant difference in the frequency of anti-dsDNA, antichromatin, antiribosomal P, anti-U1RNP, anti-Sm, anti-Ro, anti-La, and antitopoisomerase I antibodies, but not LAC or aCL antibodies among the clusters (Table 3). Only autoantibodies that were present in at least 50% of patients in a cluster were considered a strong characteristic of the cluster, and therefore although antitopoisomerase I antibodies were most frequently found in cluster 2, because of the relatively low prevalence of these autoantibodies (30% of patients in cluster 2), they were not considered an important characteristic of this or any cluster.

Cluster 1 was characterized by the presence of anti-dsDNA antibodies and a low prevalence of all other autoantibodies. The frequency of anti-dsDNA antibodies in this cluster (61.2%) was, however, lower than in cluster 2 (86.7%; p = 0.017) and in cluster 3 (87.9%; p < 0.001). Patients in cluster 2 had multiple autoantibodies: anti-dsDNA, antichromatin, antiribosomal P, anti-U1RNP, anti-Sm, anti-Ro, and anti-La antibodies. Cluster 3 was characterized by the presence of anti-dsDNA, anti-U1RNP and anti-Sm antibodies.

When the ethnic background of patients was studied in the context of autoantibody clustering, the highest proportion of Caucasians was in cluster 1 at 44.1%, as compared to 23.3% of cluster 2 (p = 0.05) and 13.8% in cluster 3 (p < 0.001). The frequency of the other ethnicities did not differ among the clusters (Table 4).

Last, the frequencies of clinical or laboratory features of pSLE were examined among the clusters. There was a statistically significant difference among the 3 clusters for malar rash (p = 0.003), serositis (p = 0.022), nephritis (p < 0.001), renal failure (p = 0.003), neuropsychiatric disease (p = 0.036), and hemolytic anemia (p = 0.018), but not for any of the other clinical or laboratory features (Table 5).

Cluster 1 was characterized by more frequent presence of a malar rash (85.3%) than cluster 2 (53.3%; p < 0.001) or cluster 3 (70.7%; p = 0.046). This cluster had the lowest

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### Table 2. Distribution of autoantibodies based on ethnic background.

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Asian, n = 40 (%)</th>
<th>South Asian, n = 25 (%)</th>
<th>Black, n = 30 (%)</th>
<th>Caucasian, n = 45 (%)</th>
<th>Other, n = 16 (%)</th>
<th>Total, n = 156 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA</td>
<td>31 (77.5)</td>
<td>19 (76.0)</td>
<td>24 (80.0)</td>
<td>30 (66.7)</td>
<td>15 (93.8)</td>
<td>119 (76.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Antichromatin</td>
<td>19 (47.5)</td>
<td>9 (36.0)</td>
<td>9 (30.0)</td>
<td>19 (42.2)</td>
<td>10 (62.5)</td>
<td>66 (42.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Antiribosomal P</td>
<td>12 (30.0)</td>
<td>5 (20.0)</td>
<td>7 (23.3)</td>
<td>8 (17.8)</td>
<td>3 (18.8)</td>
<td>35 (22.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-U1RNP</td>
<td>25 (62.5)</td>
<td>11 (44.0)</td>
<td>19 (63.3)</td>
<td>11 (24.4)</td>
<td>14 (87.5)</td>
<td>80 (51.3)         &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>19 (47.5)</td>
<td>10 (40.0)</td>
<td>16 (53.3)</td>
<td>11 (24.4)</td>
<td>10 (62.5)</td>
<td>66 (42.3)         0.032</td>
<td></td>
</tr>
<tr>
<td>Anti-SSA(Ro)</td>
<td>17 (42.5)</td>
<td>8 (32.0)</td>
<td>14 (46.7)</td>
<td>13 (28.9)</td>
<td>8 (50.0)</td>
<td>62 (39.7)         NS</td>
<td></td>
</tr>
<tr>
<td>Anti-SSB(La)</td>
<td>17 (42.5)</td>
<td>3 (12.0)</td>
<td>7 (23.3)</td>
<td>7 (15.6)</td>
<td>2 (12.5)</td>
<td>26 (16.7)         NS</td>
<td></td>
</tr>
<tr>
<td>Antitopoisomerase I</td>
<td>17 (42.5)</td>
<td>2 (8.0)</td>
<td>6 (20.0)</td>
<td>6 (13.3)</td>
<td>4 (25.0)</td>
<td>23 (14.7)         NS</td>
<td></td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>17 (42.5)</td>
<td>6 (24.0)</td>
<td>2 (6.7)</td>
<td>7 (15.6)</td>
<td>1 (6.3)</td>
<td>23 (14.7)         NS</td>
<td></td>
</tr>
<tr>
<td>Anticardiolipin</td>
<td>21 (52.5)</td>
<td>15 (60.0)</td>
<td>19 (63.3)</td>
<td>21 (46.7)</td>
<td>10 (62.5)</td>
<td>86 (55.1)         NS</td>
<td></td>
</tr>
</tbody>
</table>

NS: not statistically significant.

### Table 3. Cluster analysis: autoantibody frequencies.

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Cluster 1, n = 68 (%)</th>
<th>Cluster 2, n = 30 (%)</th>
<th>Cluster 3, n = 58 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-dsDNA</td>
<td>42 (61.2)</td>
<td>26 (86.7)</td>
<td>51 (87.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Antichromatin</td>
<td>19 (27.9)</td>
<td>21 (70.0)</td>
<td>26 (44.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Antiribosomal P</td>
<td>5 (7.4)</td>
<td>16 (53.3)</td>
<td>14 (24.1)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anti-U1RNP</td>
<td>0 (0.0)</td>
<td>25 (83.3)</td>
<td>55 (94.8)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>0 (0.0)</td>
<td>15 (50.0)</td>
<td>51 (87.9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anti-SSA(Ro)</td>
<td>12 (17.7)</td>
<td>30 (100.0)</td>
<td>20 (34.5)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anti-SSB(La)</td>
<td>5 (7.4)</td>
<td>21 (70.0)</td>
<td>0 (0.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Antitopoisomerase I</td>
<td>8 (11.8)</td>
<td>9 (30.0)</td>
<td>6 (10.4)</td>
<td>0.03</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>11 (16.2)</td>
<td>4 (13.3)</td>
<td>8 (13.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Anticardiolipin</td>
<td>31 (45.6)</td>
<td>17 (56.7)</td>
<td>38 (65.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: not statistically significant.
incidence of renal involvement (19.1%) versus cluster 2 (60.0%; p < 0.001) and cluster 3 (39.7%; p = 0.011). Patients in cluster 1 also had the lowest incidence of serositis (5.9%); however, this was statistically significant only when compared to cluster 2 (26.7%; p = 0.007) but not cluster 3 (13.8%, p = 0.132). Thrombocytopenia tended to occur more often in cluster 1 (35.3%) than in cluster 2 (16.7%; p = 0.063) and cluster 3 (19.0%; p = 0.041).

Cluster 2 was characterized by the highest incidence of hemolytic anemia (40%) versus cluster 1 (17.7%; p = 0.018) and cluster 3 (15.5%; p = 0.011). Renal involvement was also most common in cluster 2 (60%) and moreover, 26.7% of patients in cluster 2 presented with renal failure within the first year of their disease, which was statistically significant compared to cluster 1 (4.4%; p = 0.003) or cluster 3 (8.6%; p = 0.024).

Patients in cluster 3 tended to more frequently have neuropsychiatric disease (32.8%) compared to cluster 2 (10.0%; p = 0.021) and cluster 1 (19.1%; p = 0.079). The most frequent neurological manifestations in cluster 3 were psychosis and cerebrovascular disease, which represented 37% and 32%, respectively, of all neuropsychiatric syndromes in the cluster. Headaches accounted for 16%, cognitive dysfunction for 10%, and depression for 5% of all neuropsychiatric syndromes in this cluster.

**DISCUSSION**

Production of autoantibodies is the hallmark of patients with SLE. Preceding reports have demonstrated a relationship between ethnicity and autoantibody profiles in adult-onset SLE\(^2\)\(^8\). In addition, studies have suggested that there is a clustering of autoantibodies in patients with aSLE\(^10,14\). In contrast to these reports in aSLE, there are fewer reports on the role of autoantibodies in pSLE\(^15,16\), and none has evaluated antibody clustering and clinical associations. Ethnic differences in autoantibody profiles in children diagnosed with pSLE are reported in our study, and it was shown for the first time that autoantibodies occur in clusters in pSLE and that these clusters differ in their clinical characteristics.

Previous studies examining the relationship between ethnicity and autoantibody patterns in SLE reported a high prevalence of anti-Sm and anti-U1RNP antibodies in African American patients\(^2\)\(^8\). Consistent with these reports, a significantly higher prevalence of anti-Sm and anti-U1RNP antibodies was observed in patients of African and Asian origin as compared to Caucasians. To our knowledge, this is the first report to examine this issue in a single pediatric center.

Three distinct autoantibody clusters were identified: cluster 1 consisted of anti-dsDNA antibodies; cluster 2 consisted of anti-dsDNA, antichromatin, antiribosomal P, anti-U1RNP, anti-Sm, anti-Ro, and anti-La antibodies; and cluster 3 consisted of anti-dsDNA, anti-Sm, and anti-U1RNP antibodies. The distribution of aCL and LAC did not differ among the 3 clusters, and therefore it was not possible to evaluate their association with clinical presentation of SLE. This is in contrast to the findings of To and Petri\(^14\), who found that patients with aCL, LAC, and anti-dsDNA antibodies formed a distinct cluster in aSLE. Although antitopoisomerase I was most frequently found in cluster 2 (30% of patients as compared to 12% for cluster 1 and 10% for cluster 3), it did not meet our criterion of presence in ≥50% of patients to be considered a strong characteristic of this cluster. While antitopoisomerase I antibodies have been described in up to 25% of patients with aSLE\(^24\), this is the first report on the presence of antitopoisomerase I antibodies in pSLE. It would be important to validate these results on other diagnostic platforms and assays, and thus this observation requires further study.

Cluster 1, which had a very low prevalence of all autoantibodies except anti-dsDNA antibodies, represented a subset of patients with an overall mild disease. Renal involvement occurred in fewer than 20% of patients in this cluster. At first glance, these findings appear to contradict the previous evidence of the association of anti-dsDNA antibodies and renal involvement\(^9\). However, closer examination reveals that...
although cluster 1 was characterized by anti-dsDNA antibodies, patients in this cluster were less likely to have anti-dsDNA antibodies than patients in the other clusters. In addition, cluster 1 had the highest percentage of Caucasian patients, and they are less likely to have renal involvement than Black patients. It is also possible that, in addition to anti-dsDNA, other autoantibodies, such as antichromatin antibodies, are required to increase the risk of renal involvement. This is supported by the highest incidence of nephritis and renal failure in cluster 2, which was characterized by a high prevalence of 6 other autoantibodies in addition to anti-dsDNA. The association of anti-Sm and anti-U1RNP antibodies and renal disease has previously been suggested in most but not all studies. The ethnic background of the different populations studied may be at least partly responsible for the contradictory results in the literature, and it is possible that autoantibodies per se are less important than the ethnicity of the patients in determining the risk for development of renal disease.

Consistent with a previous Canadian study of aSLE, patients with malar rash were more likely to have anti-dsDNA antibodies without other autoantibodies (cluster 1). However, clustering of anti-Ro antibodies and photosensitivity, as suggested by that study, was not observed. In keeping with previous reports in adults, it was noted that hemolytic anemia was associated with anti-Ro and antiribosomal P antibodies (cluster 2). The association of antiribosomal P antibodies and neuropsychiatric disease, as suggested by other groups, was not confirmed. However, the low number of pSLE patients with depression or psychosis, the specific central nervous system (CNS) manifestations associated with antiribosomal P antibodies, did not allow us to analyze these patients separately in the cluster analysis. The highest incidence of neuropsychiatric disease in our cohort was in cluster 3, which was characterized by anti-dsDNA, anti-U1RNP, and anti-Sm antibodies. Research on the role of these antibodies in nervous system disease in SLE has yielded conflicting results. The most consistent finding in aSLE has been an association of antiphospholipid antibodies with CNS involvement, which was not evaluated in our study as there was no difference in the distribution of antiphospholipid antibodies among our clusters.

This study is an exploratory analysis of antibody clusters and their clinical correlations in patients with pSLE. We did not correct our statistical analysis for multiple testing, and therefore the p values must be interpreted with caution. However, as our study is a hypothesis-generating work, we decided not to adjust for multiple testing but rather suggest that these findings need to be confirmed in a separate pediatric cohort. All patients were screened for presence of selected autoantibodies by an autoantigen array and laser-based flow technology. The advantages of this technology include the capacity to rapidly analyze a complex array of autoantibodies in a single sample at the same time, requiring only a small amount of serum. Early studies showed a high level of agreement (> 90%) with conventional techniques, and this technology was found to be reliable, accurate, cost-effective, and highly sensitive.

Our study demonstrated ethnic differences in autoantibody profiles in a cohort of pediatric patients with SLE, and confirmed a significantly higher prevalence of anti-Sm and anti-U1RNP antibodies in non-Caucasian patients as suggested by other groups. In addition, the results confirm that autoantibodies in pSLE exist in distinct clusters. Significant associations between clusters of autoantibodies and clinical or laboratory features of pediatric lupus were noted. From the clinical perspective, this finding of an association of autoantibody clustering and clinical features suggests that determining the complete autoantibody profile may help predict the clinical course of pSLE and identify patients at risk of developing major organ involvement. Our results imply that the clinical associations of autoantibodies differ between patients with pediatric SLE and those with adult-onset SLE.

REFERENCES


