Lymphopenia is associated with neuropsychiatric manifestations and disease activity in paediatric systemic lupus erythematous patients


Introduction
Lymphopenia is a common clinical manifestation and one of the haematological criteria according to the American College of Rheumatology (ACR) classification and diagnostic criteria of systemic lupus erythematosus (SLE) [1]. Lymphopenia was observed in 24–59% of paediatric patients [2, 3] and in 62% of adult patients on initial diagnosis of SLE [4]. Cumulative percentage of the occurrence of lymphopenia in the course of disease reached >90% in the adult series [4]. In addition to aiding in the diagnosis of SLE, lymphopenia has been shown to be associated with disease activity in adult SLE patients [5].

Our previous study showed that neuropsychiatric manifestations in SLE (NPSLE), by using ACR definition of NPSLE as 19 neuropsychiatric syndromes in 1999 [6], are a major cause of morbidity and mortality in paediatric SLE patients [7]. We further investigated the association of lymphopenia and different clinical manifestations. We showed in this article that lymphopenia has strong correlation to NPSLE and disease activity in paediatric SLE.

Materials and methods
We retrospectively reviewed the charts of SLE patients (onset age <18 yrs) who attended the Paediatric Rheumatology Clinic of the National Taiwan University Hospital, a tertiary referral centre, from 1985 to 2006 and who satisfied the ACR 1997 revised criteria for SLE [1]. All patients were ethnic Chinese. Ethics approval was obtained.

Demographic, clinical features, diagnostic evaluation results, treatment and outcome of SLE patients were recorded. We recorded diagnostic evaluation results at the time of SLE diagnosis and during SLE flares. A flare is defined as change in SLEDAI of at least three points [8]. SLE flares were divided to neuropsychiatric manifestations (NPSLE), lupus nephritis (LN), neuropsychiatric manifestations and lupus nephritis at the same time (NPSLE and LN), and other type of flares (non-NPSLE/LN) groups according to the disease manifestations. NPSLE was defined by the ACR nomenclature and included a diagnostic guideline for 19 NP symptoms. Patients were excluded from the study, when their NP manifestations were secondary to other causes, such as hypertensive encephalopathy, uremia, infection or other central nervous system disease not related to SLE. The prevalence, the distribution of 19 NP symptoms, the association with anti-phospholipid antibodies and mortality of NPSLE patients have been previously described in detail [7]. LN was defined by the presence of any of the following indicators: proteinuria (>0.5 g/day), cellular cast, glomerular infiltration (<50%), abnormalities on renal biopsy and end-stage renal disease treated by transplant or dialysis.

Diagnostic evaluation included complete blood and differential counts, anti-nuclear antibody, anti-dsDNA antibody, serum C3 and C4 levels, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-extracellular antibody, anti-phospholipid antibody (APA), anti-cardiolipin antibody (ACA). APAs were assayed using IMUCLONE® aPL IgG ELISA kit. ACAs were assayed using AUTOZYM® ACL anti-cardiolipin IgG and IgM sandwich immunoassays. Lymphopenia was defined according to the ACR criteria (<1500/mm³). Global disease activity was quantified by the SLE Disease Activity Index (SLEDAI) [9]. Because leucopenia (WBC <3000/mm³) is one of the parameters included in the SLEDAI, it is excluded from the SLEDAI score and expressed as the modified SLEDAI score. Cumulative organ damage was assessed by the ACR/Systemic Lupus International Collaborating Clinics (SLICC) damage index [10].

Statistical analysis
Data with a normal distribution were expressed as the mean ± s.d., and non-normally distributed data were expressed as the median (range). Comparisons were done using the
chi-square test or Fisher exact test for categorical variables. For continuous data, a Student’s t-test or analysis of variance (ANOVA) was employed. When the data did not follow a normal distribution, the Mann–Whitney U-test was used. Linear regression was performed to investigate the association between lymphocyte counts (at SLE flares) and modified SLEDAI scores, SLICC scores and anti-dsDNA antibodies levels. Multivariate analysis was performed to investigate the relationship between marked lymphopenia (lymphocyte <500/mm³), C3 decrease, C4 decrease, anti-dsDNA elevation and the development of NPSLE or LN, adjusted for sex and age. The variables were entered by enter variable selection procedures. Statistical significance was defined as P < 0.05, two-tailed. All statistics were computed using the SPSS software program version 12.0.

**Results**

One-hundred and eighty-six SLE patients were included in this study. There were 159 (85.5%) female and 27 (14.5%) male patients. The female to male ratio was 5.9 to 1. The mean age at onset of SLE was 13.2 ± 3 yrs (range 4.3–17.9 yrs). Thirty-seven patients (19.9%) were diagnosed as SLE after 16 yrs of age. The mean duration of follow-up was 7.1 ± 5.9 yrs.

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<tr>
<th>Disease activity, organ damage, mortality and cumulative medication usage in patients grouped by lymphocyte counts at SLE flares</th>
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<td>Lymphocytes (10⁶/mm³)</td>
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<tr>
<td>Lymphocytes ≥1500 n=40</td>
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<tr>
<td>Anti-dsDNA antibodies</td>
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<td>C3 (mg/dl)</td>
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¹Anti-dsDNA was expressed by median (range), compared by Kruskal–Wallis test.
²Significant difference between lymphocyte ≥1500 and lymphocyte <1500.

Lymphopenia (<1500/mm³) and marked lymphopenia (<500/mm³) was observed in 62.8 and 12.2% at the time of SLE diagnosis. At the time of SLE diagnosis, lymphopenia was significantly associated with oral ulcers (37.2 vs 20.7%, P = 0.048), leucopenia (48.9 vs 6.9%, P = 0.000), anti-dsDNA antibodies (185.3 ±184.9 vs 94 ±97.9 IU/ml, P = 0.000) and C4 decrease (98.8 vs 77.6%, P = 0.031), compared with lymphocyte counts of more than 1500/mm³. The association of lymphopenia and NPSLE was not significant. Marked lymphopenia was also significantly associated with serositis (36.8 vs 16.8%, haemolytic anaemia (57.9 vs 31.4%), leucopenia (57.9 vs 28.5%), thrombocytopenia (57.9 vs 21.2%) and anti-Sm antibodies (47.1 vs 22.5%) (P < 0.05), compared with lymphocyte counts of more than 500/mm³.

At the time of flares, lymphopenia was significantly associated with anti-dsDNA antibodies, methylprednisolone pulse therapy (MPT) and modified SLEDAI scores (Table 1). Marked lymphopenia was significantly associated with azathioprine, cyclophosphamide pulse therapy (CyCPT), MPT, modified SLEDAI scores and SLICC scores (Table 1). Lymphocyte counts were inversely correlated with modified SLEDAI scores (R² = 0.03, P = 0.043), but there was no significant linear correlation between lymphocyte counts and SLICC scores or anti-dsDNA antibodies (figure not shown).

There were 54 (34.8%) neuropsychiatric flares (NPSLE), 82 (52.9%) lupus nephritis flares (LN), 10 (6.5%) neuro-psychiatric and lupus nephritis flares at the same time (NPSLE and LN), and 9 (5.8%) other types of flares (non-NPSLE/LN). Compared with four types of flares (Table 2), lymphopenia was highly observed in non-NPSLE/LN flares (100%). However, NPSLE and NPSLE/LN flares have a higher percentage of marked lymphopenia (28.3 and 40%, respectively) and a lower percentage of anti-dsDNA elevation (63.6 and 57.1%, respectively). LN flares have the lowest percentage of marked lymphopenia (4.2%) and highest percentage of anti-dsDNA elevation (89.5%).

Using multivariate logistic regression adjusted for sex and age, marked lymphopenia was independently associated with increased risk for NPSLE [odds ratio (OR) 7.41, 95% confidence interval (CI) 1.99–27.0, P = 0.003]. C3 decrease and anti-dsDNA elevation was significantly associated with increased risk for LN (OR 10.9, 95% CI 1.11–111.1, P = 0.041 and OR 3.38, 95% CI 1.1–10.3, P = 0.034, respectively), but protective from NPSLE (OR 0.14, 95% CI 0.02–0.87, P = 0.035 and OR 0.29, 95% CI 0.1–0.85, P = 0.021).
Discussion

In this study, we demonstrated that lymphopenia (≤1500/mm³) is significantly associated with oral ulcers, leucopenia, anti-dsDNA antibodies and disease activity (SLEDAI). Marked lymphopenia (<500/mm³) is significantly associated with neuropsychiatric flares (NPSLE), disease activity (SLEDAI), and organ damage (SLICC). Marked lymphopenia has strong inverse association with LN, which is significantly related to C3 decrease and anti-dsDNA antibodies elevation.

The association of lymphopenia with oral ulcers has not been described by other reports. We found significant association of marked lymphopenia with NPSLE, which is supported by the previous reports [11, 12]. The association of lymphopenia with anti-dsDNA antibodies is consistent with Vila’s study [13]. These anti-DNA antibodies may have lymphocytotoxic activity by cross-reactivity between nuclear antigen and lymphocyte membrane [14].

The correlation of lymphopenia during lupus flares correlates with immunosuppressant usage, disease activity (SLEDAI) and organ damage (SLICC) in our study is consistent with the previous reports [5, 15]. Vila’s study showed that moderate (500–999/mm³) and marked lymphopenia (<500/mm³) are associated with higher disease activity and damage accrual [5]. In addition, Mirzayan et al. [16] addressed that lymphopenia at disease diagnosis predicts higher disease activity and flares within 1 yr.

Several possible explanations of lymphopenia in SLE and especially in NPSLE have been proposed. First, anti-lymphocyte antibodies, including anti-DNA and anti-ribosomal P antibodies, were frequently found in SLE patients [14, 17]. Anti-ribosomal P antibodies are especially linked to NPSLE. Anti-ribosomal P antibodies induce T cell apoptosis and they can cross-react with neuron cells, which might explain marked lymphopenia in NPSLE [17, 18]. Second, lymphocyte apoptosis increased in active SLE, which may result from activation induced cell death via Fas and Fas ligand pathway [19, 20] or death by neglect [12]. Silva’s study demonstrated that lymphocytes of NPSLE patients are more susceptible to death by neglect apoptosis than non-NP SLE patients, and APA and anti-SSA/Ro antibodies may be involved in the pathogenesis [12]. Third, lymphocytes may be sequestrated at sites of inflammation or lymphoid tissues. All mechanisms are associated with active diseases in SLE.

Marked lymphopenia is an independent risk factor for NPSLE. On the contrary, it is protective from LN, which is strongly associated with decreased C3 level and elevated anti-dsDNA antibody by multivariate regression analysis. Marked lymphopenia as a powerful parameter for NPSLE have not been mentioned before. Our study also revealed patients with marked lymphopenia have higher disease activity and received more cumulative immunosuppressants (azathioprine, MPT and CyCPT). From our study it can be argued that lymphopenia could be caused by immunosuppressive agents and corticosteroid. However, in retrospective chart review, we recorded lymphopenia only if it was clinically attributable to lupus activity and not to severe infection (sepsis) or other causes. We excluded the patients with lymphopenia which occurred after immunosuppressants were added in dosage or MPT or CyCPT were given. Our study favoured that marked lymphopenia was due to higher disease activity rather than medication. Our observations should be further studied in the future.

In conclusion, lymphopenia at the time of diagnosis is significantly associated with oral ulcers, leucopenia, anti-dsDNA antibodies and C4 decrease. Marked lymphopenia at SLE flares was significantly associated with immunosuppressants usage, disease activity and organ damage. Marked lymphopenia is also an independent risk factor for NPSLE while protective from LN.

Rheumatology key messages

- Lymphopenia at SLE flares is associated with disease activity and organ damage.
- Marked lymphopenia is independently associated with neuropsychiatric manifestations.

The authors have declared no conflicts of interest.

References