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Autoantibody-based subgroups and longitudinal seroconversion in juvenile-onset systemic lupus erythematosus

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ABSTRACT

Objective To explore the clinical value of autoantibody-based subgroup framework and the trend of autoantibody fluctuation in juvenile-onset SLE (JSLE).

Methods Eighty-seven patients with JSLE were retrospectively collected and divided into subgroups via a two-step cluster based on the status of nine autoantibodies (double-stranded-DNA (dsDNA), nucleosome, histone, ribosomal P protein, Smith (Sm), u1-ribonucleoprotein (RNP), Sjögren's syndrome antigen A (SSA)/Ro52, SSA/Ro60, Sjögren's syndrome antigen B (SSB)/La). The final model selected in this study was based on adequate goodness of fit of the Silhouette coefficient and clinical interpretability. Clinical manifestations, organ involvements and disease activity were compared among the subgroups. Fluctuation in autoantibody status was also collected and analysed. Flare-free survival rates of the patients with positive/negative seroconversion and patients without seroconversion were studied by the Kaplan-Meier method and compared using a log-rank test.

Results Two clusters were identified: subgroup 1 (positive anti-Sm/RNP group) and subgroup 2 (negative anti-Sm/RNP group). There were more lupus nephritis (LN) and neuropsychiatric SLE (NPSLE) cases in subgroup 1 than in subgroup 2. Patients in subgroup 1 exhibited higher SLE Disease Activity Index scores compared with those in subgroup 2. Furthermore, anti-ribosomal P protein (61.1%), anti-nucleosome (58.3%) and anti-dsDNA (54%) were most commonly positive autoantibodies. A progressive decrease in the frequency of patients with positive results was demonstrated during the follow-up years. The decrease was notable for anti-dsDNA, anti-nucleosome and anti-ribosomal P protein (remaining 27.27%, 38.89% and 45.00% positive in the fifth year, respectively). While for those negative at baseline diagnosis, the decrease in the frequency of negative results was progressive but modest. Kaplan-Meier curve showed that the flare-free survival of patients with positive seroconversion was significantly lower than those without seroconversion and those with negative seroconversion ($p<0.001$).

Conclusions In children with SLE, subgroups based on autoantibody profile can be applied to differentiate phenotypes and disease activity. Two important organ involvements, LN and NPSLE, are more common in patients with positive anti-Sm/RNP autoantibodies. Positive seroconversion may provide a valuable perspective for assessing flare, and it is worthwhile to retest the array of autoantibodies during follow-up.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Juvenile-onset SLE (JSLE) is characterised by multiorgan involvement with great variability and data are scarce in early ages. Meanwhile, there is an extensive heterogeneity and frequency of autoantibodies in SLE.

WHAT THIS STUDY ADDS

⇒ It is the first time to report that in JSLE, subgroups based on autoantibody profile can be applied to differentiate phenotypes and disease activity. In addition, seroconversion can be observed overtime in patients with JSLE and help predict flare.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Subgroups of JSLE help in understanding the heterogeneity of clinical phenotypes and prognosis. Monitoring autoantibody seroconversion may provide information for flare.

INTRODUCTION

SLE is a systemic autoimmune disease that can affect multiple organs and result in significant organ damage and failure.[1] Approximately 15%~20% of patients with SLE develop the disease before their 18th birthday and are therefore diagnosed with juvenile-onset SLE (JSLE).[2] JSLE is characterised by multiorgan impairments with great variability and with scarce data in early ages. Although advances in the management of JSLE have led to a remarkable improvement in the survival of patients, severe organ involvement or flare over time still leads to unfavourable outcomes.[3] Therefore, to judge prognosis and determine medication, further study in JSLE is of great clinical significance.

There is an extensive heterogeneity and frequency of autoantibodies in SLE. Therefore, the diagnostic entities in SLE are delineated by sets of consensus criteria.[1] In order to improve the understanding of underlying pathogenesis, diagnostics and disease prognosis, prior findings suggest that subgroups

can be identified on the basis of the autoantibody profile in adult SLE and distinct autoantibody-defined phenotypes may exist.[4–6]

Furthermore, autoantibodies do fluctuate during treatment in the setting of adult lupus.[7 8] However, the clinical relevance of indirect immunofluorescence-ANA (IF-ANA) seroconversion remains uncertain. To our knowledge, no studies have investigated subgroups based on autoantibodies and longitudinal seroconversion in JSLE before. Therefore, this study aimed to evaluate autoantibody-based subgroup framework and to examine the clinical value of autoantibody fluctuation in JSLE.

METHODS

Study population

We conducted a retrospective study of 89 children with SLE who were diagnosed in the Department of Rheumatology & Immunology at Shanghai Children's Medical Center from January 2016 to August 2022. Two patients with incomplete medical charts were excluded. There were 87 patients (9 males and 78 females) for final analysis. The mean age at diagnosis was 11.71±2.93 years (range: 2.75~17.75 years).

Twenty-one (21 of 87, 24.14%) cases were in the prepubertal period (≤7 years) at diagnosis, 52 of 87 (59.77%) in peri-pubertal (8~13 years) and 14 of 87 (16.09%) in the adolescent age group (14~18 years). All patients fulfilled the American College of Rheumatology (ACR) criteria, with disease onset at ages before 18 years.

Two-step cluster analysis

Two-step cluster, an approach for exploring empirical groups of individuals with similar characteristics, is free hypothesis and uses the log-likelihood distance measure.[9] The Schwarz Bayesian Information Criterion and the large ratio of distance measures are considered to automatically select the optimal number of clusters.[9] The final model selected in this study was testified with adequate goodness of fit of the Silhouette coefficient and clinical interpretability.[10]

Silhouette coefficient is a cohesion and separation index which measures how similar individuals are to their cluster compared with other clusters (values >0.50 are interpreted as good fitting, between 0.30 and 0.50 as fair, and <0.30 as poor).[10]

As shown in online supplemental figure 1, a two-step cluster based on status of nine autoantibodies (double-stranded-DNA (dsDNA), nucleosome, histone, ribosomal P protein, Smith (Sm), u1-ribonucleoprotein (RNP), Sjögren's syndrome antigen A (SSA)/Ro52, SSA/Ro60, Sjögren's syndrome antigen B (SSB)/La) was performed, and two clusters (subgroups) were identified (Silhouette=0.4).

When anti-cardiolipin (CL) immunoglobulin (IgG/IgM) and anti-β2 glycoprotein I (β2GP1) IgG were included together in the model, the average Silhouette was 0.3, lower than 0.4, so these three antiphospholipid (aPL) antibodies were not included here finally.

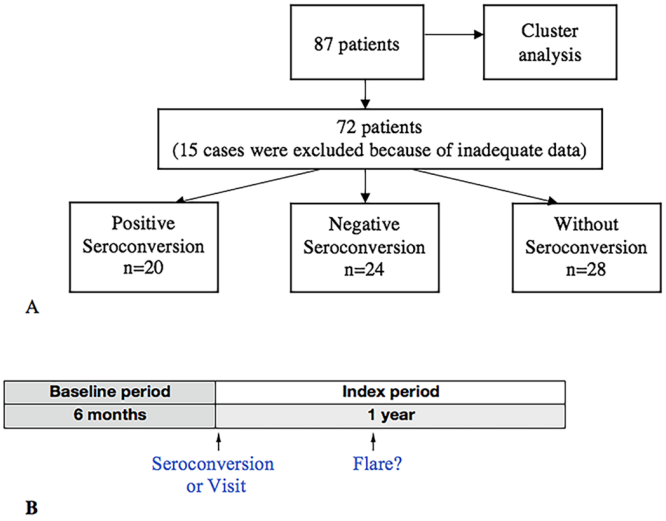


Figure 1 (A) Flow chart of 87 patients with JSLE. A cross-sectional research part with cluster analysis to understand the heterogeneity of clinical phenotypes. A longitudinal research part of 72 patients to evaluate autoantibody seroconversion. (B) Schematic of the study about how the seroconversion relates or not to flare. JSLE, juvenile-onset SLE.

Autoantibody data collection

We collected fluctuation in autoantibody status during follow-up in databases. Clinical manifestations were documented at the same time point as the autoantibody determination. An autoantibody test was considered positive in a patient if positive on ≥2 different occasions. The same was true for a negative result. Antibodies were detected by double immunodiffusion (DID). All tests were performed when blood was drawn according to standard protocol in the department.

aPL antibodies, including anti-CL IgG/IgM and anti-β2GP1 IgG, were analysed in the serum by ELISA, with aPL cut-off levels for positivity corresponding to the 99th percentile of the normal population.[6]

Four different autoantibody data patterns were observed for each autoantibody:[8] (1) always negative; (2) always positive; (3) negative at diagnosis with subsequent positive tests (positive seroconversion) and (4) positive at diagnosis with subsequent negative tests (negative seroconversion).

Autoantibody seroconversion

Seventy-two patients had regular follow-up records at an interval of 1~6 months during the study period and they had at least two visits with autoantibody results in the longitudinal cohort (figure 1A).

The baseline period and index period were defined as 6 months before and 1 year after seroconversion, respectively (figure 1B). To reduce the effects of baseline high disease activity on the subsequent disease flares in the index period, we excluded patients with flares at baseline.

Table 1 Characteristics of patients with positive/negative seroconversion and patients without seroconversion at the time of seroconversion or visits

	Positive conversion cases n=20	Negative conversion cases n=24	Patients without seroconversion n=28	P value
Age	13.89±1.97	13.86±2.11	13.65±1.99	0.902
Gender (female, %)	19 (95.0)	22 (91.7)	24 (85.7)	0.542
Disease duration (months)	24.16±14.30	28.79±15.99	21.40±10.72	0.228
Lupus nephritis, n (%)	10 (50.0)	5 (20.8)	5 (17.9)	0.032
Prior daily prednisone*	8.68±7.88	12.14±4.69	11.67±5.01	0.164
Autoantibody seroconversion events (n)			/	
Anti-La/SSB	2 (10.0%)	4 (16.7%)		
Anti-ribosomal P protein	4 (20.0%)	23 (95.8%)		
Anti-dsDNA	6 (30.0%)	20 (83.3%)		
Anti-Sm	3 (15.0%)	6 (25.0%)		
Anti-u1-RNP	6 (30.0%)	9 (37.5%)		
Anti-histone	8 (40.0%)	16 (66.7%)		
Anti-nucleosome	7 (35.0%)	24 (100.0%)		
Anti-Ro52/SSA	6 (30.0%)	5 (20.8%)		
Anti-Ro60/SSA	6 (30.0%)	6 (25.0%)		
Subgroup at diagnosis: anti-Sm/RNP subgroup, n (%)	12 (60.0)	8 (33.3)	3 (10.7)	0.001

*Prior daily prednisone: medications used during the baseline period until seroconversion/visits.
dsDNA, double-stranded DNA; RNP, ribonucleoprotein; Sm, Smith; SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B.

Patients who were treated with rituximab or plasma exchange within 1 year before negative seroconversion were also excluded.

We identified 20 patients with positive seroconversion, 24 patients with negative seroconversion and 28 patients without seroconversion (table 1).

Statistical analysis

Continuous variables were expressed as mean±SD and median (range). Categorical variables were presented as an absolute number (frequency). Two-step cluster analysis was used to identify groups of patients with similar autoantibody profiles. The result of two clusters was interpretable and clinically meaningful. The clusters were referred to as disease subgroups in this study. Logistic regression was performed to assess the association between clinical variables and each subgroup, including sex and age at inclusion as covariables. To evaluate number (frequency), categorical variable comparisons were first assessed by Pearson's $\chi 2$ test, which requires that at least 80% of the cells must have an expected frequency of ≥5 and no cell must have an expected frequency <1. Fisher's exact test (n≤5) was used. Flare-free survival rates of the patients with positive/negative seroconversion and patients without seroconversion were studied by the Kaplan-Meier method and compared using a log-rank test. All statistical analyses were performed using the SPSS V.26.0. The statistical tests were two sided and a $p<0.05$ was considered statistically significant.

RESULTS

Subgroups defined by autoantibody status

Two-step cluster analysis based on autoantibody status grouped patients with JSLE into two subgroups (table 2).

Subgroup 1 (n=31, 35.6%) was dominated by positive anti-Sm/RNP, and subgroup 2 (n=56, 64.4%) was characterised by negative anti-Sm/RNP.

There were no significant differences in age and gender at baseline. There were more lupus nephritis (LN) and neuropsychiatric SLE (NPSLE) cases in subgroup 1 than in subgroup 2. Cases in subgroup 1 exhibited higher SLE Disease Activity Index (SLEDAI) scores compared with subgroup 2 at diagnosis. There were no significant differences in serositis, arthritis or mucocutaneous diseases (malar rash, discoid lupus, alopecia and oral ulcers) (table 3).

ANA fine specificities and organ involvements

Association between autoantibodies and organ involvements is presented in online supplemental table 1. Among the ANA fine specificities, positive anti-dsDNA, anti-histone and anti-nucleosome were associated with LN. Positive anti-ribosomal P protein was associated with

Table 2 Characteristics of autoantibody pattern in JSLE subgroups at diagnosis

	Subgroup 1 n=31 (35.6%)	Subgroup 2 n=56 (64.4%)
Anti-Sm	22 (71.0%)	0 (0.0%)
Anti-u1-RNP	31 (100.0%)	0 (0.0%)
Anti-dsDNA	23 (74.2%)	31 (55.4%)
Anti-nucleosome	22 (71.0%)	27 (48.2%)
Anti-ribosomal P protein	22 (71.0%)	26 (46.4%)
Anti-histone	21 (67.7%)	27 (48.2%)
Anti-Ro52/SSA	20 (64.5%)	14 (25.0%)
Anti-Ro60/SSA	24 (77.4%)	16 (28.6%)
Anti-La/SSB	7 (22.6%)	11 (19.6%)
Anti-CL IgG	8 (25.8%)	3 (5.36%)
Anti-CL IgM	6 (19.4%)	2 (3.57%)
Anti-β2GP1 IgG	8 (25.8%)	3 (5.36%)

CL, cardiolipin; dsDNA, double-stranded DNA; JSLE, juvenile-onset SLE; RNP, ribonucleoprotein; Sm, Smith; SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B; β2GP1, β2 glycoprotein I.

NPSLE. No significant difference was found in the other ANA fine specificities with organ involvements.

ANA staining patterns

All patients fulfilled the ACR-1997 criteria[11] with IF-ANA positive at diagnosis (n=87, 100%). The distribution of staining patterns was detailed according to the International Consensus on ANA Patterns nomenclature. There were homogeneous (H, AC-1) (n=41, 47.13%), speckled (S, AC-4) (n=40, 45.98%), homogeneous/speckled (AC-1/4) (n=6, 6.90%) at diagnosis.

The majority of patients (n=75, 86.21%) kept their type of staining pattern during follow-up. The switched pattern was all found in subgroup 1. Twelve cases with H (13.79%) switched patterns at least once. Among them, nine cases with H switched to S and three cases with H switched to a dense, fine-speckled pattern (AC-2).

Decreasing IF-ANA titres were found in 55 cases (63.22%). Increasing IF-ANA titres were found in 15 cases (17.24%). Titres remained in 17 cases (19.54%).

Two patients in subgroup 1 (2 of 31, 6.45%) lost ANA positivity over time. The initial ANA titres of these two cases were in the range of 320. One had LN (International Society of Nephrology/Renal Pathology Society: IV+V), and the other had macrophage activation syndrome at diagnosis. Both were prescribed antimalarials, steroid and cyclophosphamide, one in combination with rituximab. At month 8, when data from two cases were available, they were IF-ANA negative and stayed negative during the follow-up.

Autoantibody occurrence and seroconversions

All patients were ever positive regarding ≥1 of the analysed SLE-related fine specificity autoantibodies. Fifteen patients with incomplete autoantibody follow-up data were excluded. Online supplemental table 2 illustrates the prevalence of IF-ANA fine specificities and the number of patients seroconverting for each autoantibody specificity. Antibodies, which were ever positive and most stable over time, included anti-Ro52/SSA, Ro60/SSA, La/SSB, Sm and u1-RNP.

Anti-ribosomal P protein (n=44, 61.1%), anti-nucleosome (n=42, 58.3%) and anti-dsDNA (n=39, 54.2%) were most commonly autoantibodies positive at least once during follow-up. Over half of these cases seroconverted from positive to negative (online supplemental table 2).

The time to negative and positive seroconversion is shown in online supplemental table 3A,B.

Among patients with JSLE positive for each autoantibody at diagnosis, a progressive decrease in the frequency of patients with positive results was demonstrated during the follow-up years. The decrease was notable for anti-dsDNA, anti-nucleosome and anti-ribosomal P protein (remaining 27.27%, 38.89% and 45.00% positive in the fifth year, respectively) (figure 2A). Meanwhile, for those negative at diagnosis, the decrease in the frequency of negative results was progressive but modest (figure 2B).

Table 3 Clinical manifestations in each JSLE subgroup at diagnosis

	Subgroup 1 n=31 (35.6%)	Subgroup 2 n=56 (64.4%)	P value	HR	OR (95% CI)
Age	10.98±2.79	11.91±3.72	0.228	1.085	0.950~1.239
Gender (female, %)	28 (90.3)	50 (89.3)	0.879	1.120	0.260~4.828
SLEDAI	15.85±4.51	12.81±6.29	0.035	1.102	1.007~1.207
Mucocutaneous diseases	23 (74.2%)	43 (76.8%)	0.787	1.515	0.417~3.177
Lupus nephritis	17 (54.8%)	16 (28.6%)	0.017	3.036	1.216~7.576
Neuropsychiatric lupus	15 (48.4%)	7 (12.5%)	0.001	6.562	2.274~18.940
Serositis	8 (25.8%)	11 (19.6%)	0.506	1.423	0.563~4.026
Arthritis	11 (35.4%)	11 (19.6%)	0.108	2.250	0.838~6.042

JSLE, juvenile-onset SLE; SLEDAI, SLE Disease Activity Index.

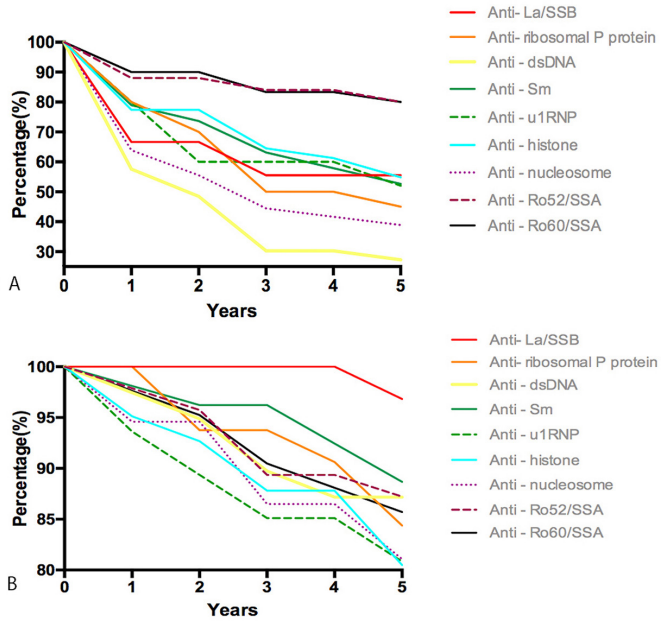
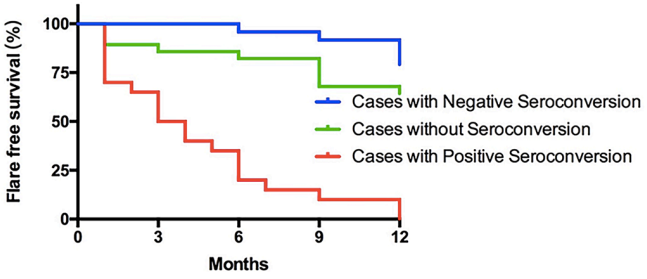


Figure 2 (A) Decrease in the percentage of patients with JSLE remaining positive for autoantibodies within 5 years (patients with an initial positive test result at the first visit were analysed). (B) Decrease in the percentage of patients with JSLE remaining negative for autoantibodies within 5 years (patients with an initial negative test at the first visit result were analysed). dsDNA, double-stranded DNA; JSLE, juvenile-onset SLE; RNP, ribonucleoprotein; Sm, Smith; SSA, Sjögren's syndrome antigen A; SSB, Sjögren's syndrome antigen B.



	3m	6m	9m	12m	P(vs Cases without Seroconversion)
Cases with Negative Seroconversion	24 (100.0%)	23 (95.8%)	22 (91.7%)	19 (79.2%)	0.179
Cases with Positive Seroconversion	10 (50.0%)	4 (20.0%)	2 (10.0%)	0 (0%)	< 0.001
Cases without Seroconversion	24 (85.71%)	23 (82.14%)	19 (67.86%)	18 (64.29%)	

Figure 3 Lupus flare-free survival curve of seroconversion or visits. Kaplan-Meier curve of patients from three groups within the index period (flare: defined by SELENA-SLEDAI Flare Index). SLEDAI, SLE Disease Activity Index.

In two cases in this study, positive seroconversion of anti-Sm/RNP happened at 3 years after the first diagnosis of JSLE, followed by the occurrence of newly onset LN at the fifth year's follow-up.

Concordance of ANA fine specificities was observed. Thirty-seven patients had Ro52/SSA and/or Ro60/SSA antibodies, overlapping in 30 cases. Ten of 11 anti-La/SSB-positive patients were also positive with anti-Ro52/SSA and Ro60/SSA. Co-occurrence was also observed for antibodies against Sm and u1-RNP, whereas isolated appearance was only found for anti-u1-RNP (n=9). The presence of anti-histone (n=39) mainly overlapped with anti-dsDNA; only five cases were not anti-dsDNA positive.

Autoantibody seroconversions and flare

Kaplan-Meier curve showed that the flare-free survival of patients with positive seroconversion was significantly lower than those without seroconversion and those with negative seroconversion (p<0.001) (figure 3).

DISCUSSION

Accurate assessment of disease status is important for the management of JSLE.[12] Through analysis of autoantibody-based subgroups and longitudinal seroconversion, a deeper understanding of JSLE can be attained. We found differences existed in clinical manifestations and disease activity in the subdivision of JSLE based on autoantibodies. Seroconversion overtime was common in JSLE. Negative seroconversion rates were rather frequent for anti-DNA antibodies, while anti-Ro/La antibodies were relatively persistent. Positive seroconversion existed and may be followed by flare.

IF-ANA has a capital role and serves as an entry criterion in the 2019 SLE classification criteria from the European Alliance of Associations for Rheumatology and ACR,[1] but data on switched patterns and titre fluctuation were scarce. The distribution in the present study was dominated by homogeneous (H, AC-1) (n=41, 47.13%) and speckled (S, AC-4) (n=40, 45.98%) at diagnosis, similar to that found in previous SLE cohorts.[8 13] The switched pattern from H to S was found and decreasing IF-ANA titres were observed during follow-up. According to the previous study, anti-DNA and anti-nucleosome antibodies produced by plasmablasts lead to the H staining, whereas anti-RNP antibodies generated by long-lived plasma cells, less susceptible to conventional immunosuppressive therapy, lead to the S pattern.[14] We also found that negative seroconversion rate was rather frequent for anti-nucleosome and anti-dsDNA. Thus, it seems that the switched pattern from H to S during treatment may be related to the easier elimination of the plasmablasts than the long-lived plasma cells in the bone marrow.[14] However, the staining pattern was stable overtime in the majority of our cases (84.21%). Furthermore, both the pattern and titres were not correlated with SLEDAI scores, in agreement with prior studies.[8] This finding may be related to the impact of various autoantibodies on ANA staining patterns and titres.[14 15] Thus, evaluating IF-ANA staining patterns and titres may not be helpful during follow-up and could not be applied to evaluate the disease activity of JSLE. It seems that the analysis of ANA fine specificities is an essential complement to the IF-ANA study.

Through two-step cluster analysis with autoantibody-defined phenotypes, our study showed that the cases

could be categorised based on autoantibody-defined phenotypes and the subgroups bear differential patterns of clinical manifestations in JSLE. Two important organ impairments, LN and NPSLE, differed between the groups at diagnosis. SELENA-SLEDAI scores in subgroup 1 were significantly higher than in subgroup 2. The phenomenon of renal involvement and lower SELENA-SLEDAI in the anti-nucleosome/Sm/DNA/RNP group was also put forward among adults with SLE.[6] This may be explained by the results that positive anti-dsDNA, anti-histone and anti-nucleosome were associated with LN. Furthermore, the higher prevalence of LN found in the anti-Sm/RNP group corresponded with previous studies.[4 6] On the other hand, positive anti-ribosomal P protein was associated with NPSLE. In addition, more positive seroconversion events were observed in subgroup 1 than in subgroup 2. Therefore, the division of the JSLE into distinct disease subsets may help to predict important organ involvement and to further design treatment strategies.

The cluster of SLE on the basis of antibodies varied from studies.[4 6] This may be due to the different onset of disease, population and experimental methods of testing the antibodies. The final model selected in this study was based on adequate goodness of fit of the Silhouette coefficient and clinical interpretability. In our study, aPL antibodies were not included in the final cluster analysis. This may be related to the small sample size. According to previous studies, the prevalence of SLE with positive aPL antibodies varies from 20% to 50% in adult patients with SLE and from 11% to 87% in patients with JSLE (depending on different aPL subtypes and study cohorts), though aPL syndrome is rare.[16 17] However, the interplay between aPLs and JSLE remains uncertain. Prior research of aPL analysis in patients with JSLE did not include the other autoantibodies, and thus the results may not be convincing enough because the impact of the other autoantibodies was neglected in the disease course.[18–20] Further studies with the increasing sample size and the prospective design will be necessary.

Most measurements of subdivisions based on autoantibodies were performed in a cross-sectional design.[6] However, autoantibodies vary over time. To our knowledge, seroconversion study for JSLE was scare. In our study, two cases (2.63%) lost ANA positivity over time. Ever-positive rate (15.3%–61.1%, respectively, depending on autoantibody specificity) and seroconversion rates fell within the range of prior findings detected by ELISA,[8] a more sensitive technique than DID. Thus, the fluctuation in this study was not a consequence of the low sensitivity of the DID assay. From a practical point of view, it is worthwhile to validate fluctuation by DID, a widely used assay, during follow-up.

Our study found that negative seroconversion rate was rather frequent for anti-nucleosome (57.1%) and anti-dsDNA (51.3%). The same phenomenon was found among adults with SLE.[7 8] It may be related to their origin from newly generated plasmablasts that require proliferation for their differentiation and maintenance,[14] and therefore, are more susceptible to immunosuppressive therapy. On the other hand, the relatively persistent expression pattern of anti-Ro/La/RNP/Sm antibodies was considered to be related to their origin from long-lived plasma cells in the bone marrow.[14] As a result of the less frequent seroconversion rate of anti-Ro/La/RNP/Sm antibodies, whether it is necessary or cost-effective to repeat testing for these antibodies against extractable nuclear antigens (anti-ENA), remains controversial.[21] However, anti-Ro/ SSA and anti-dsDNA co-positivity was recently reported to be associated with progression to end-stage renal disease in juvenile LN,[22] suggesting the necessity to retest anti-Ro/SSA regardless of its relatively low seroconversion rate. Furthermore, the higher prevalence of LN found in the anti-Sm/RNP group corresponded with previous studies.[4 6] Therefore, analysis of serological conservations of both anti-DNA and anti-ENA may provide some information for assessing and guiding treatment, despite different conservation rates among autoantibodies.[23]

Although not as frequent as negative seroconversion, we found that the phenomenon of positive seroconversion still existed (negative seroconversion rates: 16.1%~57.1% vs positive seroconversion rates: 9.1%~19.4% of cases, respectively, depending on autoantibody specificity). The median interval to positive seroconversion varied from 2 to 5 years for the nine autoantibodies. In two cases of our study, positive seroconversion of anti-RNP/Sm happened 3 years after the first diagnosis of JSLE, followed by the occurrence of newly onset LN at the fifth year's follow-up. This finding of the production of IgG autoantibodies several years before organ involvement may be concordant with the prior study by Arbuckle *et al*[24] that ANA fine specificities were reported to be detected several years before the symptoms of SLE onset. The phenomenon was considered to be related to the years auto-reactive B cell needed to mature through episodic exposure to immunogenic autoantigens.[25] On the other hand, for patients already diagnosed with JSLE, we found that flare-free survival of patients with positive seroconversion was significantly lower than those without seroconversion and those with negative seroconversion. Therefore, it is worthwhile to retest the array of autoantibodies during follow-up, and positive seroconversion may provide a valuable perspective for assessing flare.

The limitations of our study were as follows. First, the size of our cohort was small. We will increase the sample size in future study. A prospective study will be necessary. Second, quantitative detection through ELISA will be applied, and autoantibody titres will be gathered and analysed. Third, due to the retrospective study design, there was an absence of the British Isles Lupus Assessment group index (BILAG) or the Systemic Lupus International Collaborating Clinics damage index (SDI), genomic variants[26] and serological data, such as cytokines.[27] Appropriate advanced biomarkers[28] may be introduced in further study to provide more insights into the pathogenic mechanisms of JSLE. Nevertheless, our study

shed light on better understanding of JSLE subphenotypes and seroconversions.

Contributors SB and YLJ designed experiments. SB wrote the manuscript. YLJ critically revised the article. HH, YYJ, FD and ZY assisted with data collection. SB, XX, CL and JL analysed the data. YLJ is the guarantor. All authors have approved the final version of the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Parental/guardian consent obtained.

Ethics approval This was a study based on a retrospective chart review. The trial protocol was approved by the ethics committee of Shanghai Children's Medical Center (SCMCIRB-K2022068-1) and the study was carried out in accordance with the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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