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Approval Sheet

Severity of Childhood-onset Systemic Lupus Erythematosus: Impact of Preceding and Co-existing Autoimmune Cytopenias

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Severity of Childhood-onset Systemic Lupus Erythematosus: Impact of Preceding and Co-existing Autoimmune Cytopenias

By

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Abstract

Severity of Childhood-onset Systemic Lupus Erythematosus: Impact of Preceding and Co-existing Autoimmune Cytopenias

By

Ekemini Akan Ogbu, MBBS

Background: Autoimmune cytopenias may precede or occur with childhood-onset systemic lupus erythematosus (cSLE). Adult studies suggest that lupus patients with concurrent autoimmune cytopenias have relatively lower prevalence of lupus nephritis (LN) and are a unique sub-population. Therefore, the objectives of our study were to assess whether in cSLE, autoimmune cytopenias decrease the 2-year risk and severity of LN; to assess associated serological differences in those with and without autoimmune cytopenias and the effect of prior immune therapy for autoimmune cytopenia on 2-year risk of LN; and to perform descriptive analyses of these pediatric patients without LN at cSLE diagnosis.

Methods: Ours was a retrospective cohort study of incident cSLE cases over a 16-year period. We included patients aged less than 17 years who met the classification criteria for SLE. We excluded patients diagnosed outside our institution and those with LN at cSLE diagnosis. Our follow-up period was 2 years. We defined autoimmune cytopenia as either autoimmune hemolytic anemia, Coombs positive anemia without hemolysis, immune thrombocytopenia or Evan's syndrome.

Results: Our study included 130 incident cSLE patients. Of these, 43 (33%) had autoimmune cytopenia before or at cSLE diagnosis. Those with autoimmune cytopenia had significantly more neuropsychiatric symptoms and higher mean ESR versus those without autoimmune cytopenia. However, they had less arthritis, malar rash and myositis. 2-year incidence of LN was 12% in our cohort. Patients with autoimmune cytopenia had lower 2-year risk of LN compared to other cSLE patients (7% vs 15%). Of the 16 patients who developed LN, those with autoimmune cytopenia had mostly class V (2 of 3 patients) versus mostly class III and IV in those without autoimmune cytopenia (6 of 12 patients). None of the 13 patients pre-treated for autoimmune cytopenia prior to cSLE diagnosis developed LN.

Conclusion: Patients with autoimmune cytopenia before or at cSLE diagnoses have significant and clinically relevant differences in their presentation from other cSLE patients. Our findings call for further studies on the immunologic and genetic bases of these differences.

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Table of Contents

INTRODUCTION

Systemic lupus erythematosus (SLE) is a leading cause of autoimmune disease in children. Up to 20% of patients with SLE have disease onset in childhood (1). Although it has been suggested that childhood-onset SLE (cSLE) and adult-onset disease have similar pathogenesis, cSLE has a more severe clinical phenotype and is associated with higher morbidity and mortality (2). This risk of morbidity and mortality is heightened by the presence of end-organ involvement such as lupus nephritis (LN). LN is a particularly debilitating indicator of severe cSLE with a prevalence of 50 - 67% (3, 4). Among those with cSLE who develop LN, most (80%) develop LN within the 1st year of cSLE diagnosis. The remaining 20% mostly develop LN between 1 – 2 years after cSLE diagnosis (4).

Despite advances in therapeutics and care for LN, remission rates post-induction therapy and while on maintenance therapy are still sub-optimal.(5) There is still a high rate of renal flares while on therapy and one-third of LN patients who develop end-stage renal disease (ESRD) will need to be transplanted within the first 5 years of LN diagnosis (6). Therefore, research in cSLE has focused not only on improving therapeutics, but also on identifying risk factors and predictors of cSLE severity. There has been growing recognition of the positive impact of early recognition and diagnosis of this disease in improving outcomes (7-9). There has also been a focus on identifying populations at risk of developing cSLE and sub-populations of cSLE patients whose phenotype differ from others and add to our understanding of this disease and its outcomes (10).

Autoimmune cytopenias including autoimmune hemolytic anemia (AIHA), Coombs positive anemia without active hemolysis, idiopathic thrombocytopenic purpura (ITP) and Evans syndrome (ES) may precede SLE or occur at the time of diagnosis (4, 11-13). Surprisingly, some studies suggest that patients with coexisting autoimmune cytopenias and SLE have lower incidence and prevalence of LN compared to those SLE patients without autoimmune cytopenias

and thus, may represent a unique sub-population (14-16). These prior studies were conducted in predominantly adult populations in Europe, Asia and Latin America. However, there has been no clear explanation for this observation and this phenomenon has not been well studied in children. It is still unclear if coexisting autoimmune cytopenia is associated with better clinical outcome in cSLE (2, 17). Since autoimmune cytopenias are more common in cSLE and often occur early in this disease, assessing if they are a unique sub-population and their impact on cSLE phenotype is important. In addition, some patients with idiopathic autoimmune cytopenia, prior to cSLE diagnosis, undergo immunosuppressive or immunomodulatory therapy similar to the treatment for cSLE. It is unclear if this treatment for idiopathic autoimmune cytopenia has an effect on subsequent cSLE phenotype and developing LN. This has not previously been explored.

Therefore, our objective was to conduct a retrospective cohort study to assess whether the presence of preceding or co-existing autoimmune cytopenias in children with SLE decreases the 2-year risk and severity of LN compared to those without autoimmune cytopenia. We assessed serologic differences in patients with autoimmune cytopenia before or at cSLE diagnosis in comparison to other cSLE patients without autoimmune cytopenias. Specifically, we examined differences between the presence of anti-double-stranded deoxyribonucleic acid (anti-dsDNA) and anti- ribonucleoprotein (anti- RNP) which have been associated with increased and decreased LN risk respectively (18-20). We also evaluated whether receiving treatment for autoimmune cytopenia prior to cSLE diagnosis decreases the 2-year risk of LN compared to other cSLE patients with autoimmune cytopenia who were not pre-treated and those without autoimmune cytopenia. Finally, as our study was conducted on one of the largest single-center cohorts of diverse cSLE patients in the United States, we performed a descriptive analysis of our population of pediatric patients without LN at cSLE diagnosis and estimated differences in race, sex and age at diagnosis associated with the 2-year risk of LN.

BACKGROUND

cSLE is the prototypic autoimmune disease characterized by multisystem immune-dysregulation and auto-antibody formation. It is the second most common pediatric rheumatologic disease with a worldwide incidence of 0.36 to 2.5 per 100,000 children and prevalence of 1.89 to 25.7 per 100,000 children (21-23).

cSLE is a severe clinically heterogeneous disease whose management is often challenging. Factors contributing to the severity in children compared to adults are not well understood. Lupus nephritis (LN) occurs more frequently in cSLE and is a particularly debilitating complication of this disease (23). The reason for this increased frequency of LN in cSLE compared to adults is unclear. However, the underlying pathoetiology of LN is similar in cSLE and adults (2, 24). In LN there is immune complex formation in the kidney with aberrant clearance of apoptotic debris, leading to complement activation and consequent chronic inflammation in the glomeruli and interstitium (25). These changes can be seen on renal biopsy, which is currently the gold standard for diagnosing and determining LN severity.

LN has a pathognomonic pattern of immune deposits. There are 6 distinct classes (I - IV) described in the 1995 World Health Organization and 2003 International Society of Nephrology/Renal Pathology Society criteria although class V can overlap with class II, III and IV(6, 26, 27). Class III, IV, V and IV are severe forms. However, patients with Class III and IV LN have a higher risk of progressing to ESRD than class V LN (25).

For poorly understood reasons, cSLE tends to be associated with more aggressive LN than adult disease. Similarly, black, Hispanic and Asian race/ethnicity are associated with more aggressive LN compared to non-Hispanic white race/ethnicity (28, 29). Factors such as age at onset of cSLE, duration of poorly controlled cSLE activity, physiologic age-related changes in the kidney, genetics, sex and socio-economic factors have been considered. However, none of these factors

independently or collectively fully explains the differences in LN manifestation. Rather, they emphasize the need for a better understanding of the processes that contribute to improving long-term outcomes.

Our study focuses on patients with primary autoimmune cytopenias who developed cSLE and cSLE patients with autoimmune cytopenias at time of cSLE diagnosis as a distinct sub-group. Extrapolating from adult studies, these patients could have lower risk of renal disease (14-16). These autoimmune cytopenias occur more frequently in children and include autoimmune hemolytic anemia (AIHA), Coombs positive anemia without active hemolysis, immune thrombocytopenic purpura (ITP) and Evans syndrome (ES) (2). Zhang et al reported a significantly lower incidence of LN in patients with concurrent ES and systemic lupus erythematosus (15). This was primarily an adult study of an Asian population, which did not specifically look at cSLE. Lavelle et al found less severe renal disease in patients with AIHA, ITP or ES in their population (16). Alger et al noted lower overall disease severity and nephropathy in their patients with systemic lupus erythematosus and concurrent AIHA or ITP (14). The prospective cohort study by Nossent et al found that patients with AIHA were likely to have serositis but found no difference in incidence of renal or cerebral manifestations in their patients with AIHA or ITP (30).

Conversely, there have been studies that showed an association between autoimmune cytopenias and more severe SLE. Costallat et al found a higher incidence of LN in patients with ES and SLE versus SLE alone (31). Aleem et al demonstrated that, in their Saudi-Arabian population, AIHA at the time of presentation was not associated with renal disease but predicted neurologic involvement (32). They also found that thrombocytopenia at the time of presentation was associated with neurological involvement. As adult studies do not always concur with findings in pediatric disease, our study will be very relevant in examining if the presence of autoimmune

cytopenias is associated with a less severe cSLE phenotype particularly as it relates to renal disease.

Much as the association of autoimmune cytopenias and lower occurrence of LN has been described, there is no clear explanation for this. It could be multifactorial and include an alteration of the known mechanisms of inflammation in LN directly affected by the red blood cells (RBCs) and platelets (33). It is known that RBCs and platelets play a role in the systemic inflammation in cSLE. In particular, RBCs are active in clearing circulating immune complexes (CIC). These CIC bind to the C3b receptor (CR1) of RBCs, which deposit them in the liver and spleen. This action is thought to mitigate the effects of immune complex disease on other end organs such as the kidney (34-36). The nature of this process in the presence of Coombs positive anemia without active hemolysis and AIHA is not clearly defined. This buttresses the question of whether there are inherent protective mechanisms in the sub-group of patients with autoimmune cytopenias or if there are external contributing factors that impact their clinical phenotype.

Treatment for isolated autoimmune cytopenia such as AIHA or ITP often includes the use of therapies which suppress or modulate the immune system.(37) These immune-directed therapies are also used to treat cSLE. Therefore, we think that the use of these medications could potentially influence a subsequent cSLE phenotype and risk of LN. Rituximab, cyclophosphamide, corticosteroids and intravenous immunoglobulin (IVIG) are such immune-directed therapies. Rituximab is an anti-CD20 chimeric monoclonal antibody and cyclophosphamide is a potent alkylating agent. Rituximab, cyclophosphamide and IVIG can have particularly long-lived effects up to 6 months of more. The duration of effect of corticosteroids is variable depending on the dose, frequency and duration of therapy. However, regardless of duration of exposure, in mouse models, it has been demonstrated that early immune modifying therapy can impact SLE phenotype by preventing development of endothelial dysfunction and reducing progression of nephritis (38, 39).

cSLE is a serologically heterogeneous disease. The implication of the various serum biomarker patterns in end-organ involvement is still being investigated. However, it has been determined that some of the serologic markers are associated with risk of LN. Though these biomarkers have limited sensitivity and specificity, they are still helpful in clinically distinguishing patients that need increased surveillance or earlier intervention. Positive anti-dsDNA occurs in 72 – 93% of cSLE patients and anti-RNP occurs in 27 – 62% of cSLE patients (1, 6, 28, 40). In some studies, they have been associated with increased and decreased risk of LN respectively (18-20). As both of these markers are commonly tested, assessing if there is a difference in their frequency in patients with cSLE and autoimmune cytopenia relative to other cSLE patients will be important in understanding the risk of LN.

Our study has been conducted on one of the largest single-center cohorts of cSLE patients. We have a predominantly black pediatric population, but also a substantial percentage of Hispanic and non-Hispanic white patients. Our results will inform further studies on autoimmune cytopenias in cSLE as well as potential age, sex, racial differences in cSLE overall.

METHODS

Specific Aims and Hypotheses

Aim 1: Perform a descriptive analysis of our population of pediatric patients without LN at cSLE diagnosis by age, race, sex and autoimmune cytopenia status.

Aim 2: Conduct a retrospective cohort study to assess whether the presence of preceding or co-existing autoimmune cytopenias at diagnosis of cSLE decreases the 2-year risk and severity of LN compared to those cSLE patients without autoimmune cytopenia.

Hypothesis #2a: Patients with preceding or co-existing autoimmune cytopenias at time of cSLE diagnosis have decreased 2-year risk of lupus nephritis compared to other cSLE patients without autoimmune cytopenias.

Hypothesis #2b: Patients with cSLE and co-existing or preceding autoimmune cytopenias who develop LN within 2 years of cSLE diagnosis have less severe lupus nephritis compared to other cSLE patients who develop lupus nephritis.

Aim 3: Conduct a cross-sectional analysis of patients with and without autoimmune cytopenia at cSLE diagnosis, to assess differences in baseline serological markers known to be associated with LN risk.

Hypothesis #3a: Patients with cSLE and co-existing or preceding autoimmune cytopenias have a lower prevalence of anti-dsDNA antibodies at diagnosis compared to other cSLE patients without co-existing or preceding autoimmune cytopenias.

Hypothesis #3b: Patients with cSLE and co-existing or preceding autoimmune cytopenia have a higher prevalence of anti-RNP antibodies at diagnosis compared to other cSLE patients without co-existing or preceding autoimmune cytopenias.

Aim 4: Conduct a retrospective cohort study to **e**xamine whether receiving treatment for autoimmune cytopenia prior to cSLE diagnosis decreases the 2-year risk of LN compared to other cSLE patients with autoimmune cytopenia who were not pre-treated and cSLE patients without autoimmune cytopenia.

Hypothesis #4: Patients with cSLE and co-existing or preceding autoimmune cytopenias who had treatment with immunomodulatory or immunosuppressive therapy (IVIG, corticosteroids, rituximab or cyclophosphamide) prior to diagnosis of cSLE have decreased 2-year risk of LN compared to other cSLE patients with autoimmune cytopenias.

Study design and setting

This was a retrospective cohort study of incident cSLE patients at the Emory Children's Center and the Children's Healthcare of Atlanta pediatric rheumatology service over a 16-year period. Approval of the study protocol with waiver of informed consent was obtained from the Children's Healthcare of Atlanta IRB.

Characteristics of Study Population

We extracted patient data from electronic medical records and paper charts with ICD 9 or 10 codes corresponding to a diagnosis of SLE between January 1, 2000 and June 30, 2016. We included patients who were diagnosed at age 2 to less than 17 years and who met at least 4 of the 11 American College of Rheumatology (ACR) and/or Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE (41, 42). We excluded patients with a pre-existing diagnosis of cSLE who transferred care to our center and those with LN at time of cSLE diagnosis. Our follow-up period was 2 years from time of cSLE diagnosis.

Measurements

We defined time of cSLE diagnosis (baseline) as time of initial evaluation for cSLE by a pediatric rheumatologist at our institution. Variables defined at diagnosis included data at initial evaluation up to 1-month post cSLE diagnosis. Autoimmune cytopenia referred to AIHA (Coombs positive anemia with active hemolysis), Coombs positive anemia without active hemolysis, thrombocytopenia and/or Evans syndrome. We defined the presence of autoimmune cytopenia as preceding diagnosis of a primary autoimmune cytopenia and/or the presence of an autoimmune cytopenia at the time of initial evaluation for cSLE and up to 1-month post cSLE diagnosis. We defined AIHA as hemoglobin ≤10 g/dl with positive direct Coombs and/or reticulocytosis. Coombs positive anemia was defined as hemoglobin ≤10 g/dl with positive direct Coombs but without evidence of active hemolysis. We defined ITP as thrombocytopenia <100,000/mm^3 and Evans syndrome as concurrent or sequential AIHA and ITP.

Demographic, clinical and laboratory data were obtained at baseline. We used age greater than 9 years as a proxy for puberty. We defined positive ANA as titers ≥ 1:40 (41, 42). We included the SLEDAI-2K which is measurement of overall cSLE activity (43). SLEDAI-2K scores greater than or equal to 6 indicate active cSLE and higher scores indicate more disease activity. Neuropsychiatric symptoms were classified using ACR nomenclature and case definitions (44). Renal parameters (urinalysis, urine microscopy, urine protein to creatinine ratio (UPCr) and estimated glomerular filtration rate (eGFR)) were extracted at baseline and at LN diagnosis. UPCr and eGFR were collected as continuous variables. UPCr was sub classified as a dichotomous outcome with ≥ 2mg/mg as nephrotic range proteinuria and < 2 as sub-nephrotic proteinuria. eGFR was calculated using the modified Schwartz formula which is a validated measure for patients aged 2 to 18 years (45). Decreased eGFR < 90mL/min/1.73m^2 is indicative of decreased renal function.

We defined LN as the presence of persistent UPCr > 0.5 and/or ≥ 3+ proteinuria in a patient with cSLE and/or renal biopsy demonstrating LN. UPCr was sub classified as a dichotomous outcome with ≥ 2 as nephrotic range proteinuria and < 2 as sub-nephrotic proteinuria. Time of LN diagnosis was categorized in reference to time of cSLE diagnosis as: LN diagnosed within the first 6 months from baseline, LN diagnosed between > 6 months to ≤ 12 months from baseline and diagnosis of LN > 12 months to ≤ 24 months from baseline. LN classification was derived from the 1995 World Health Organization and/or the 2003 International Society of Nephrology/Renal Pathology Society criteria for renal biopsy (46). We sub-classified the six broad classes into mild renal disease (Class I and II only) and severe (Class III, IV, V, VI and any combination of Class V with other classes). The use of immune-directed therapy prior to time of cSLE diagnosis was obtained as a dichotomous variable for rituximab, cyclophosphamide, IVIG and corticosteroids.

Statistical Analyses

We carried out descriptive statistics for all variables of interest including demographic, clinical, and laboratory variables. Continuous variables were summarized using means and standard deviations and/or medians and interquartile ranges. Counts and percentages were calculated for categorical variables. Differences in continuous variables were tested using two sample t-tests or Wilcoxon rank sum test for non-parametric measures. Differences in categorical variables were tested using chi-square tests or Fisher's exact test for expected counts less than 5. All analyses were performed in SAS v 9.4 (Cary, NC). We used a statistical significance level of $p < 0.05$.

Descriptive analyses

We described characteristics of all patients with cSLE at baseline and compared those with autoimmune cytopenia to other cSLE patients without autoimmune cytopenia. We also compared

characteristics by age less than 9 or greater/equal to 9 years, by sex and by race (black versus other).

Association of autoimmune cytopenia at baseline and 2-year LN risk

Among all cSLE patients, we compared characteristics between patients with autoimmune cytopenia at baseline to other cSLE patients. Univariate and multivariable logistic regression models were developed with the outcome of LN to identify the independent and adjusted association between the presence of autoimmune cytopenias and LN. Variables with univariate associations with incident LN showing p-values < 0.2 as well as our covariates of interests (age at cSLE diagnosis, sex, ethnicity, race, the presence of anti-dsDNA, and anti-RNP, and prior use of immune-directed therapy) were included in the initial model. Manual model selection strategies were used to produce the final model using a priori list of confounders.

Association of autoimmune cytopenia at baseline and LN severity

Among patients who developed LN, we compared characteristics by the autoimmune cytopenia status at baseline. Specifically, we compared the frequency of mild LN (Class 1and II only) by autoimmune cytopenia status.

Differences in known serologic markers of LN risk at baseline by autoimmune cytopenia status

Among all cSLE patients at baseline, proportions of positive anti-dsDNA and anti-RNP were compared between those with and without autoimmune cytopenias.

Association of immune-directed treatment for autoimmune cytopenia prior to cSLE diagnosis and 2-year LN risk

Among all patients with cSLE, we compared the 2-year risk of LN among three groups. i) those who had prior immune-directed treatment for autoimmune cytopenia; ii) those who had

autoimmune cytopenia but did not receive prior immune-directed treatment; iii) cSLE patients without autoimmune cytopenia.

Sensitivity Analyses

We planned a sensitivity analysis a priori to re-analyze our data excluding those patients with less than 2 years of follow-up to assess if the observed association of autoimmune cytopenia and 2-year LN risk still held.

Potential Pitfalls

We anticipated missing data as this was a retrospective study. We conducted complete case analyses to account for missing data.

We also expected that renal biopsy would not be done in all patients at time of LN diagnosis, which could limit histologic diagnosis and classification. But we anticipated that this would involve a small minority of patients as renal biopsies are often obtained at time of LN diagnosis.

We anticipated that our study would be underpowered for some of our proposed analyses. However, we expected to still demonstrate clinically significant differences to inform further clinical and immunologic studies.

RESULTS

We identified 397 patients with ICD-9 or ICD-10 codes corresponding to SLE between January 1, 2000 and June 30, 2016 from our medical records. We excluded a total of 267 of these patients who were aged 17 years or more at baseline (n = 40), who did not meet ACR or SLICC criteria for systemic lupus erythematosus (n = 24), who transferred care to our center (n = 91) or who had LN at baseline (n = 112). Our final study population had 130 incident cases of cSLE (Figure 1). Forty-three (33%) of these patients had autoimmune cytopenia before or at the time of cSLE diagnosis.

Demographic, clinical and laboratory characteristics of final study population at baseline (Aim 1):

Table 1 summarizes the demographic, clinical and laboratory characteristics of 130 incident cSLE patients of which 107 were female. There were 81 (66%) black, 23 (19%) white, 7 (6%) Asian and 10 (8%) Hispanic patients. The mean age at cSLE diagnosis was 12 (SD 3) years.

Arthritis (50%), fever (42%), and malar rash (36%) were the most commonly occurring clinical features. Neuropsychiatric symptoms (6%), angioedema (6%), nasal ulcers (3%) and discoid rash (2%) were the least common. Pericardial effusion was seen in 17 of 53(32%) patients. Pleural effusion and myositis were seen in 26 of 85 (31%) and 20 of 69 (29%) the patients respectively.

One-hundred and thirty patients had positive ANA ≥ 1:40, 88 (70%) had positive anti-dsDNA and 72 (58%) had positive anti-RNP. Positive Coombs test and anemia (hemoglobin ≤ 10g/dl) was seen in 18% of patients while 15% had thrombocytopenia < 100,000/uL. A total of 9 (7%), 4 (3%) and 1 (1%) had a prior diagnosis of ITP, AIHA and ES respectively. Frequency of lymphopenia, low C3 complement and low C4 complement was 49%, 53% and 65% respectively. Median overall disease activity index (SLEDAI-2K) was 9 while 30% of patients had eGFR less than 90ml/min/1.73m^2.

Comparison of final study population at baseline by autoimmune cytopenia status:

Table 2 shows the comparison of 35 (81%) of the 43 patients with autoimmune cytopenia were female. This was similar in the group without autoimmune cytopenia i.e. 72 (83%). There was no difference in race or mean age at T^0 comparing those with autoimmune cytopenia to those without. Patients with autoimmune cytopenia had more neuropsychiatric symptoms (14% versus 2% p 0.02) but less arthritis, malar rash and myositis (31% versus 59% p 0.003, 24% versus 43% p 0.04 and 10% versus 37% p 0.03 respectively). When we compared differences in serologic markers, there was no meaningful difference in the frequency of positive anti-dsDNA and anti-RNP between those with autoimmune cytopenia and those without (72% versus 69% and 56% versus 59% respectively). Patients with autoimmune cytopenia had a higher frequency of elevated erythrocyte sedimentation rate (45% versus 36% p 0.003). There were no statistically significant differences in eGFR, SLEDAI-2K or low C3 and c4 complements.

Comparison of final study population at baseline by race:

As shown in Table 3, when we compared black patients versus patients of other races, females were still the predominant sex in both groups and there was no statistically significant difference in mean age at baseline. Black patients had fewer oral ulcers (16% versus 34% p 0.03) but higher frequency of positive anti-RNP (67% versus 39% p 0.003), anti-Smith (70% versus 33% p 0.0002) antibodies.

Comparison of final study population at baseline by age:

Table 4 shows that 15 of 129 children were aged less than 9 years. Mean age of patients less than 9 years at baseline was 7 (SD 1) and 13 (SD 2) in patients 9 years or older. The younger patients, less than 9 years, had a higher frequency of fever (73% versus 38% p 0.01). There were no statistically significant differences in eGFR or SLEDAI-2K.

Comparison of final study population at baseline by sex

As shown in Table 5, there was no statistically significant difference in race, age, clinical or laboratory features when we compared females to males.

Association of autoimmune cytopenia at baseline and 2-year LN risk (Aim 2):

Overall 2-year risk of LN was 12% in our study population. As shown in Table 2, the 2-year risk of LN was less in patients with autoimmune cytopenia at 7% compared to 15% in those without autoimmune cytopenias.

In our univariate analysis, examining the association of patient characteristics with incident LN as shown in Table 6, the odds ratio comparing patients with autoimmune cytopenia at baseline to those without autoimmune cytopenia was 0.43 (95% CI 0.12, 1.60). The odds ratio of developing LN was 3.81 comparing those with low C3 complement at baseline to those without (95% CI 1.01, 14.42).

Autoimmune cytopenia at baseline and low C3 complement at baseline were included in our final multivariable logistic regression model. As shown in Table 7, the odds ratio of developing LN was 4.24 comparing those with low C3 complement at baseline to those without low C3 complement at baseline adjusting for the presence of autoimmune cytopenia (95% CI 1.10, 16.34).

Association of autoimmune cytopenia at baseline and LN severity (Aim 3):

Tables 8 and 9 summarize the characteristics of the 16 patients who developed lupus nephritis within the 2-year follow-up period. This occurred mostly in the first 6 months and between the 1st and 2nd year. At baseline, 3 of these 16 patients had autoimmune cytopenia. When we compared these patients to those without autoimmune cytopenia at baseline, we found no statistically significant difference in age, baseline eGFR or SLEDAI-2K. We also found no statistically

significant difference in eGFR or UPCr at the time of LN diagnosis. None of the patients with autoimmune cytopenia who developed LN had mild LN compared to 3 (25%) of those without autoimmune cytopenia. Two of the three patients with autoimmune cytopenias had class V LN compared to those without autoimmune cytopenia who had mostly (67%) class III and IV LN.

Association of immune-directed treatment for autoimmune cytopenia prior to cSLE diagnosis and 2-year LN risk (Aim 4):

Of the 43 patients with autoimmune cytopenia at baseline, 10 (23%), 7 (16%) and 2 (5%) were treated with corticosteroids, IVIG and rituximab respectively, prior to cSLE diagnosis. Mean interval from Rituximab administration to cSLE diagnosis was 6 months (SD 5.30). Median interval from IVIG administration to cSLE diagnosis was 4 months (IQR 51). Corticosteroids were mostly administered with Rituximab or IVIG. None of the patients with or without autoimmune cytopenias received cyclophosphamide. Table 10 shows the comparison of the 2-year risk of LN for those who had prior immune-directed treatment for autoimmune cytopenia, those who had autoimmune cytopenia but did not receive prior immune-directed treatment and other cSLE patients without autoimmune cytopenia. None of the 13 patients who had prior treatment developed LN compared to 10% of those who received treatment and 15% of other cSLE patients.

Sensitivity analysis

When we re-analyzed our data excluding those patients with less than 2-year follow-up, the 2-year risk of LN was 8% in patients with autoimmune cytopenia compared to 15% in those without autoimmune cytopenia (p 0.0362).

DISCUSSION

Our primary objective was to compare cSLE patients with autoimmune cytopenias to those without autoimmune cytopenias, to assess whether the presence of autoimmune cytopenias in cSLE decreased the 2-year risk and severity of LN, and whether there were differences in commonly tested serological markers, known to be associated with LN risk, by autoimmune cytopenia status at time of cSLE diagnosis. We also aimed to assess whether receiving treatment for autoimmune cytopenia prior to cSLE diagnosis decreases the 2-year risk of lupus nephritis compared to other cSLE patients with autoimmune cytopenias who did not receive prior treatment and those cSLE patients without autoimmune cytopenias.

As none of the patients included in our final study population would have LN at time of cSLE diagnosis, our secondary objective was to describe this population of patients and also compare differences by race, age and sex.

Overall, our study found clinically relevant differences to support prior reports that cSLE patients with autoimmune cytopenias should be viewed as a distinct sub-population from other patients with cSLE alone (14-16). We found a lower 2-year risk of LN in cSLE patients with autoimmune cytopenias versus other cSLE patients (OR = 0.43 95% CI 0.12, 1.60). We also found that cSLE patients with autoimmune cytopenias who developed LN within the first 2 years, had mostly Class V LN, compared to those without autoimmune cytopenias who had mostly Class III and IV LN. Also, patients with autoimmune cytopenias who were treated with immune-directed therapy prior to cSLE had a lower risk of LN within the first 2 years compared to those with autoimmune cytopenias who did not have prior immune-directed treatment and to cSLE patients without autoimmune cytopenias.

Our study specifically looked at the first 2 years after cSLE diagnosis because this is when LN is more likely to develop. The relatively lower 2-year risk of LN we found in cSLE patients with

autoimmune cytopenias is similar to earlier reports from adult studies that showed lower incidence and prevalence of LN in this subset of patients. We did not find that the presence of anti-dsDNA, anti-RNP or other tested serologic markers at the time of cSLE diagnosis were associated with LN risk. While 30% of our study population had low eGFR levels at cSLE diagnosis, and low C3 at cSLE diagnosis was associated with an increased 2-year risk of LN, we did not find differences in eGFR or low C3 complement between patients with autoimmune cytopenias and those without autoimmune cytopenias to suggest that these factors are associated with their differential LN risk. Similarly, we did not find a difference in both SLEDAI-2K index or low C4 complements, at time of cSLE diagnosis, between the 2 subgroups to explain the difference in LN risk. However, we found other clinical and laboratory findings that further distinguished cSLE patients with autoimmune cytopenias from those without autoimmune cytopenias. Those with autoimmune cytopenias had more neuropsychiatric disease but less arthritis, malar rash and myositis than those without autoimmune cytopenias. ESR is a non-specific marker of ongoing systemic inflammation and was higher in the patients with autoimmune cytopenias compared to the other patients. It is known that ESR may be elevated in the presence of anemia which likely contributed to our observation here because at the time of cSLE diagnosis, the SLEDAI-2K which is a measure of overall disease activity was similar between the two groups.

Prior reports showed that adult patients with autoimmune cytopenias had less severe renal involvement (14, 16). Our study showed that of the 16 cSLE patients who developed lupus nephritis, those with autoimmune cytopenia at cSLE diagnosis had mostly Class V while those without autoimmune cytopenia had mostly Class III and IV LN. While our inference is limited by the small number of patients who developed LN, this observation showed be further explored as Class III and IV are associated with a higher risk of progression to ESRD.

We examined whether some of the decreased risk of LN in autoimmune cytopenia could be attributed to prior immune-directed treatment. Interestingly, our study showed that none of the 13 cSLE patients with autoimmune cytopenia who received immune-directed treatment prior to cSLE diagnosis developed LN. In comparison, the 2-year risks of LN for those with autoimmune cytopenia and cSLE who did not receive pre-treatment and for cSLE patients without any autoimmune cytopenia were 10% and 15% respectively.

We were able to describe our population of 130 patients without LN at cSLE diagnosis. Two-thirds (66 %) of our patients were black. Our population had a mean age at cSLE diagnosis of 12 years which is similar previous reports (28, 40). Our female: male ratio of 4.65:1 was similar to reports from the large cSLE cohort studies from France and Toronto (1, 40). Also, arthritis, fever and malar rash were the most commonly occurring clinical features. Nasal ulcers and discoid rash were uncommon. Black patients had statistically significantly higher frequency of positive anti-RNP and anti-Smith antibodies as has been previously reported (24, 47-49). However, we did not find higher positive anti-SSA/SSB antibodies as reported in others (28). We did not find many differences in cSLE manifestation by age. Younger children, aged less than 9, had more fever. It is thought that factors such as younger age and age-related physiologic changes in the kidney may independently contribute to increased risk of LN. However, there was no difference in the 2-year risk of LN or eGFR at cSLE diagnosis in our study when we compared the two age groups. We also found no statistically significant sex differences in clinical and laboratory features or 2-year risk of LN. Interestingly, 30% of our patients presented with some renal insufficiency though they did not have persistent proteinuria to suggest LN. This renal insufficiency did not differ by autoimmune cytopenia status, age at cSLE diagnosis, race or sex. We think our findings are relevant in understanding the contributing factors to LN risk in cSLE and specifically in patients with autoimmune cytopenias.

A major strength of our study is that it was conducted on a predominantly black pediatric lupus population. We were also able to examine age, sex and racial/ethnic differences. Our study also approached renal outcomes in cSLE from a hematologic perspective. To the best of our knowledge, we are the first to examine the association of pre-treatment with immune-directed therapy for autoimmune cytopenia on renal outcomes within the first 2 years of cSLE diagnosis.

Our study has some limitations. While it was a relatively large single-center study on cSLE, we had a low incidence of LN (2-year risk of 12%) and so our study was likely underpowered to detect statistically significant differences in risk and severity of LN and prior therapy effects. Also, it was a retrospective study and so were limited to using the available data. The first and last patients were separated by 16 years and there were differences in physician practice over this period in obtaining laboratory and imaging data to evaluate for cSLE. This was a single center study and so generalizability may be limited. However, there is a dearth of pediatric rheumatologists and so our institution is the regional center for most of the southeastern US states. Therefore we have a representative sample population. Also our center does not routinely perform activity or chronicity scoring of kidney biopsies which would have added more information about LN severity. Finally, we had a short follow-up period of 2 years but more than 80% of LN develop within the first 2 years of cSLE diagnosis (4).

In conclusion, our findings indicate that patients with autoimmune cytopenia before or at cSLE diagnoses have significant and clinically relevant differences in their presentation from other cSLE patients. Larger longitudinal studies are needed in this subset of patients, collectively and by disease subtype, to understand the factors contributing to these differences and the long-term outcomes. We highlighted the need for more specific serum biomarkers and further immunologic and genetic studies to explain these clinical differences and to understand the impact of prior exposure to immune suppressing therapy.

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TABLES / FIGURES

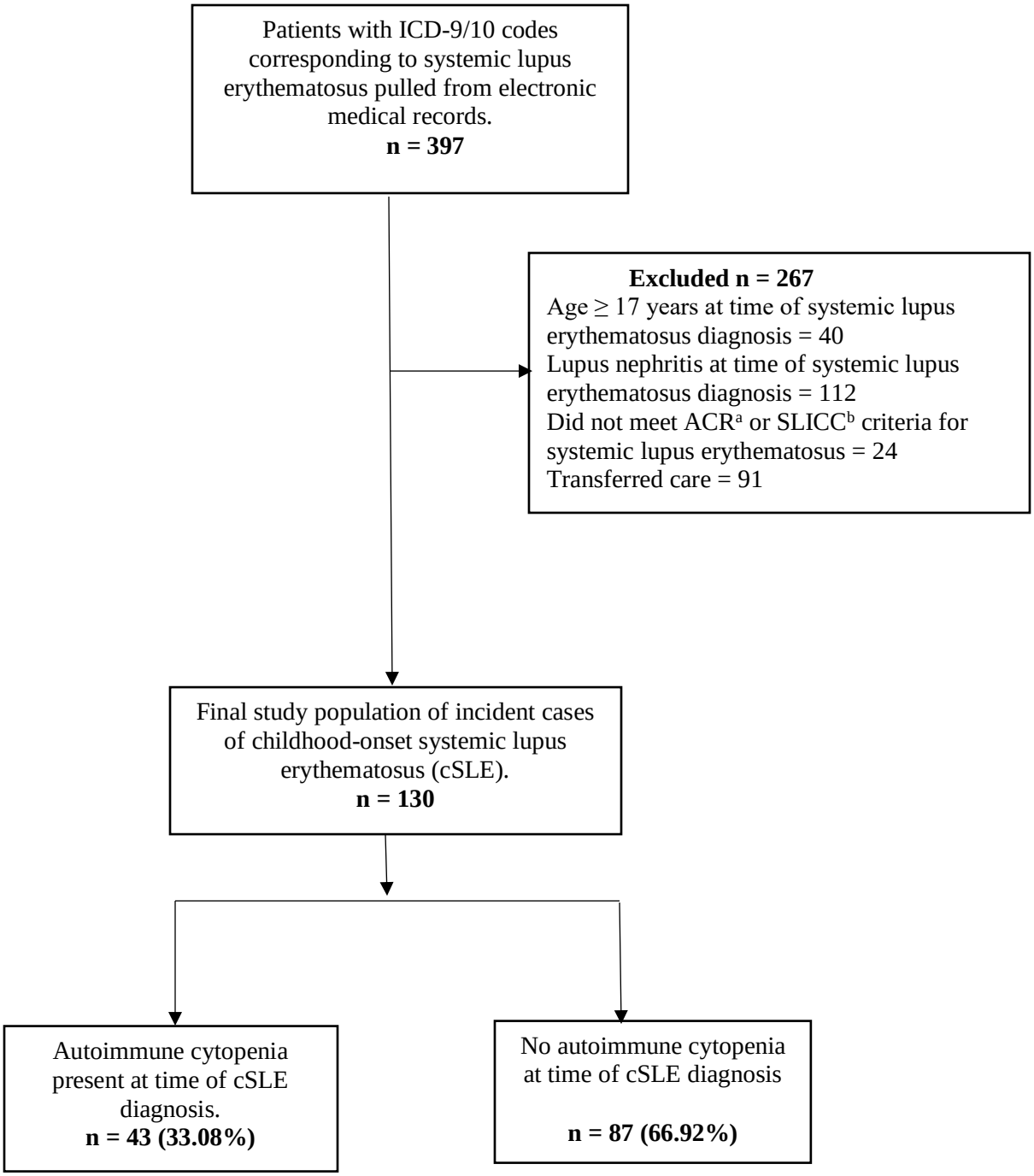

Figure 1: Flow diagram showing selection of study population

Abbreviations: [a]ACR = American College of Rheumatology; [b]SLICC = Systemic Lupus International Collaborating Criteria for Systemic Lupus Erythematosus

Table 1. Comparison of demographic, clinical and laboratory characteristics of 130 incident patients with childhood-onset systemic lupus erythematosus diagnosed between January 1, 2000 and June 30, 2016.

	All cSLE (n=130) N (%)	Missing
Demographics		
Female	107 (82)	
Race		8
Black	81 (66)	
Other	41 (34)	
Age in years at cSLE diagnosis (mean (SD))	12 (3)	
Clinical features		
Fever	54(42)	1
Malar rash	47(37)	1
Discoid lupus	3(2)	1
Oral ulcers	31(24)	1
Nasal ulcers	4(3)	1
Arthritis	64(50)	2
Pleural effusion	26 (31)	45
Pericardial effusion	17(32)	77
Neuropsychiatric symptoms	8(6)	
Myositis*	20(29)	61
Laboratory features		
Positive ANA ≥ 1:40	130(100)	
Positive anti-dsDNA ≥ 1:10	88(70)	4
Positive anti-RNP*	72(58)	6
Positive anti-Smith*	71(57)	6
Positive anti-SSA*	56(45)	6
Positive anti-SSB*	20(16)	7
Lymphopenia ≤ 1,500/uL	81(66)	7
Positive Coombs test and anemia (hemoglobin ≤ 10g/dl)	23 (18)	3
Thrombocytopenia <100,000/uL	19(15)	4
Low C3 complement*	63(53)	10
Low C4 complement*	78(65)	10
ESR in mm/hr (mean(SD))	63 (41)	
eGFR < 90mL/min/1.73m2	35(30)	
SLEDAI-2k (median(IQR, range))	9(5, 2 - 32)	
Prior Treatment		
Corticosteroids[a]	10(8)	
Cyclophosphamide	0	
IVIG[a]	7(5)	
Rituximab[a]	2(2)	
2 year risk of lupus nephritis after cSLE diagnosis	16(12)	
Follow-up period in years (mean (SD))[c]	4 (2)	

Abbreviations: ANA = Anti-nuclear antibody; cSLE = childhood-onset systemic lupus erythematosus; dsDNA = double-stranded DNA; ESR = Erythrocyte sedimentation rate; eGFR = Estimated glomerular filtration rate; IVIG = intravenous immunoglobulin; RNP = ribonucleoprotein; SLEDAI-2k = Systemic Lupus Erythematosus Disease Activity Index 2000; SS = Sjogren syndrome-related antigen; * By laboratory reference range

Table 2. Comparison of demographic, clinical and laboratory characteristics of 130 incident patients with childhood-onset systemic lupus erythematosus diagnosed between January 1, 2000 and June 30, 2016 by baseline autoimmune cytopenia status.

	cSLE with AC (n=43) N(%)	cSLE without AC (n=87)N(%)	P-value	Missing
Demographics				
Sex			0.85	
Female	35(81)	72(83)		
Race			0.62	8
Black	26/41(63)	55/81(68)		
Other	15/41(37)	26/81(32)		
Age in years at cSLE diagnosis (mean (SD))[c]	12.65(3)	12.10(3)	0.32	
Clinical features				
Fever	21/42(50)	33/87(38)	0.19	1
Malar rash	10/42(24)	37/87(43)	0.04[S]	1
Discoid lupus[a]	1/42(2)	2/87(2)	1	1
Arthritis	13/42(31)	51/86(59)	0.00[S]	2
Neuropsychiatric symptoms[a]	6/43(14)	2/87(2)	0.02[S]	
Myositis*	2/20(10)	18/49(37)	0.03[S]	61
Laboratory features				
Positive anti-dsDNA ≥ 1:10	31/43(72)	57/83(69)	0.69	4
Positive anti-RNP*	24/43(56)	48/81(59)	0.71	6
Positive anti-Smith*	22/43(51)	49/81(61)	0.32	6
Positive anti-SSA*	20/43(47)	36/81(44)	0.83	6
Positive anti-SSB*	10/43(23)	10/80(13)	0.12	7
Low C3 complement*	25/41(61)	38/79(48)	0.18	10
ESR in mm/hr (mean(SD))[c]	79.8(45)	55.21(36)	0.00[S]	
eGFR < 90mL/min/1.73m2	16/41(39)	19/74(26)	0.14	
SLEDAI-2k (median(IQR, range))[b]	9 (5, 4 - 32)	10 (5, 2 - 23)	0.66	
2 year risk of lupus nephritis after cSLE diagnosis	3/43(7)	13/87(15)	0.19	

Abbreviations: AC = Autoimmune cytopenia; cSLE = childhood-onset systemic lupus erythematosus; dsDNA = double-stranded DNA; ESR = Erythrocyte sedimentation rate; eGFR = Estimated glomerular filtration rate; IVIG = intravenous immunoglobulin; RNP = ribonucleoprotein; SLEDAI-2k = Systemic Lupus Erythematosus Disease Activity Index 2000; SS = Sjogren syndrome-related antigen; S = significant with p value < 0.05; * By laboratory reference range, Comparisons were by chi square test except otherwise stated, [a] Fisher's exact test, [b]Wilcoxon rank sum test, [c]Student's t-test

Table 3. Comparison of baseline demographic, clinical and laboratory characteristics of 130 incident patients with childhood-onset systemic lupus erythematosus diagnosed between January 1, 2000 and June 30, 2016 by race.

	Black (n=81)N(%)	Other (n=41)N(%)	P-value	Missing (n=8)
Demographics				
Sex			0.49	8
Female	65/81(80)	35/41(85)		
Age in years at cSLE diagnosis (mean (SD))[c]	13 (3)	12 (3)	0.49	
Clinical features				
Malar rash	26/80(33)	18/41(44)	0.22	9
Discoid lupus[a]	2/80(3)	1/41(2)	1	9
Oral ulcers	13/80(16)	14/41(34)	0.03[S]	9
Nasal ulcers[a]	2/80(3)	2/41(5)	0.60	9
Arthritis	40/79(51)	19/41(46)	0.66	10
Neuropsychiatric symptoms[a]	5/81(6)	3/41(7)	1	8
Myositis	17/45(38)	3/19(16)	0.08	66
Laboratory features				
Positive anti-dsDNA ≥ 1:10	54/80(68)	27/39(69)	0.85	11
Positive anti-RNP*	53/79(67)	15/39(39)	0.00[S]	12
Positive anti-Smith*	55/79(70)	13/39(33)	0.00[S]	12
Positive anti-SSA*	39/79(49)	13/39(33)	0.10	12
Positive anti-SSB*	14/78(18)	5/39(13)	0.48	13
Positive Coombs test and Anemia (hemoglobin ≤ 10mg/dl)	17/80(21)	6/40(15)	0.41	10
Thrombocytopenia <100,000/uL	9/79(11)	8/40(20)	0.21	11
Low C3 complement*	40/78(51)	19/35(54)	0.77	17
Low C4 complement*	49/78(63)	24/35(69)	0.55	17
Elevated ESR > 20mm/hr	61/70(87)	26/35(74)	0.10	25
ESR in mm/hr (mean(SD))[c]	67(39)	61(44)	0.52	
eGFR mL/min/1.73m2(median(IQR, range))[b]	103(34)	101(32)	0.67	
SLEDAI-2k (median(IQR, range))[b]	9(6)	10(4)	0.77	
Autoimmune cytopenia at cSLE diagnosis	26/81(32)	15/41(37)	0.62	8
Prior Treatment				
Corticosteroids[a]	5/81(6)	4/41(10)	0.48	8
IVIG[a]	4/81(5)	2/41(5)	1	8
Rituximab[a]	2/81(3)	0/41(0)	0.55	8
2 year risk of lupus nephritis after cSLE diagnosis	12/81(15)	3/41(7)	0.23	8

Abbreviations: cSLE = childhood-onset systemic lupus erythematosus; dsDNA = double-stranded DNA; ESR = Erythrocyte sedimentation rate; eGFR = Estimated glomerular filtration rate; IVIG = intravenous immunoglobulin; RNP = ribonucleoprotein; SLEDAI-2k = Systemic Lupus Erythematosus Disease Activity Index 2000; SS = Sjogren syndrome-related antigen; S = significant with p value < 0.05; * By laboratory reference range, Comparisons were by chi square test except otherwise stated, [a] Fisher's exact test, [b]Wilcoxon rank sum test, [c]Student's t-test

Table 4. Comparison of baseline demographic, clinical and laboratory characteristics of 130 incident patients with childhood-onset systemic lupus erythematosus diagnosed between January 1, 2000 and June 30, 2016 by age.

	< 9 years (n=15)N(%)	≥ 9 years (n=115)N(%)	P-value	M
Demographics				
Sex[a]			0.73	
Female	12/15(80)	95/115(83)		
Race[a]			0.76	8
Black	8/13(62)	73/109(67)		
Other	5/13(39)	36/109(33)		
Age in years at cSLE diagnosis (mean (SD))[c]	7(1)	13(2)	<0.00[S]	
Clinical features				
Fever	11/15(73)	43/114(38)	0.01[S]	1
Malar rash	8/15(53)	39/114(34)	0.15	1
Oral ulcers	6/15(40)	25/114(22)	0.19	1
Arthritis	7/15(47)	57/113(50)	0.78	2
Angioedema[a]	3/15(20)	5/112(5)	0.05[S]	3
Neuropsychiatric symptoms[a]	0/15(0)	8/115(7)	0.60	
Myositis[a]	3/9(33)	17/60(28)	0.71	61
Laboratory features				
Positive anti-dsDNA ≥ 1:10[a]	10/15(67)	78/111(70)	0.77	4
Positive anti-RNP*	8/14(57)	64/110(58)	0.94	6
Positive Coombs test and Anemia (hemoglobin ≤ 10mg/dl)[a]	1/15(7)	22/112(20)	0.30	3
Thrombocytopenia <100,000/uL	0/15(0)	19/111(17)	0.12	4
Low C3 complement*	8/15(53)	55/105(52)	0.95	10
Elevated ESR > 20mm/hr[a]	10/11(91)	80/100(80)	0.69	19
eGFR < 90mL/min/1.73m2	3/14 (21)	32/101(32)	0.55	
SLEDAI-2k (median(IQR, range))[b]	11(7, 4 - 17)	9(5, 2 - 32)	0.63	
Autoimmune cytopenia at cSLE diagnosis[a]	2/15(13)	41/115(36)	0.14	
2 year risk of lupus nephritis after cSLE diagnosis	2/15(13)	14/115(12)	1	

Abbreviations: cSLE = childhood-onset systemic lupus erythematosus; dsDNA = double-stranded DNA; ESR = Erythrocyte sedimentation rate; eGFR = Estimated glomerular filtration rate; M = missing; RNP = ribonucleoprotein; SLEDAI-2k = Systemic Lupus Erythematosus Disease Activity Index 2000; S = significant with p value < 0.05; * By laboratory reference range, Comparisons were by chi square test except otherwise stated, [a] Fisher's exact test, [b]Wilcoxon rank sum test, [c]Student's t-test

Table 5. Comparison of baseline demographic, clinical and laboratory characteristics of 130 incident patients with childhood-onset systemic lupus erythematosus diagnosed between January 1, 2000 and June 30, 2016 by sex.

	Female (n=107)N(%)	Male (n=23)N(%)	P-value	Missing
Demographics				
Race				
Black	65/100(65)	16/22(73)	0.48	8
Other	35/100(35)	6/22(27)		
Age in years at cSLE diagnosis (mean (SD))[c]	12(3)	13(3)	0.13	
Clinical features				
Fever	47/106(44)	7/23(30)	0.22	1
Malar rash	39/106(37)	8/23(35)	0.86	1
Discoid lupus[a]	1/106(1)	2/23(9)	0.08	1
Oral ulcers	25/106(24)	6/23(26)	0.80	1
Arthritis	53/105(51)	11/23(48)	0.82	2
Pleural effusion	21/69(30)	5/16(31)	1	45
Pericardial effusion	15/41(37)	2/12(17)	0.30	77
Neuropsychiatric symptoms[a]	7/107(7)	1/23(4)	1	
Myositis	18/55(33)	2/14(14)	0.32	61
Laboratory features				
Positive anti-dsDNA ≥ 1:10	76/104(73)	12/22(55)	0.09	4
Positive anti-RNP*	58/103(56)	14/21(67)	0.38	6
Positive anti-Smith*	57/103(55)	14/21(67)	0.34	6
Positive anti-SSA*	44/103(43)	12/21(57)	0.23	6
Positive anti-SSB*[a]	18/103(18)	2/20(16)	0.52	7
Low C3 complement*	56/99(57)	7/21(33)	0.05	10
Low C4 complement*	67/99(68)	11/21(52)	0.18	10
Elevated ESR > 20mm/hr	77/94(82)	13/17(77)	0.74	19
eGFR < 90mL/min/1.73m2	27/96(28)	8/19 (42)	0.23	15
SLEDAI-2k (median(IQR, range))[b]	10(5)	8(4)	0.12	
Autoimmune cytopenia at cSLE diagnosis	35/107(33)	8/23(35)	0.85	
Prior Treatment				
Corticosteroids[a]	7/107(7)	3/23(13)	0.39	
IVIG[a]	6/107(6)	1/23(4)	1	
Rituximab[a]	1/107(1)	1/23(4)	0.32	
2 year risk of lupus nephritis after cSLE diagnosis	13/107(12)	3/23(13)	1	

Abbreviations: cSLE = childhood-onset systemic lupus erythematosus; dsDNA = double-stranded DNA; ESR = Erythrocyte sedimentation rate; eGFR = Estimated glomerular filtration rate; IVIG = intravenous immunoglobulin; RNP = ribonucleoprotein; SLEDAI-2k = Systemic Lupus Erythematosus Disease Activity Index 2000; SS = Sjogren syndrome-related antigen; S = significant with p value < 0.05; * By laboratory reference range, [a] Fisher's exact test, [b]Wilcoxon rank sum test, [c]Student's t-test

Table 6: Univariate logistic regression analysis examining the association of characteristics of cSLE patients with incident lupus nephritis.

Characteristics	Odds ratio	95% CI for Odds ratio
Presence of autoimmune cytopenia at cSLE diagnosis	0.43	0.12, 1.60
Age at cSLE diagnosis (in years)	0.99	0.83, 1.18
Sex (female versus male)	0.92	0.24, 3.54
Race (Black versus other)	2.20	0.59, 8.29
Arthritis	0.56	0.19, 1.64
Myositis*	3.07	0.85, 11.07
Positive anti-double-stranded DNA*	1.84	0.49, 6.94
Positive anti-ribonucleoprotein*	1.52	0.49, 4.73
Positive anti-Smith*	2.25	0.67, 7.49
Positive anti-SSB*	0.77	0.16, 3.71
Positive anti-SSA*	1.98	0.66, 5.95
low C3 complement at cSLE diagnosis*	3.81	1.01, 14.42
ESR >20mm/hr at cSLE diagnosis	1.46	0.30, 7.09
eGFR < 90mL/min/1.73m2 at cSLE diagnosis	2.61	0.84, 8.11
SLEDAI -2K at cSLE diagnosis (per 1 point in score)	0.98	0.86, 1.11

Abbreviations: cSLE = childhood-onset systemic lupus erythematosus; ESR = Erythrocyte sedimentation rate; eGFR = Estimated glomerular filtration rate; SLEDAI-2k = Systemic Lupus Erythematosus Disease Activity Index 2000; SS = Sjogren syndrome-related antigen; * By laboratory reference range

Table 7: Final multivariable logistic regression model examining the association of patient characteristics with incident lupus nephritis.

Characteristics	Odds ratio	95% CI for Odds ratio
Presence of autoimmune cytopenia at cSLE diagnosis	0.40	0.10, 1.58
low C3 complement at cSLE diagnosis*	4.24	1.10, 16.34

Abbreviations: cSLE = childhood-onset systemic lupus erythematosus; * By laboratory reference range

Table 8. Comparing demographics and characteristics at time of cSLE diagnosis of patients who developed lupus nephritis within the first 2 years of diagnosis by baseline autoimmune cytopenia status.

Characteristics	All cSLE (n = 16)N(%)	cSLE with AC (n = 3)N(%)	cSLE without AC (n =13)N(%)	P	M
Female[a]	13/16(81)	3/3(100)	10/13(77)	1	
Race				1	1
Black[a]	12/15(80)	3/3(100)	9/12(80)		
Other	3/15(20)	0	3/12(25)		
Age in years at cSLE diagnosis (median(IQR))[b]	13(4)	11(3)	14(3)	0.15	
Renal characteristics at time of cSLE diagnosis					
eGFR < 90mL/min/1.73m^{2}[a]	7/7(50)	1/3(33)	6/11(55)	1	2
Hematuria >5RBC/hpf[a]	2/15(13)	0/3(0)	2/12(17)	1	1
SLEDAI - 2K at time of cSLE diagnosis (median(IQR)[b]	8(7)	5(4)	12(7)	0.08	

Abbreviations: AC = Autoimmune cytopenia; cSLE = Childhood-onset systemic lupus erythematosus; eGFR= Estimated glomerular filtration rate; LN= Lupus nephritis; M = Missing; P = P-value; SLEDAI-2k = Systemic Lupus Erythematosus Disease Activity Index 2000; [a] Fisher's exact test, [b]Wilcoxon rank sum test

Table 9. Comparing characteristics of patients who developed lupus nephritis within the first 2 years of diagnosis by baseline autoimmune cytopenia status.

Characteristics	All cSLE (n = 16)N(%)	cSLE with AC (n = 3)N(%)	cSLE without AC (n =13)N(%)	P
LN by class[a]				0.30
II	3/15(20)	0/3(0)	3/12(25)	
III	2/15(13)	0/3(0)	2/12(17)	
IV	5/15(33)	1/3(33)	4/12(33)	
V	3/15(20)	2/3(67)	1/12(8)	
IV/V	2/15(13)	0/3(0)	2/12(17)	
LN severity[a]				
Mild i.e. Class I and II only	3/15(20)	0/3(0)	3/12(25)	1
Renal characteristics at time of LN diagnosis				
eGFR < 90mL/min/1.73m^{2a}	6/15(40)	1/3(33)	5/12(42)	1
UPCr ≥2 mg/mg[a]	10/16(63)	3/3(100)	7/13(54)	0.25
RBC casts at time of LN diagnosis[a]	1/16(6)	0/13(0)	1/13(8)	1
Hematuria >5RBC/hpf[a]	12/16(75)	1/3(33)	11/13(85)	0.14
Interval of LN from time of cSLE diagnosis in months				1
>1 to ≤ 6	6/16(38)	1/3(33)	5/13(39)	
> 6 to ≤ 12	3/16(19)	0/3(0)	3/13(23)	
> 12 to ≤ 24	7/16(44)	2/3(67)	5/13(39)	

Abbreviations: AC = Autoimmune cytopenia; cSLE = Childhood-onset systemic lupus erythematosus; eGFR= Estimated glomerular filtration rate; LN= Lupus nephritis; M = Missing; P = P-value; UPCr = Urine protein to creatinine ratio; [a] Fisher's exact test

Table 10. Comparing 2-year risk of lupus nephritis in 130 patients with childhood-onset systemic lupus erythematosus.

	Pre-treated autoimmune cytopenia (n=13)	Autoimmune cytopenia without pre-treatment (n= 30)	No autoimmune cytopenia (n= 87)	P-value
Risk of lupus nephritis(%)	0/13 (0)	3/30(10)	13/87(15)	0.41*

*Fisher's exact test