

The Expression pattern of NK cells in systemic lupus erythematosus patients with different disease activities

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Abstract

Data demonstrated the role of natural killer (NK) cells in systemic lupus erythematosus (SLE). We aimed to determine the immunophenotype and frequency of NK cells and their subsets, CXCR3, CD161 expression in blood and renal tissue of SLE patients with and without lupus nephritis and their relationship with disease activity. The study included 31 SLE patients and 11 controls. Study participants underwent full history and thorough clinical examination. SLE patients underwent routine laboratory investigations. Renal tissue biopsies were taken from patients with lupus nephritis. The frequency of NK cell subsets in blood of patients and controls and renal tissue from patients was performed by flow cytometry. An increase in circulatory CD56^{bright} NK cells and its CD56^{bright} CD16^{dim} subtype was associated with the severity of systemic manifestations in SLE patients. Total CD56^{bright} NK cells and its CD56^{bright} CD16^{bright} subtypes in renal tissues were related to renal damage. We detected decreased CD161 expression on NK cells related to renal damage and severity of systemic manifestations in SLE patients. Decreased expression of CXCR3 on NK cell surface in renal tissues causes misdirected trafficking of NK cells that seems to reduce the severity of lupus nephritis. In conclusion, our results paved the way to understanding the role of NK cells in the pathogenesis of SLE which may represent a future target for immune therapy of SLE. NK and CD56^{dim} subset were decreased in the blood and increased in renal tissue of SLE patients, while the CD56^{bright} subset was increased in blood and decreased in renal tissue reflecting their effects on renal damage and severity of manifestations in SLE.

Keywords: CD16; CD56, CXCR3; Natural killer cells, Systemic lupus erythematosus (SLE).

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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease affecting a wide range of organs and characterized by the breakdown of self-tolerance of innate and adaptive immune responses against self-antigen.¹ The European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) declared that SLE criteria are weighted from 2 to 10 and a total score of 10 points is necessary to be classified as having SLE.²

Lupus nephritis is a severe organ manifestation of SLE that can affect up to 70% of the SLE population. About 10–30% of patients with lupus nephritis will progress to end-stage renal failure.³ In humans, natural killer (NK) cells constitute approximately 10% of peripheral blood lymphocytes.⁴ Human NK cells are conventionally defined as CD3⁺CD56⁺ lymphocytes.⁵ NK cells can be split into two major subsets, based on the relative densities of CD56 surface expression.

The CD56^{dim} NK cells, comprise 90% of the peripheral blood NK cells, have a high cytolytic capacity (cytotoxic), and secrete low levels of cytokines.⁶ Conversely, CD56^{bright} NK cells are the main type of NK cells in secondary lymphoid tissues and sites of inflammation, secrete a greater number of cytokines (cytokine producer) including interferon (IFN) and tumor necrosis factor alpha (TNF- α) but acquire cytotoxicity only after prolonged activation.⁷ The predominant type of NK cells in inflammatory lesions is CD56^{bright}, but CD56^{dim} NK cells also express chemokine receptors that may lead them to sites of inflammation.⁵ Several studies exploring the contribution of NK cells in SLE demonstrated a numerical decrease in circulating NK cells associated with clinical symptoms and disease activity of SLE patients.^{8,9,10} Despite of absolute reduction of NK cells in patients with SLE, various studies also demonstrated an augmented CD56^{bright} NK cell proportion, especially during the active phase of the disease, compared to decreased cytotoxic CD56^{dim} NK cells.^{9,11,12} Studies analyzing renal NK cells suggested that CD56^{bright} NK cells are associated with the degree of fibrosis and loss of renal function.^{13,14} These

cells were localized to sites of tubule-interstitial injury and they express interferon gamma (IFN- γ), suggesting that NK cells, particularly CD56^{bright} NK cells, play important roles in the disease progression of renal fibrosis.¹³ CD161 is a NK cell receptor that can deliver an inhibitory signal for the cytotoxicity function of human NK cells. CD161 is a C-type lectin-like receptor expressed by the majority of NK cells and 24% of T cells.¹⁵ Chemokine receptors such as receptor for INF- γ -inducible 10-kd protein (IP-10) (CXCR3) [also designated CXCL10], whose expression is inducible rather than constitutive, plays an important role in the control of the inflammation process.¹⁶ In recent years, there has been strong clinical and experimental evidence supporting the concept that CXCR3 is involved in the development of autoimmune diseases, through the amplification of the inflammatory process in target organs, thus causing a worsening of clinical manifestations of diseases.¹⁷ The present study was carried out to determine the immunophenotype and frequency of the major NK cell subsets (CD56^{dim} and CD56^{bright}; CD16^{dim} and CD16^{bright}), and the level of CXCR3 and CD161 expression in peripheral blood of SLE patients compared to controls, and in renal tissue of SLE patients with lupus nephritis and relate them with disease activity.

Subjects and Methods

This was a hospital-based case-control study that was conducted from May 2021 to July 2022. The study included 31 patients with SLE (16 patients with lupus nephritis and 15 patients without lupus nephritis) admitted to the Rheumatology Unit of the Department of Internal Medicine in Assiut University Hospitals and 11 normal control subjects that were selected from health care workers at Assiut University Hospitals. Eligible patients were selected from patients who fulfilled at least four criteria of SLE according to ACR¹⁸. The exclusion criteria included: age <18 years or >60 years old, pregnancy or lactation, coexistence of other autoimmune diseases, viral infection, and malignancies.

Clinical and routine laboratory assessments

Full medical history was taken from the study participants according to a pre-designed questionnaire. Then they underwent a thorough clinical examination. Patients assessed for disease activity using the SLE disease activity index (SLEDAI).¹⁹ We also performed routine laboratory investigations that included complete blood count (CBC), liver function tests, kidney function tests, erythrocyte sedimentation rate (ESR), antinuclear antibodies (ANA) testing by ELISA, and urine analysis according to routine methods of the Clinical Pathology laboratory at the South Egypt Cancer Institute, Assiut.

Blood samples for flow cytometry

A peripheral venous blood sample (3 ml) was collected from each patient and control individual in EDTA tubes labeled with the subject's name, sex, age, and the date of collection.

Renal tissue biopsy from SLE patients with lupus nephritis

Renal tissue biopsies were collected by a specialist from SLE patients with nephritis. A percutaneous true cut needle was used to obtain a core of renal tissue guided by ultrasound or computed tomography scan to direct the needle. Collected tissue samples were placed in cups containing formalin and labeled with the patient's name, sex, age, and the date of collection. A part of the specimen was sent routinely to the histopathology laboratory at Assiut University Hospitals for histopathological grading of lupus nephritis (from grade I to grade V). The other part of the collected renal biopsy sample was transferred to the Department of Clinical Pathology, South Egypt Cancer Institute, Assiut University for laboratory processing and flow cytometry analysis.

Detection of the frequency of CD56⁺NK cell subsets and their surface expression levels of 161 and CXCR3 by flow cytometry

CD56⁺NK cell subsets were detected in peripheral blood samples of patients and controls, and in renal tissue biopsy samples from patients with lupus nephritis. We used the

following fluorochrome-conjugated antibodies: Allophycocyanin (APC-H7) conjugated anti-CD3 to stain surface CD3 (to exclude CD3+ve cells) (BD Bioscience, USA), Phycoerythrin (PE) conjugated anti-CD56 to stain surface CD56 (DAKO), Phycoerythrin (PE-Cy7) conjugated anti-CD16 to stain surface CD16 (BD Bioscience), Fluorescein 6-isothiocyanate (FITC) conjugated anti-CXCR3 to stain surface CXCR3 (BD Bioscience), and Allophycocyanin (APC) conjugated anti-CD161 to stain surface CD161 (EXBIO Praha). Flow cytometry was carried out by a flow cytometer (BD FACSCanto™ clinical flow cytometer, Bioscience, USA), and data were analyzed using software (BD FACS Diva™ software, version 8.0.1), according to the manufacturer's instructions.

The lymphocyte population was identified by the size and granularity of cells using forward scatter and side scatter. The percentage of NK cells was expressed as a proportion of total gated lymphocytes. The CD56^{bright} and CD56^{dim} subsets were identified according to the level of CD56 expression. Gates were set by using isotype control antibodies.

Statistical Analysis

Data were verified, coded by the researcher, and analyzed using the Statistical Package for the Social Sciences (SPSS) version 24.0 (SPSS-IBM Inc., Chicago, IL, USA). Descriptive statistics: means, standard deviations, medians, ranges [interquartile range (IQR)], frequency, and percentages were calculated. The normality of continuous variables was tested using the Kolmogorov–Smirnov test or Shapiro–Walk test as appropriate. Fisher's exact test was used to compare the difference in distribution of frequencies among different groups. Mann Whitney U test analysis was carried out to compare the medians of non-parametric data. Significant test results were considered when the *p* value was ≤ 0.05.

Results

Demographic data, clinical manifestations, and treatment regimen

A total of 31 SLE patients were enrolled in this study. They were 21 (68%) females and 10

(32%) males. The mean ($\pm$ SD) age of the patients was 26.6 ± 7.5 years and the mean disease duration was 3.65 ± 2.4 years. The control group comprised of 11 normal subjects and were 9 females (82%) and 2 (18%) males with a mean age of 35.73 ± 5.8 years. There was a statistically significant difference in the age between patients and controls ($p=0.001$).

According to the SLEDAI score, two (6.5%) patients showed mild disease activity (i.e. score: 1-5), 10 (32%) patients showed moderate disease activity (score: 6-12), 17 (55%) patients showed high disease activity (score: 13-20), and two (6.5%) patients showed very high disease activity (score above 20). The patients were clinically evaluated to determine the frequency of clinical manifestations. The most frequent reported clinical manifestations were oral-nasal ulceration (in 74% of patients), fatigue (68%), malar rash (58%), photosensitivity (58%), alopecia (55%) and arthralgia/arthritis (52%). Other clinical manifestations included fever (12 patients; 39%), skin rash (10 patients; 32%), headache and weight loss (each in 8 patients; 26%), serositis (4 patients; 13%), and vasculitis (2 patients; 6.5%). SLE patients were treated with steroids (22 patients; 71%) and immunosuppressive drugs including hydroxychloroquine (24 patients; 77%), azathioprine (10 patients; 32%), methotrexate (4 patients; 13%), and cyclophosphamide (2 patients; 6.5%).

Results of the renal biopsy for SLE patients with lupus nephritis

The study included 31 SLE patients. Of these, 15 (48%) patients did not have lupus nephritis (i.e., grade 0) and the remaining 16 (52%) patients had variable grades of lupus nephritis. Of these, 6 (19%) patients had grade II, 2 (6.5%) patients had grade IIIA, 6 (19%) patients had grade IV, and 2 (6.5%) patients had mixed grades IV/V.

Routine laboratory tests for SLE patients

Most SLE patients (29 patients; 94%) tested ANA positive, while the other two (6.5%) patients tested ANA negative. Compared to SLE patients without nephritis, SLE patients with lupus nephritis suffered significantly higher ESR (74.4 ± 47.4 vs 25 ± 32.7 mmHg; $p=0.002$), higher

serum alanine aminotransferase enzyme levels (23 ± 8.8 vs 17 ± 6.5 U/L; $p=0.044$), elevated serum creatinine levels (83.5 ± 25 vs 53.9 ± 5.3 μ mol/L; $p \leq 0.001$), higher levels of 24 h protein in the urine (1952.5 ± 1557.8 vs 253.8 ± 160.6 mg; $p \leq 0.001$), increased counts of pus cells in urine (11.6 ± 9.4 vs 5.9 ± 2.7 cell per high power field; $p=0.031$), and increased red blood cells counts in urine (6 ± 5.8 vs 1.2 ± 1.8 cell per high power field; $p=0.005$). Other routine laboratory investigations did not show significant differences between SLE patients with and without nephritis.

Flow cytometric analysis for detection of NK cells, NK cell subsets, CD161, and CXCR3 expression levels

The detected NK cell subsets were: CD56^{dim} CD16^{dim} (CD3⁻, CD161⁺, and CXCR3⁻), CD56^{dim} CD16^{bright} (CD3⁻, CD161⁺, and CXCR3⁻), CD56^{bright} CD16^{dim} (CD3⁻, CD161⁺, and CXCR3⁺), CD56^{bright} CD16^{bright} (CD3⁻, CD161⁺, and CXCR3⁺) (Figure 1). In peripheral blood, the frequency of total NK cells and CD56^{dim} NK cells of SLE patients were not lower than that in controls.

The frequency of CD56^{bright} NK cells of SLE patients was not different than that in controls. The frequencies of other NK cells subsets were similar in SLE patients and in controls. However, the level of CXCR3 expression was significantly lower in SLE patients than in controls ($p=0.007$). However, the level of CD161 surface expression was not different in patients and in controls ($p=0.516$).

In the renal tissues of patients, the frequency of total NK cells was not different than in patients' blood ($p=0.301$). The frequency of CD56^{dim} was significantly higher in renal tissue biopsy than in peripheral blood ($p \leq 0.001$), while the frequency of total CD56^{bright} was significantly lower in renal tissue biopsy than in peripheral blood ($p=0.003$). The frequency of CD56^{dim} CD16^{dim} and CD56^{bright} CD16^{dim} NK cells was significantly lower in renal tissue than in peripheral blood ($p=0.001$ and 0.004 , respectively). However, the frequency of both CD56^{dim} CD16^{bright} and CD56^{bright} CD16^{bright} NK cells was significantly higher in renal tissue than in peripheral blood ($p=0.002$ and 0.037 ,

respectively). The level of CD161 and CXCR3 surface expressions were significantly lower in

renal tissue than in peripheral blood ($p \leq 0.001$ and 0.033, respectively) (Table 1).

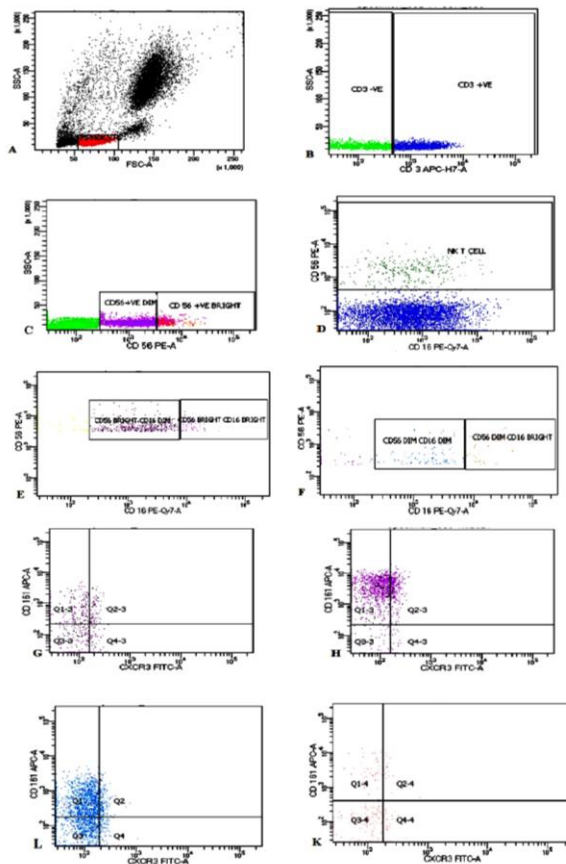


Figure 1. Flow cytometric detection of Natural killer lymphocytes and Neutral killer subsets and their expression of CD161 and CXCR3. A: Forward and side scatter histogram was used to define the lymphocyte population, B: The expression of CD3 was assessed on the lymphocytes, and then, the CD3⁺ and CD3⁻ were gated for further analysis, C: the expression of CD56 on CD3⁺ cell was detected and then CD56⁺ CD56⁻ cells were gated, D: The expression of CD56 and CD16 was assessed on CD3⁺ lymphocytes to detect Natural killer T cells, E-F: Natural killer cell was divided into CD56^{bright} CD16^{bright}, CD56^{bright} CD16^{dim}, CD56^{dim} CD16^{bright} and CD56^{dim} CD16^{dim} cells, G- H: The expression of CD161 and CXCR3 on CD56^{bright} CD16^{bright} and CD56^{bright} CD16^{dim}, and I-K: The expression of CD161 and CXCR3 on CD56^{dim} CD16^{bright} and CD56^{dim} CD16^{dim}.

Table 1. Frequency of NK cells, NK cell subsets, and expression levels of CD161 and CXCR3 in peripheral blood of SLE patients compared to healthy controls and in renal tissue in patients with lupus nephritis.

Cell subset	Peripheral blood of SLE patients (n=31)	Controls (n=11)	<i>p</i> value*	Renal biopsy tissue of SLE patients (n=16)	<i>p</i> value**
	Median value (IQR)			Median value (IQR)	
Total NK cells	3.6 (7)	4.7 (6)	NS	5.1 (3)	NS
Total CD56 ^{dim}	6 (14)	6.5 (7)	NS	57.5 (37)	≤0.001
Total CD56 ^{bright}	5.5 (12)	1.2 (1.1)	NS	0.5 (3)	0.003
CD56 ^{dim} CD16 ^{dim}	65.5 (20)	73 (21)	NS	40.5 (33)	0.001
CD56 ^{dim} CD16 ^{bright}	4.8 (11)	6.8 (6.5)	NS	43 (53)	0.002
CD56 ^{bright} CD16 ^{dim}	56.5 (24)	64.5 (25)	NS	28.7 (33)	0.004
CD56 ^{bright} CD16 ^{bright}	18.5 (28)	21.5 (37)	NS	33.8 (34)	0.037
CD161 expression level	48.5 (25)	56 (28)	NS	1.8 (8)	≤0.001
CXCR3 expression level	2.8 (5.5)	8 (5)	0.007	1.0 (4)	0.033

Data are represented as median (interquartile range; IQR) as analyzed by Mann Whitney U test was used to compare differences in medians between groups. * Differences between SLE patients and controls, ** Differences between peripheral blood and renal tissues in SLE patients with lupus nephritis. Abbreviations: NK=natural killer; SLE=systemic lupus erythematosus. $p > 0.05$ is not significant (NS).

Frequency of NK cells, NK cell subsets, CD161, and CXCR3 and relations to SLEDAI

The frequencies of NK cells and cell subsets, CD161, and CXCR3 in blood and renal tissues according to different SLEDAI are shown in Figures 2 and 3. Total NK cells showed no correlation in the peripheral blood of SLE patients and SLEDAI, while such correlation was significant in renal tissue. On the other hand, there was no correlation between the frequency of CD56^{dim} NK cells in peripheral blood and SLEDAI, while there was a significant moderate positive correlation between CD56^{dim} NK cells in renal tissue with SLEDAI. The

frequency of CD56^{bright} NK cells in peripheral blood and renal tissue showed a significant moderate positive correlation with SLEDAI. There was a significant moderate positive correlation between the level of CD161 surface expression and SLEDAI in peripheral blood, but such a positive correlation did not reach significance in renal tissue. In addition, there was no correlation between the level of CXCR3 surface expression and SLEDAI in the peripheral blood of SLE patients. However, there was a significantly high negative correlation between the level of CXCR3 surface expression and SLEDAI in renal tissue of SLE patients (Table 2).

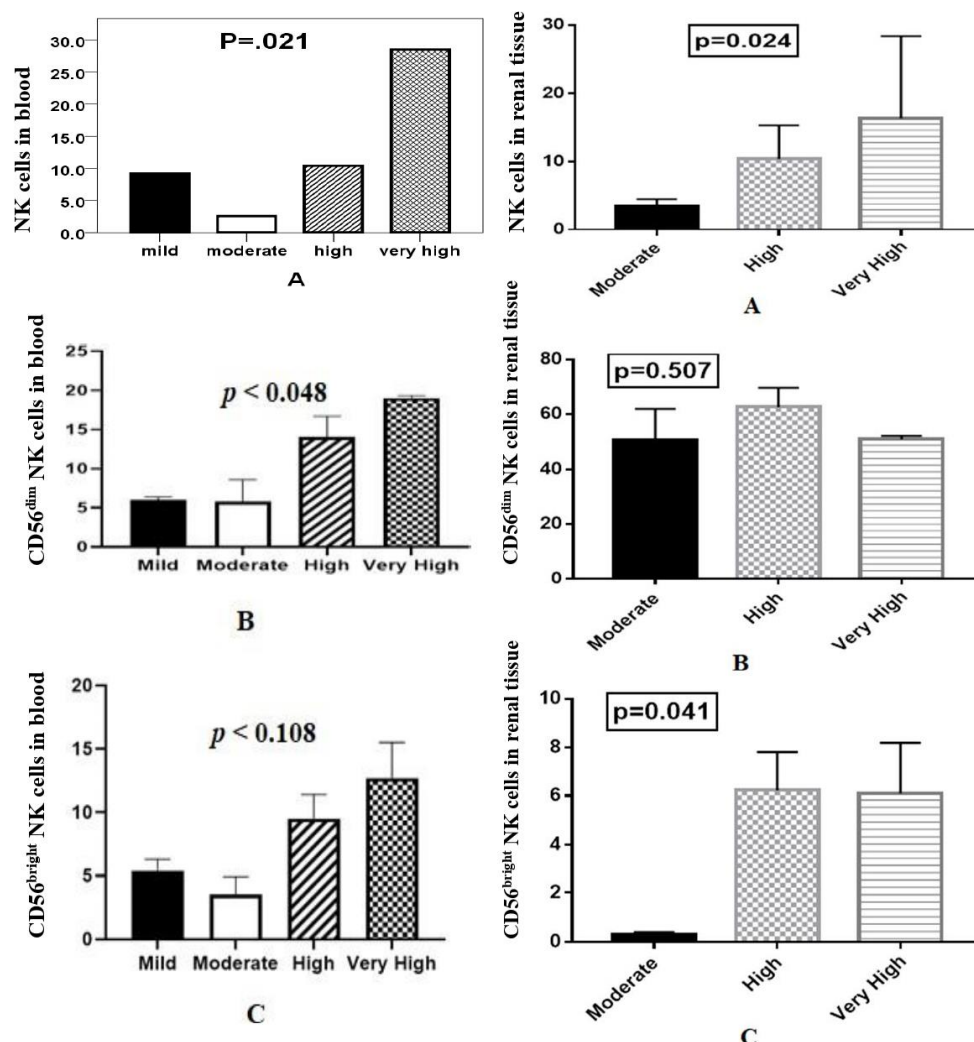


Figure 2. Frequency of A: NK cells, B: CD56^{dim} NK cells, and C: CD56^{bright} NK cells in peripheral blood and renal biopsy tissue of SLE patients with different SLEDAI. NK=natural killer; SLE=systemic lupus erythematosus; SLEDAI=systemic lupus erythematosus disease activity index.

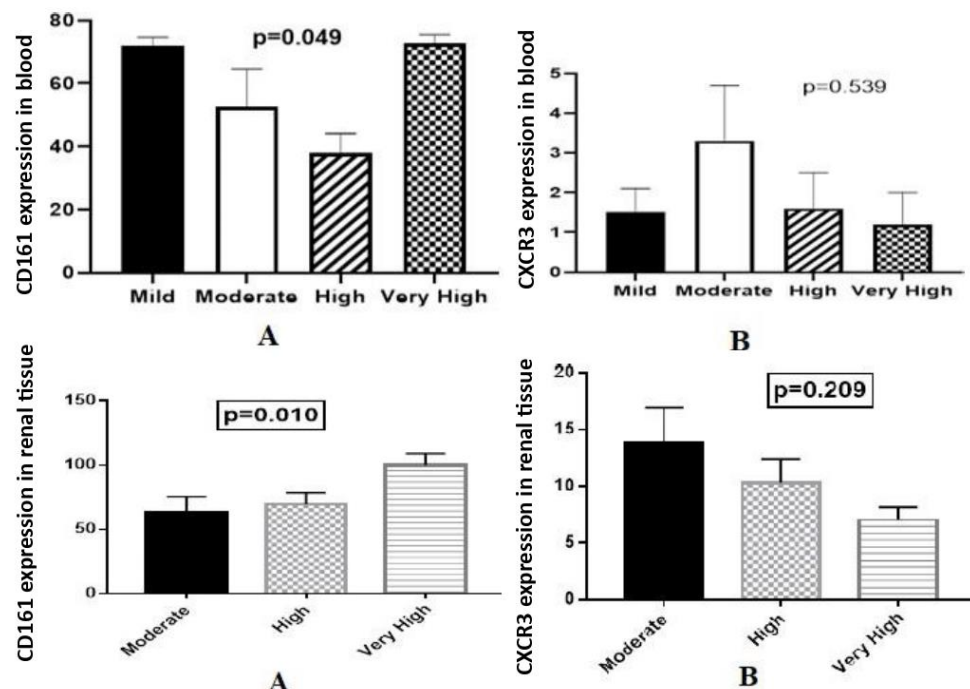


Figure 3. CD161 and CXCR3 surface expression in the peripheral blood and renal tissue of SLE patients with different SLEDAI. SLE=systemic lupus erythematosus; SLEDAI=systemic lupus erythematosus disease activity index.

Table 2. Correlations between the frequency of total NK cells, CD56^{dim}, CD56^{bright} NK cells, CD161 and CXCR3 surface expression in peripheral blood and renal tissue of SLE patients and SLEDAI.

	Correlations with different SLEDAI			
	Peripheral blood of SLE patients (n=31)		Renal biopsy tissue of SLE patients (n=16)	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
Total NK cells	0.185	NS	0.443	0.006
CD56 ^{dim} NK cells	-0.073	NS	0.301	0.049
CD56 ^{bright} NK cells	0.469	0.033	0.341	0.030
CD161 expression level	0.589	0.008	0.249	NS
CXCR3 expression level	-0.368	NS	-0.636	0.001

NK=natural killer; SLE=systemic lupus erythematosus; SLEDAI=systemic lupus erythematosus disease activity index.

p > 0.05 is not significant (NS).

Differences in frequency of total NK cells, CD56^{dim} NK cells, CD56^{bright} NK cells, CD161, and CXCR3 surface expression in different histopathological grades of lupus nephritis

The frequency of NK cells was significantly high in grade III-A lupus nephritis followed by grade II, grade IV, and finally grade IV/V. The frequency of CD56^{dim} NK cells was not different

in all grades of lupus nephritis. The frequency of CD56^{bright} was not different in all grades of lupus nephritis. The level of CD161 surface expression was not different in all grades of lupus nephritis. The level of CXCR3 surface expression was significantly higher in grade III-A, while it was lower in grades IV/V, IV, and II, respectively (Figure 4).

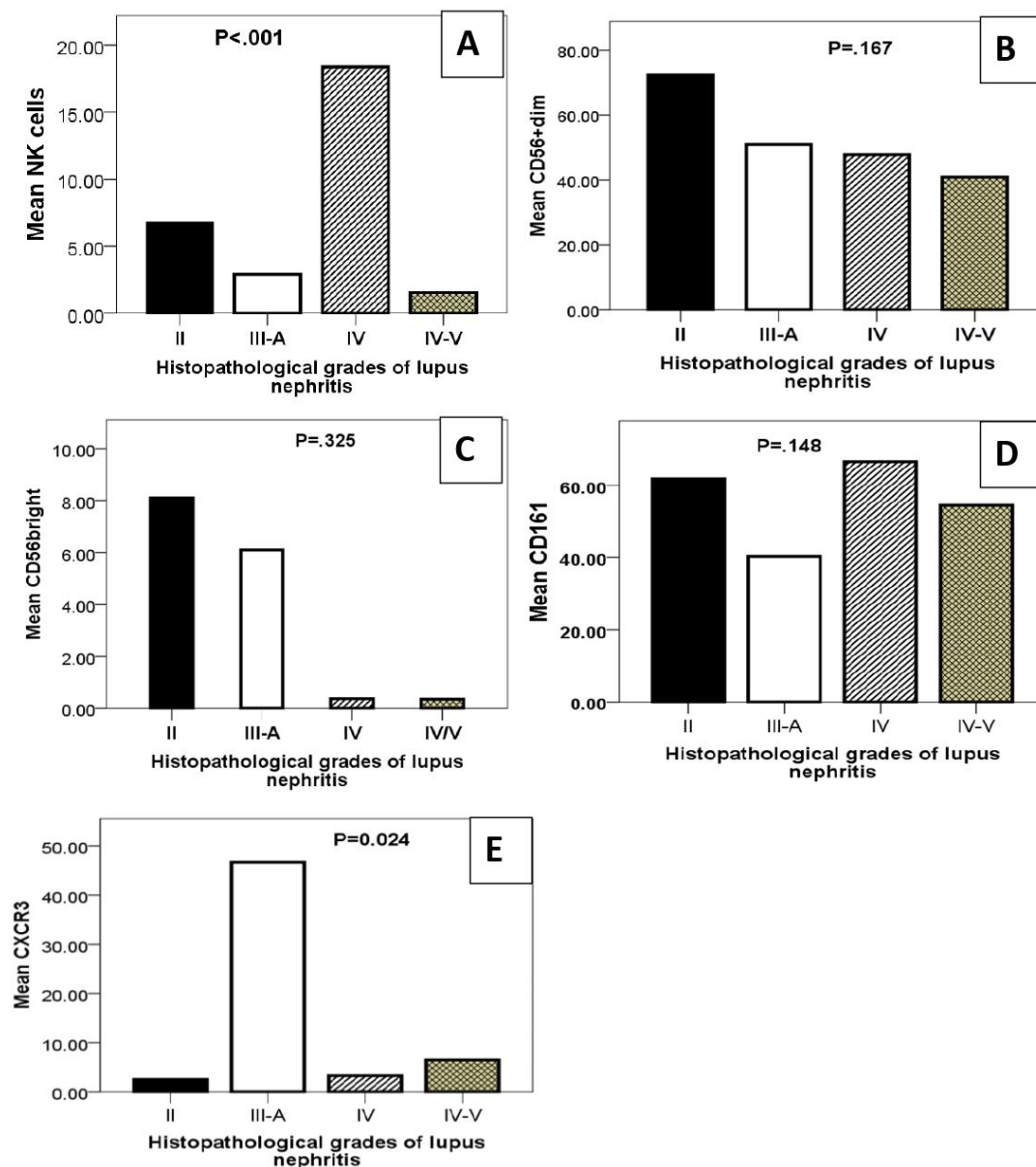


Figure 4 (A-E). Differences in frequency of total NK cells, CD56^{dim} NK cells, CD56^{bright} NK cells, and the level of CD161 and CXCR3 surface expression in different histopathological grades of lupus nephritis. NK=natural killer.

Discussion

In this study we aimed to determine the immunophenotype and frequency of NK cell and cell subsets (CD56^{dim} and CD56^{bright}; CD16^{dim} and CD16^{bright}), and the level of CXCR3 and CD161 expression in peripheral blood of SLE patients compared to normal controls, and in renal tissue of SLE patients with lupus nephritis and relate them with disease activity. According to our findings, the frequency of total NK cell

was increased in relation to the grade of renal damage in SLE patients. Also, increased total CD56^{dim} and its CD56^{dim}CD16^{bright} subtype in renal tissue and its CD56^{dim}CD16^{dim} subtype in peripheral blood are related to renal damage and severity of systemic manifestations in SLE patients. In this study we proved that increased circulatory total CD56^{bright} NK cells and its CD56^{bright}CD16^{dim} subtype was associated with severity of systemic manifestations in SLE patients. Total CD56^{bright} NK cells and its

CD56^{bright}CD16^{bright} subtype in renal biopsy tissues was related to renal damage in SLE patients. It is worthy to note in this study that decreased CD161 surface expression on NK cells is related to both renal damage and severity of systemic manifestations in SLE patients. Also, decreased surface expression of CXCR3 on NK cell surface in renal tissues causes misdirected trafficking of NK cells that seems to protect the kidney and reduce the severity of lupus nephritis. The results of our study paved the way to understand the role of NK cells in pathogenesis of SLE that may represent a future target for immune therapy of this disease.

Despite the improved understanding of the adaptive immune mechanisms that lead to organ damage in SLE, the contribution of other innate immune cells, including NK cells, needs further studies.²⁰ As previously reported, SLE is a disease that affects multiple systems and its symptoms vary widely,²¹ cases included in our study showed a wide variety of symptoms. As previously recommended,²² SLEDAI was used in our study to determine the pattern of disease activity. The disease activity pattern of our cases varied from mild to very high activity. More than half of our SLE patients suffered from lupus nephritis which coincided with other reports.^{3, 23} In our study, as expected, laboratory investigations showed marked abnormalities in patients with lupus nephritis compared to SLE patients with no nephritis. Although, the EULAR and ACR concluded that the positivity of ANA is required for diagnosis of SLE²⁴, two of our patients tested ANA negative. Rare cases with negative ANA could be encountered, so the test must be repeated several times in suspected patients.²⁵

To overcome the misdirected immune response in SLE, most of our patients received steroid therapy in addition to immune-suppressive drugs in the form of hydroxychloroquine, azathioprine, methotrexate, and cyclophosphamide. Tailoring of treatment regimens was based on disease severity as evaluated by SLEDAI.¹ Our results revealed a low frequency of NK cells and CD56^{dim} subset and expansion of total CD56^{bright} NK cells and its CD56^{bright}CD16^{dim} subtype in peripheral blood were marked in our patients

and are related to the severity of systemic manifestations in SLE. In the present study, the decreased frequency of total NK and CD56^{dim} cells in patients' blood than controls and the negative association to SLEDAI were also reported in earlier studies.^{26,27,28} In agreement with our findings, the study by Zahran *et al.*, 2019, showed a significant correlation between the decrease in circulating NK cells and the impairment of renal function and active renal affection (clinically and by renal biopsy).¹¹ Other reports also showed a direct relation between the drop of circulating NK cells and the onset of lupus nephritis.^{10,29} Many factors may contribute to depletion of circulating NK cells in SLE patients, such as a reduction in the number of NK cell precursors and their susceptibility to ligand-induced apoptosis via signaling molecules such as CD16 linked to the formation of immune complexes. In addition, influence of serum cytokines can also mediate the activation-induced apoptosis of NK cells as high serum IFN- α levels, secreted mainly by plasmacytoid dendritic cells, cell apoptosis induced by reactive oxygen species and the use of medication.^{8,10} According to the study by Spada *et al.*, 2015,²⁹ and by Stratigou *et al.*, 2017,²⁷ this decrease in circulatory NK cells, specifically the mature CD56^{dim} subset, in SLE could reflect the migration of these highly toxic cells from the peripheral blood to target organs; thus, aggravating local tissue damage. Our study also showed increased total NK cell, CD56^{dim}, and its CD56^{dim}CD16^{bright} subtype and low frequency of CD56^{bright} subset in renal biopsy to grade of renal damage with a significant positive correlation between frequency of NK cells in renal tissue of SLE patients and SLEDAI. According to the study by Law *et al.*, 2017,¹³ kidneys from patients with different forms of renal diseases contain a substantial number of CD56⁺ (including CD56^{bright}) NK cells. In contrast to CD56⁺ cells in peripheral blood, these CD56⁺ cells in the kidney may cause pathogenic effects. The study by Amand *et al.*, 2017,³⁰ concluded that the CD56^{dim}CD16^{bright} NK cell subtype represents the most cytotoxic subset (with the highest content of lytic granules and KIRs). In the present study, we detected increased total CD56^{bright} NK cells and their

CD56^{bright}CD16^{dim} subtype in peripheral blood than renal tissue and were positively correlated to SLEDAI. The study by Zahran *et al.*, 2019, explained this increase on several bases including their release in high numbers from the bone marrow and/or the lymphoid tissue (as the precursor of CD56^{dim} NK cells), the described resistance of CD56^{bright} NK cells to oxidant-induced cell death, and the possibility that CD56^{bright} NK cells might selectively expand as a result of their essential role as cytokines producers.⁷ The same study by Zahran *et al.*, 2019, demonstrated a significant direct association between the increase in circulating CD56^{bright} NK cells in the peripheral blood and disease activity, the impairment of renal function, activity, and chronicity indices of renal biopsy in the SLE patient group.¹¹ Despite the shortage of previous reports about circulating CD56^{bright} NK cells, the study by 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 proinflammatory pathogenic effect of this NK cell subset.¹³ CD161 seems to function as an inhibitory receptor in NK cells (can deliver an inhibitory signal for the cytotoxicity function of human NK cells). The study by Lin *et al.*, 2017, reported that the expression level of CD161 was not affected in SLE patients.²⁶ The study by Park *et al.*, 2020, reported non-significant downregulation of CD161 expression on NK cells in SLE patients that may contribute to the SLE pathogenesis.³¹ Our results showed that CD161 expression level in NK cells in SLE patients was lower than in controls and increases in SLE patients with increased disease activity. In agreement with the study by Kurioka *et al.*, 2018,³² who stated that CD161 expression identifies NK cells that may contribute to inflammatory disease pathogenesis and correlates with an innate responsiveness of NK cells to cytokines. Our study observed that CD161 surface expression on NK cells was related to severity of systemic manifestations in SLE patients because of the following significant findings. The level of CD161 surface expression was significantly higher in peripheral blood than in renal tissues of SLE patients. The frequency of CD161 surface expression in the peripheral

blood of SLE patients with very high activity and mild activity was significantly the highest followed by those with moderate activity, and finally patients with high activity (this difference in frequency of CD161 in SLEDAI could be attributed to different number of patients in each grade). The frequency of CD161 surface expression in renal biopsy tissue of SLE patients with very high activity was significantly the highest followed by those with high activity and finally patients with moderate activity, and the significant moderate positive correlation between level of CD161 surface expression and SLEDAI in peripheral blood of SLE patients. The study by Henriques *et al.*, 2013, reported that SLE patients were associated with reduced CXCR3 expression in NK cells and NK cell subsets.⁹ According to the study by Castriconi *et al.*, 2018, the CXCR3 G protein-coupled receptor belongs to a family of chemokine receptors. It is expressed by CD56^{bright} NK cells and mediates its homing to secondary lymphoid tissues.³³ In this regard, the results of our study are consistent with the possibility that decreased CXCR3 expression on NK cell surface appears to cause misdirected trafficking of NK cells that seems to protect the kidney and reduce the severity of lupus nephritis as the level of CXCR3 surface expression was significantly lower in SLE patients than that in controls and the level of CXCR3 surface expression is significantly lower in renal tissue biopsy than peripheral blood, the significant high negative correlation between level of CXCR3 surface expression and SLEDAI in renal biopsy tissue of SLE patients. This decrease may correspond to a sustained CXCR3 internalization resultant from higher serum IP-10 levels described in SLE and from redistribution and accumulation of these active cells at sites of inflammation (due to high concentrations of ligands for CXCR3 circulating in the serum of patients).⁹

In conclusion, the low frequency of NK cells and CD56^{dim} subset and expansion of CD56^{bright} subset in peripheral blood are marked in SLE patients. The high frequency of NK cells and CD56^{dim} subset and low frequency of CD56^{bright} subset in renal tissue may reflect their effects on renal damage and severity of systemic manifestations in SLE patients. Because CD161

expression on NK cells may contribute to inflammatory disease pathogenesis and correlates with the innate responsiveness of NK cells to cytokines, decreased CD161 surface expression on NK cells may be related to the severity of systemic manifestations in SLE patients. Decreased surface expression of CXCR3 on NK cell surface in tissues of renal biopsy causes misdirected trafficking of NK cells that seems to protect the kidney and reduce the severity of lupus nephritis.

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Author Contributions

OMB, MSB, SGE, AMZ, and MEM; shared in the design, methodology, analysis, and writing the manuscript. MRA and MMAH; provided the study's resources. All authors reviewed and edited the manuscript, and approved the submitted version.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Assiut University (IRB approval dated September 2022).

Informed consent

An informed consent was obtained from each participant in the study and all patient data were kept confidential.

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