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ORIGINAL ARTICLE

Natural Killer and Natural Killer T Cells in Juvenile Systemic Lupus Erythematosus: Relation to Disease Activity and Progression

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Abstract

The contribution of innate immune cells, including natural killer (NK) and natural killer T (NKT) cells, in systemic lupus erythematosus (SLE) is still unclear. Herein, we examined the frequency of peripheral NK cells, $CD56^{dim}$ and $CD56^{bright}$ NK cells, and NKT cells in patients with juvenile SLE and their potential relations to SLE-related clinical and laboratory parameters. The study included 35 SLE children and 20 apparently healthy controls. After baseline clinical and lab work, SLE Disease Activity Index (SLEDAI-2K) and Pediatric Systemic Lupus International Collaborative Clinics/American College of Rheumatology (SLICC/ACR) Damage Index (Ped-SDI) scores were assessed. The frequency of peripheral NK cells, $CD56^{dim}$ and $CD56^{bright}$ NK cells, and NKT cells was examined using flow cytometry. SLE patients showed significantly lower frequency of NK cells and NKT cells and higher frequency of $CD56^{bright}$ NK cells compared to controls. Disease activity, urea, and creatinine correlated negatively with NK, but positively with $CD56^{bright}$ NK cells. NK and NKT cells exhibited inverse correlation with the renal biopsy activity index; however, $CD56^{bright}$ NK cells showed direct correlations with both activity and chronicity indices. Regarding Ped-SDI, renal, neuropsychiatry disorders, and growth failure correlated inversely with NK but directly with $CD56^{bright}$ NK cells. NKT cell inversely correlated with renal damage and delayed puberty. In conclusion, low frequency of NK and NKT and expansion of $CD56^{bright}$ NK cells are marked in juvenile SLE, particularly with activity. These changes have direct effect on renal impairment and growth failure, reflecting their potential influence on disease progression.

Keywords NK cells · NKT cells · SLE

Abbreviations

ANA	Antinuclear antibodies
anti-dsDNA	Antidouble-stranded DNA
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
NK	Natural killer
NKT	Natural killer T
Ped-SDI	Pediatric Systemic Lupus International Collaborative Clinics/American College of Rheumatology (SLICC/ACR) Damage Index
SD	Standard deviation
SLE	Systemic lupus erythematosus
SLEDAI-2K	Systemic Lupus Erythematosus Disease Activity Index
γδT	Gamma delta T cells

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Introduction

Systemic lupus erythematosus (SLE) is a progressive autoimmune inflammatory disease associated with chronic stimulation of various components of the immune system, involving multiple organs. The disease has variable clinical manifestations with exacerbations and remissions. Although

the etiology of SLE is assumed to be multifactorial, tissue damage in SLE is due to the production of autoantibodies and complement fixing immune complex deposition, leading to permanent organ destruction (de Leeuw et al. 2006; Horak et al. 2001).

In vivo and in vitro studies have verified that pathogenic production of autoantibodies against autoantigens by B cells is promoted by the help of T cells, especially $CD4^+$ T cells. The possible explanation for this helper activity is that conventional $CD4^+$ T cells, identifying a specific MHC class II associated autoantigen, interact with B cells having receptors for the exact autoantigen, leading to shared stimulation of T and B cells and the production of autoantibody (La Cava et al. 2005).

In addition to B cells and T cells, natural killer (NK) cells of the innate immune system have also been reported to play a role in the development of pathogenic autoantibodies in lupus by helping B cells (Gray and Horwitz 1995). According to the relative expression of the markers CD16 and CD56 on NK cell surface, two subsets of NK cells can be distinguished. $CD56^{dim}CD16^+$ NK cells constitute about 90% of circulating NK cells and are responsible for cytotoxic function. $CD56^{bright}CD16^+$ NK (10–15% of circulating NK cells, but predominant in secondary lymphoid organs), release abundant cytokines, and have prominent immunomodulatory functions (Caligiuri 2008; Poli et al. 2009).

Natural killer T (NKT) lymphocytes are innate-like minor subset of T lymphocytes that possess characteristics of both NK cells (CD161) and conventional T lymphocytes (CD3 and TCRα/β) (Godfrey et al. 2010). In the thymus, generation and differentiation of NKT lymphocytes occur by positive selection, negative selection, and VDJ recombination (Taniguchi et al. 2003).

Natural killer T cells can play a role in many immune responses, including inflammatory diseases by interaction with other immune cells (Wu and Van Kaer 2009). NKT cells can interact with other immune cells, such as T cells, B cells, NK cells, and dendritic cells (Lang 2009). This interaction between NKT cells and other immune cells is mediated not only by cytokines, but also by cell-to-cell (Godfrey et al. 2010; Kronenberg and Gapin 2002). Various studies established a suppressive role of NKT cells in a wide range of autoimmune diseases (Cho et al. 2011; Gutowska-Owsiak et al. 2014; van der Vliet et al. 2004).

Emerging evidence suggests that NK cells and NKT cells are important not only for protection against infection, but also for their ability to directly kill target cells, interact with antigen-presenting cells as well as T cells and regulate autoimmune responses (Baxter and Smyth 2002; Johansson et al. 2005).

The aim of the current study was to examine the frequency of NK cells, $CD56^{dim}$ and $CD56^{bright}$ NK cells, and NKT cells in peripheral blood of patients with juvenile SLE, and to investigate the potential relations between the levels of these cells and some SLE-related clinical and laboratory parameters.

Subjects and Methods

This cross-sectional study was conducted from June 2017 to January 2018 on children in the Pediatric Immunology and Rheumatology Unit, Assiut University Children Hospital, Assiut, Upper Egypt. The study was approved by the Ethical Committee of the Faculty of Medicine, Assiut University, Assiut, Egypt, according to the code of ethics of the World Medical Association (Declaration of Helsinki). A written informed consent was taken from the caregiver of each participant after explaining the study to them.

A total of 35 SLE children (28 girls and 7 boys) were enrolled in this study. The inclusion criteria were children below 18 years of age who satisfied the American College of Rheumatology Revised classification Criteria for SLE (Hochberg 1997). Patients with infectious diseases, malignances, other connective tissue diseases, and end organ failure were excluded. Twenty apparently healthy sex- and age-matched children were enrolled as controls in the study.

A unified data collection protocol which included demographic data, medical history, and clinical examinations was used for all participants in the study. Baseline investigations for hematological and biochemical parameters were done for all SLE patients, including complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum albumin, serum urea and creatinine, 24 h protein in urine, urine analysis, antinuclear antibodies (ANA), antidouble-stranded DNA (anti-dsDNA) antibodies, and complement level (C3, C4).

All patients with impaired renal function were subjected to renal biopsy for stage classification, and confirmation of the activity and chronicity of lupus nephritis. Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K) score was recorded for measuring the overall disease activity with score of 0: no activity, 1–5: mild activity, 6–10: moderate activity, 11–19: high activity, and 20 and more: very high activity (Rao and Gordon 2014).

Organ damage was assessed with the pediatric adaptation of systemic lupus International Collaborating clinic/ACR damage index score (Ped-SDI) which includes 14 different organ systems (ocular, neuropsychiatric, renal, pulmonary, cardiovascular, peripheral vascular, gastrointestinal, musculoskeletal, skin, gonad, endocrine, growth failure, delayed puberty, and malignancy) (Gutierrez-Suarez et al. 2006). Growth failure was defined as height below the mean for age by more than 2 standard deviation (SD), depending on the National Center for Health Statistics–World Health Organization growth charts (Ogden et al. 2002; WHO Working

Group on Infant Growth 1994; World Health Organization 1995). Delayed puberty was defined as delay in development of secondary sexual features below the mean for age by more than 2 SD, assessed by Tanner staging (Pozo and Argente 2002; Tanner and Davies 1985). Tanner staging was obtained by physical examination of the patients. Using previously available follow-up data of the patients with growth failure and delayed puberty, we were able to confirm that those patients had a significant delay in growth and/or in development of secondary sexual characteristics that persisted for 6 months at least.

Flow Cytometric Detection of NKT and NK Lymphocyte Subsets

50 µl of blood sample was stained with 5 µl fluoroisothiocyanate-conjugated CD3, phycoerythrin-conjugated CD16, **peridinin**-chlorophyll-protein-conjugated CD56 (Becton Dickinson (BD) Bioscience, CA, USA). After incubation for 20 min, red blood cells lysis was done. Then, washing with phosphate buffer saline and analysis by FACS Calibur flow cytometry with CellQuest software (BD Biosciences, USA) were done. An isotype-matched negative control was used with each sample. About 20,000 cells were acquired. Forward and side scatter histogram was used to define the lymphocytes population. The lymphocyte population was gated first to identify $CD16^+$ lymphocyte populations that were analyzed into $CD3^+$ and $CD3^-$ populations. Then, the expression patterns of CD56 were analyzed on $CD3^-$ population. The identified cells included NKT cells ($CD3^+$ $CD16^+$ $CD56^+$), NK cells ($CD3^-$ $CD16^+$ $CD56^+$), $CD56^{bright}$ NK cells ($CD3^-$ $CD16^+$ $CD56^{+bright}$), and $CD56^{dim}$ NK cells ($CD3^-$ $CD16^+$ $CD56^{+dim}$), as shown in Fig. 1.

Statistical Analysis

Data analysis was performed with the Statistical Package for Social Sciences (SPSS version 17). Data are presented as mean ± SD for all parameters. For comparisons between patients and controls, the independent sample *t* test was used. Spearman's correlation was used to determine the correlations between studied parameters. A probability value of <0.05 denoted a statistically significant difference.

Results

Demographic and Clinical Presentation of Studied Participants

The demographic and clinical characteristics of SLE children are summarized in Table 1. The age of the SLE children ranged 6–17 (mean 8.4 ± 2.6) years. The mean disease duration was 3.7 ± 2.5 (range 1–9) years with Ped-SDI score range 0–6. All patients had active disease according to SLEDAI-2K score (range 2–25). The mean age of the controls was 8.2 ± 2.3 (range 5.5–17) years, and male-to-female ratio was 4:16. All patients were on hydroxychloroquine, 85.7% of them were on corticosteroid, and 57.5% were on azathioprine and/or methotrexate.

Laboratory Data of SLE Children and Controls

More than 50% of the patients were positive for anti-dsDNA and ANA. Highly significant differences ($P<0.0001$) between the study and the control group were found in the blood count (except platelet count), albumin level, renal function, and ESR, as shown in Table 2.

Frequency of NK, NK Subsets, and NKT Cells in Patients and Controls

This study showed that all the studied cells except the $CD56^{bright}$ NK cells were found in smaller numbers in the diseased group compared with the controls, as shown in Table 3.

Correlations Between Number of NK, $CD56^{bright}$ NK and NKT Cells and Some SLE Clinical and Laboratory Findings

Table 4 shows that NK and NKT cells had significant negative correlation with high and very high SLE disease activity as detected by SLEDI-2K. Meanwhile, $CD56^{bright}$ NK cells showed highly significant positive correlation with moderate and high SLE disease activity (both $P<0.0001$). NK cells showed significant negative correlations with anti-dsDNA, urea ($P=0.01$), and creatinine ($P=0.009$). In contrast, $CD56^{bright}$ NK cells showed strong positive correlations with anti-dsDNA, urea, creatinine, and CRP, ($P=0.001$, <0.0001, <0.0001, and 0.01, respectively) and significant negative correlation with C3 and C4 ($P=0.008$ and 0.02, respectively). NK and NKT cells exhibited significant negative correlation with the disease activity index of renal biopsy. However, $CD56^{bright}$ NK cells showed significant positive correlation with both activity and chronicity indices of the renal biopsy.

Correlations Between NK, $CD56^{bright}$ NK, and NKT Cell Numbers and Ped-SDI

According to Ped-SDI, renal, neuropsychiatric disorders, and growth failure were the most prevalent disorders among our SLE children (53.3, 46.6, and 40%, respectively) (see Table 5). Numbers of NK cells showed significant negative correlation with renal and neuropsychiatry disorders in

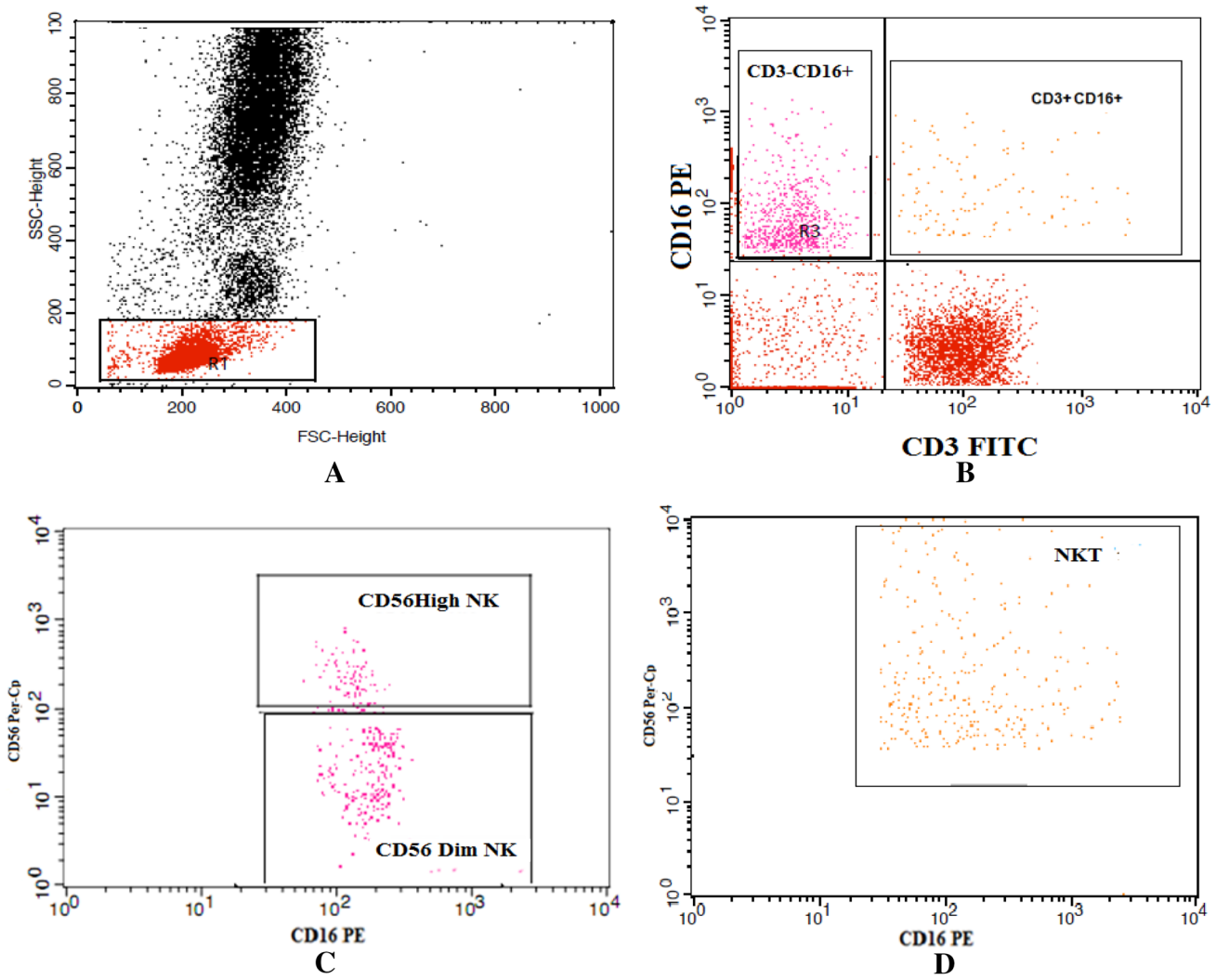

Fig. 1 Flow cytometric detection of NK cells, NK cell subsets and NKT cells. **a** Forward and side scatter histogram was used to define the lymphocytes populations. **b–d** The expression of CD3, CD16 and CD56 was assessed in the lymphocytes population to detect NKT cells ($CD3^+$ $CD16^+$ $CD56^+$), NK cells ($CD3^-$ $CD16^+$ $CD56^+$), $CD56^{bright}$ NK cells ($CD3^-$ $CD16^+$ $CD56^{+bright}$), and $CD56^{dim}$ NK cells ($CD3^-$ $CD16^+$ $CD56^{+dim}$)

addition to growth failure. However, $CD56^{bright}$ NK cells revealed significant positive correlation with renal and neuropsychiatry disorders, premature gonad failure and growth failure. NKT cell showed significant positive correlation with musculoskeletal and skin damage and premature gonad failure, but negative correlation with renal damage and delayed puberty.

Discussion

Systemic lupus erythematosus is an autoimmune disease characterized by defects of the immune cell subsets. Despite the improved understanding of the adaptive immune mechanisms that lead to organ damage in SLE (La Cava 2009), the contribution of other innate immune cells, including NK and NKT cells, needs further studies.

To our knowledge, this is the first study to investigate the frequency of the NK, NK subsets, and NKT cells in juvenile SLE patients and their relation to clinical and laboratory parameters in these patients.

In agreement with our results, several studies documented the striking reduction of the percentage of NK cells, mainly the mature $CD56^{dim}$ subset, in SLE patients compared to the healthy controls (Henriques et al. 2013; Lin et al. 2017; Spada et al. 2015; Stratigou et al. 2017), and more with disease activity (Henriques et al. 2013; Lin et al. 2017). In fact, many factors may contribute to depletion of circulating NK cells in SLE, such as exposure of NK cell precursors to ligand-induced cell death via signaling molecules such

Table 1 Demographic and clinical presentation of the studied SLE children

Parameter	SLE patients ($n=35$)
Male/female	7/28
Age (years) Range (mean ± SD)	6–17 (8.4 ± 2.6)
Disease duration (years) Range (mean ± SD)	1–9 (3.7 ± 2.5)
Ped-SDI *n* (%)	15 (42.9%)
Score (range)	0–6
SLEDAI-2K Range (mean ± SD)	2–25 (10.8 ± 6.7)
Classification of SLEDAI-2K *n* (%)	
No activity	0
Mild activity	7 (20%)
Moderate activity	16 (45.7%)
High activity	11 (31.4%)
Very high activity	1 (2.9%)
Clinical presentation *n* (%)	
Malar rash	11 (31.4%)
Discoid rash	5 (14.3%)
Photosensitivity	4 (11.4%)
Oral ulcers	2 (5.7%)
Serositis	10 (28.6%)
Arthritis	20 (57.1%)
Hematological disease	21 (60%)
Cognitive disorders	12 (34.3%)
Seizures	5 (14.3%)
Renal disorders	20 (57.1%)
Histological class of nephritis *n* (%)	
Class II	1 (5%)
Class III	8 (40%)
Class IV	5 (25%)
Class V	5 (25%)
Class VI	1 (5%)
Current medication history *n* (%)	
Hydroxychloroquine	35 (100%)
Corticosteroid	30 (85.7%)
Azathioprine	20 (57.5%)
Methotrexate	20 (57.5%)
Cyclophosphamide	7 (20%)
Mycophenolate mofetil	5 (14.3%)
Rituximab	3 (8.6%)

Data are represented as number (percentage) *n* (%) or mean ± SD

SLE systemic lupus erythematosus, *n* number, *SD* standard deviation, *Ped-SDI* pediatric systemic lupus international collaborating clinic/ACR damage index, *ACR* American College of Rheumatology, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index

as CD16 linked to the formation of immune complexes, in addition to influence of serum cytokines that can also mediate the activation-induced apoptosis of NK cells as high serum IFN-α levels, secreted mainly by plasmacytoid dendritic cells (Huang et al. 2011; Park et al. 2009). Actually, this decrease in NK cells, specifically the mature $CD56^{dim}$ subset, could reflect the migration of these highly toxic cells from the peripheral blood to target organs; thus aggravating local tissue damage (Spada et al. 2015; Stratigou et al. 2017).

The decrease in the percentage of NKT cells in SLE patients has been reported in many studies (Chen et al. 2015; Cho et al. 2011; Green et al. 2007; Riccieri et al. 2000). This may be attributed to a defect of co-stimulatory pathways where activation and proliferation of NKT cells in SLE could be impeded by reduced expression of the co-stimulatory molecule CD26. Moreover, activated NKT cells potentially secrete several cytokines (Mallevaey et al. 2011).

Consistent with Schepis et al. (2009), we detected significant increase in the numbers of $CD56^{bright}$ NK cells among SLE patients compared to the healthy controls. The expansion of $CD56^{bright}$ NK cells could be explained by a number of mechanisms including their release in high numbers from the bone marrow and/or the lymphoid tissue, as the precursor of $CD56^{dim}$ NK cells, to overcome the high turnover of $CD56^{dim}$ NK cells in several clinical conditions (Poli et al. 2009). An additional possibility is the described resistance of $CD56^{bright}$ NK cells to oxidant induced cell death (Harlin et al. 2007). In addition, $CD56^{bright}$ NK cells might selectively expand as a result of their essential role, along with their produced cytokines, in certain diseases (Poli et al. 2009). In consistence with a previous work (Henriques et al. 2013), this study found that the decreased NK cell levels in peripheral blood was correlated with parameters of lupus activity, as anti-dsDNA and the SLEDAI-2K. Our results showed significant correlation between the decrease in NK cell count and the impairment of renal function and active renal affection (clinically and by renal biopsy). This agrees with Park et al. (2009), who showed a direct relation between the drop of circulating NK cells and the onset of lupus nephritis. A recent study has shown that the reduction of NK cell numbers is initiated at the early stages of kidney affection (Tsakas et al. 2016).

The current results verified an inverse correlation between numbers of NKT cells and lupus activity (SLEDAI-2K and anti-dsDNA), similar to Cho et al. (2011), in addition to creatinine level. Yang et al. (2008) showed that NKT cells had a renoprotective effect against antibody-mediated inflammation through active engagement of NKT cells at the inflammation site and several cytokines expression. The migration of NKT cells to the inflammation site was linked to the intraglomerular expression of CXCL16; thus aiding the immune regulatory function (Yang et al. 2008). Consistent with the previous reports (Chen et al. 2015; Cho et al. 2011; Yang et al. 2008), our findings revealed that decrease in NKT cells numbers and activities may be involved in the progression of SLE.

Table 2 Laboratory data of SLE children and controls

Parameter Mean ± SD	SLE patients ($n=35$)	Controls ($n=20$)	P value
Hemoglobin (g/dl)	10.2 ± 1.9	12.1 ± 0.9	< 0.0001
WBCs (10^3/µl)	3.9 ± 1.5	7.0 ± 1.0	< 0.0001
Platelets (10^3/µl)	200.6 ± 50.3	200.6 ± 67.9	0.51
Albumin (g/dl)	3.2 ± 0.8	4.1 ± 0.3	< 0.0001
Urea (mg/dl)	83.1 ± 44.7	19.1 ± 10.8	< 0.0001
Creatinine (mg/dl)	2.4 ± 1.1	0.7 ± 0.3	< 0.0001
ESR (mm/h)	67.2 ± 39.3	6.7 ± 1.7	< 0.0001
CRP (mg/l)	3.4 ± 1.4	< 1.0 ± 1.3	< 0.005
Protein in urine 24 h (mg/dl)	500.5 ± 131.2	Negative	NA
Urine RBCs casts	3.3 ± 1.2	Negative	NA
Urine granular casts	6.1 ± 8.7	Negative	NA
C3 (mg/dl)	0.8 ± 0.5	12 ± 3.0	< 0.005
C4 (mg/dl)	0.6 ± 0.6	9 ± 2.3	< 0.005
Positive ANA	19 (54.3%)	3 (15%)	< 0.005
Positive anti-dsDNA antibodies	28 (80%)	2 (10%)	< 0.005

Data are represented as mean ± SD or number (%)

Independent sample t test. $P < 0.05$ is significant

SLE systemic lupus erythematosus, *n* number, *SD* standard deviation, *WBCs* white blood cells, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *RBCs* red blood cells, *ANA* antinuclear antibodies, *anti-dsDNA* antidouble-stranded DNA, *NA* not applicable

Table 3 NKT and NK cells' subsets in SLE patients and controls

	SLE patients ($n=35$)	Controls ($n=20$)	P value
NK cells	10.6 ± 1.5	12.9 ± 1.8	< 0.0001
NKT cells	7.4 ± 2.2	9.9 ± 3.4	0.002
$CD56^{dim}$ NK cells	78.8 ± 7.8	82.1 ± 5.5	0.07
$CD56^{bright}$ NK cells	21.8 ± 7.7	17.2 ± 5.2	0.009

Data are represented as means ± SD. Independent sample t test

$P < 0.05$ is significant

SLE systemic lupus erythematosus, *NK* natural killer cells, *NKT* natural killer T lymphocyte

The findings of this study demonstrated significant direct association between the increase in the circulating $CD56^{bright}$ NK cells and disease activity, the impairment of renal function, activity, and chronicity indices of renal biopsy in the SLE patient group. Despite shortage of previous reports about circulating $CD56^{bright}$ NK cells, Law et al. (2017) found a striking association between the tubule-interstitial $CD56^{bright}$ NK cells producing IFN-γ and deterioration of kidney function, supporting the pro-inflammatory pathogenic effect of this NK cell subset.

As regards the Ped-SDI (Dayal et al. 2002), comparable to previously reported (Gutierrez-Suarez et al. 2006), renal damage was the commonest encountered finding, followed by neuropsychiatric damage, but no malignancies were detected. Growth failure and delayed puberty were detected in 17 and 11.4% of the patients, respectively. On investigating the correlation between the frequency of the studied immune cells and the 14 organ systems/domains, renal damage was found to be significantly correlated with the low frequency of NK and NKT cells and the increased $CD56^{bright}$ NK cells. Low NK cell number was also correlated with neuropsychiatric damage and growth failure. Meanwhile, the increase in $CD56^{bright}$ NK cells correlated directly with neuropsychiatric damage, premature gonad failure, and growth failure. On the other hand, NKT cell number was directly correlated with musculoskeletal and skin damage, premature gonad failure, and delayed puberty. These data indicate that the frequency of NK, $CD56^{bright}$ NK, and NKT cells might have influence on system affection and disease progression.

Although the research was carefully prepared, there were some unavoidable limitations. We have not monitored the effect of different immunosuppressive drugs and their duration of usage on NK and NKT cell numbers. γδT cells as well as $CD16^-$ NK and NKT cells were not included in the results. Peripheral NK and NKT cells levels were correlated with the disease activity and chronicity indices of renal biopsy, but we did not study these cells locally in the renal tissue. The small sample size and the cross-sectional nature of the study are also limiting factors.

In conclusion, low frequency of NK and NKT cells and expansion of $CD56^{bright}$ NK cells are marked in juvenile SLE, particularly with activity. In addition, changes

Table 4 Correlations between NK, CD56^{bright} NK, and NKT cell numbers, and laboratory findings, biopsy parameters, and activity index

Parameter	NK cells		CD56^{bright} NK cells		NKT cells	
	r	*P*	*r*	*P*	*r*	*P*
Laboratory findings						
WBCs count	0.5	< 0.0001	− 0.3	0.06	0.3	0.06
Positive ANA	− 0.05	0.8	− 0.1	0.4	− 0.2	0.5
Positive anti-dsDNA	− 0.4	0.01	0.6	0.001	− 0.07	0.8
Serum albumin (g/dl)	0.3	0.09	− 0.3	0.06	0.4	0.01
Urea (mg/dl)	− 0.4	0.01	0.7	< 0.0001	− 0.2	0.1
Creatinine (mg/dl)	− 0.4	0.009	0.6	< 0.0001	− 0.4	0.02
CRP (mg/l)	0.3	0.09	0.6	0.01	0.4	0.02
C3 (mg/dl)	− 0.3	0.1	− 0.4	0.008	− 0.1	0.5
C4 (mg/dl)	− 0.1	0.7	− 0.4	0.02	− 0.2	0.2
Renal biopsy parameters						
Activity index	− 0.6	0.001	0.6	< 0.0001	− 0.5	0.001
Chronicity index	− 0.09	0.6	0.4	0.01	− 0.05	0.8
SLEDAI-2K						
Mild activity	− 0.1	0.3	0.2	0.09	− 0.1	0.2
Moderate activity	− 0.2	0.07	0.7	< 0.0001	− 0.1	0.4
High activity	− 0.4	0.001	0.5	< 0.0001	− 0.4	0.01
Very high activity	− 0.5	0.001	0.4	0.01	− 0.4	0.001

Spearman's correlation. *r* correlation coefficient. $P < 0.05$ is significant

NK natural killer cells, *NKT* natural killer T lymphocyte, *WBCs* white blood cells, *ANA* antinuclear antibodies, *anti-dsDNA* antidouble-stranded DNA, *CRP* C-reactive protein, *RBC* red blood cells, *SLEDAI-2K* Systemic Lupus Erythematosus Disease Activity Index

Table 5 Correlation between organ system involvement and NK, CD56^{bright} NK, and NKT cell numbers

Ped-SDI domain	Number of SLE patients	NK cells		CD56^{bright} NK cells		NKT cells	
		r	*P*	*r*	*P*	*r*	*P*
Ocular	1	− 0.08	07	0.1	0.09	− 0.08	0.9
Neuropsychiatry	7	− 0.5	0.01	0.5	< 0.0001	− 0.07	0.4
Renal	8	− 0.4	0.005	0.7	< 0.001	− 0.6	0.02
Pulmonary	2	− 0.05	0.2	− 0.1	0.4	− 0.1	0.5
Cardiovascular	1	− 0.07	0.3	− 0.2	0.07	− 0.03	0.6
Peripheral vascular	1	− 0.05	0.5	− 0.03	0.4	− 0.03	0.1
Gastrointestinal	0	0	0	0	0	0	0
Musculoskeletal	3	− 0.04	0.2	− 0.1	0.07	0.4	0.03
Skin	5	− 0.09	0.3	− 0.1	0.08	0.4	0.01
Premature gonad failure	4	− 0.08	0.09	0.5	0.01	0.5	0.02
Diabetes regardless of treatment	4	− 0.06	0.1	− 0.4	0.008	− 0.07	0.1
Malignancy	0	0	0	0	0	0	0
Growth failure	6	− 0.4	0.02	0.7	0.001	− 0.1	0.07
Delayed puberty	4	− 0.07	0.09	− 0.09	0.5	0.4	0.01

Spearman's correlation. *r* correlation coefficient $P < 0.05$ is significant

Ped-SDI pediatric systemic lupus international collaborating clinic/ACR damage index, *SLE* systemic lupus erythematosus, *NK* natural killer cells, *NKT* natural killer T lymphocyte

in these cells have direct effect on renal impairment and growth failure in those patients, which may reflect their potential influence on disease progression. Future works on the role of NK and NKT cells subsets should have special focus on studying these cells locally in the renal tissue biopsies from patients with active renal disease, as this

will allow potential directions for management of SLE nephritis.

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Compliance with ethical standards

Conflict of interest None.

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