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Analysis of interleukin-17 and interleukin-23 for estimating disease activity and predicting the response to treatment in active lupus nephritis patients

Huang Dedong[a], Zhu Feiyan[a], Shi Jie[a], Lai Xiaowei[a], Wang Shaoyang[b,c,*]

[a] Department of Infectious Diseases, the 903rd Hospital of the PLA of China, Hangzhou, China
[b] Department of Infectious Diseases, the 900th Hospital of the PLA of China, No.156 North Road West 2nd Ring Road Fuzhou, 350013, China
[c] Clinical educational Institute of the 900th Hospital of the PLA affiliated Fujian Medical University, Fuzhou, China

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ABSTRACT

Renal biopsy is a "gold standard" for establishing the diagnosis and assessing prognosis and monitoring therapy in lupus nephritis (LN) patients, but it is an invasive and inconvenient procedure. Evidences showed that interleukin-17(IL-17) and interleukin-23(IL-23) may be as alternative biomarkers for diagnosing LN, monitoring LN activity and predicting the response to treatment of LN. To analyze the roles of IL-17 and IL-23 in evaluation activity of LN and predicting active LN response to immunosuppressive treatment, by comparison between IL-17, IL-23 and clinical data of LN. Eighty patients with LN and 20 healthy volunteers were enrolled in this study. Plasma levels of IL-17 and IL-23 were detected by ELISA and clinical data were collected in patients with LN. Thirty-seven patients with active LN accepted immunosuppressive therapy and followed up to 6 months. The roles of IL-17 and IL-23 in evaluation the activity of LN and the predictability for active LN response to immunosuppressive treatment were analyzed. The ages or gender rations between LN patients and healthy controls were not significant difference at baseline. Baseline levels of IL-17 and IL-23 were higher in patients with active LN compare to them in patients with inactive LN or controls ($P < 0.001$) and IL-23 in patients with inactive LN was higher than its in controls ($P = 0.004$). IL-17 and IL-23 decreased significantly in active LN patients after 6 months therapy ($P < 0.001$). The baseline level of IL-23 was significantly different in subgroups response to the immunosuppressive treatment in patients with active LN ($P = 0.0014$). Baseline level of IL-23 in complete response group was lower than its in partial response group ($P = 0.0015$) or nonresponse group ($P = 0.013$). IL-17 was negative correlation with C3 ($r = -0.44$, $P < 0.001$). IL-17 and IL-23 correlated with systemic lupus erythematosus (SLE) disease activity index ($P < 0.001$). The correlation between IL-17 and LN pathological acute index (AI) was higher than the correlation between IL-23 and AI. ($r = 0.52$, $P < 0.001$ vs. $r = 0.41$, $P < 0.001$). Receiver Operation Characteristics (ROC) showed that IL-17 and IL-23 could be used to evaluate SLE disease activity index. IL-17 could be used as biomarker to evaluate pathological AI. IL-23 could be used as a predictor for predicting response to immunosuppressive treatment in patients with active LN. IL-17 and IL-23 may involve and contribute to LN. IL-17 could be used as a biomarker for LN clinical and pathological AI. IL-23 could be used as a predictor for predicting response to immunosuppressive treatment in patients with active LN.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder disease involving both the innate and the adaptive immune systems. The pathogenesis of SLE is complex and confusing, involving multiple organ systems with alternating clinical exacerbations and remissions [1,2]. Lupus nephritis (LN) is one of the most serious manifestation of SLE, affecting up to 60% of patients with SLE [3,4]. Although the precise pathophysiological mechanisms and underlying development of LN remain unclear, the hallmark of this condition is autoantibodies and immune complex deposition with subsequent infiltration by inflammatory cells and cytokines in the renal tissue and plasma. Many cytokines, including interleukin-17 (IL-17) and interleukin-23 (IL-23), are involved in and contribute to both the onset and progression of LN pathology [5,6].

Significant evidence indicates a key role for IL-17 in the pathogenesis of LN [5–10]. IL-17 is a pleiotropic proinflammatory cytokine primarily produced by T helper-17 (Th17) cells [6,10]. IL-17 (commonly referred to as IL-17 A) is a member of the IL-17 family of cytokines: IL-17A–F. IL-17 acts on various cell types to regulate the

* Corresponding author.
E-mail address: wsyang2005@163.com (W. Shaoyang).

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production of multiple proinflammatory molecules [11], promotes recruitment of inflammatory cells and facilitates T-cell infiltration [12,13]. IL-17 and IL-17-producing cells are increased in LN patients and associated with increased Ig deposition and complement activation in the kidney [5,8,6–10]. IL-17 has been detected in LN patients' glomeruli, and IL-17-producing cells are present in the kidney tissue from LN patients [9].

IL-23 is mainly released from tissue-resident or recruited dendritic cells and macrophages [14,15]. The basic role of IL-23 in LN is the activation and maintenance of the T-helper 17 pathway [5]. Previous studies found increased IL-23 serum levels in LN patients [13,17] and an expansion of T-cells expressing both increased IL-17 and IL-23 receptor in lupus-prone mice [5,16,17]. IL-23 receptor deficiency prevents the development of LN in murine lupus models [5,9,16]. Interestingly, a previous report documented the beneficial effects of treatment of subacute cutaneous lupus with a human monoclonal antibody that binds to the p40 subunit of IL-23 and inhibits its biological effects, indicating that blocking IL-23 may be useful in lupus patients [18]. Ustekinumab, a monoclonal antibody targeting IL-12 and IL-23, has been used in many autoimmune diseases. Ustekinumab treatment also resulted in better efficacy in clinical and laboratory parameters than placebo in the treatment of active systemic lupus erythematosus and had a safety profile in a multicenter, double-blind, phase 2, randomized, placebo-controlled trial [19].

Renal biopsy is not only a "gold standard" for establishing the diagnosis but also a useful tool for assessing prognosis and monitoring therapy in LN patients [4,20–22]. However, it is an invasive and inconvenient procedure. Thus, IL-17 and IL-23 as alternative biomarkers for diagnosing LN, monitoring LN activity and predicting the response to treatment of LN were assessed in this study by investigating their association with LN activity and their correlation with the prognosis of LN patients after treatment.

2. Subjects and methods

2.1. Study population

Eighty patients with LN and 20 healthy volunteers as controls were recruited from the outpatient clinics and inpatient section of the 903rd Hospital of the PLA in China between 2010 and 2017. Eligible patients were between 18 and 60 years old, fulfilled the updated revised American College of Rheumatology (ACR) classification criteria for SLE and were diagnosed with LN according to the International Society of Nephrology/Renal Pathology society (ISN/RPS) classification criteria for LN [4,17,23]. Demographic data were collected and SLE disease activity indexes (SLEDAIs) were evaluated. Patients were excluded if they had any of the following: other rheumatic or autoimmune diseases, renal diseases other than LN, infections, drug addiction, any other comorbidities or malignant tumors. Patients were excluded if they had received immunosuppressive therapy within the previous 12 months, or accepted SLE treatment. Women who were pregnant or breastfeeding were also excluded. The study protocol was approved by the ethics committee of the 903rd Hospital of the PLA. Written informed consent was obtained from all patients and healthy controls after explanation of the purpose and procedures of this study.

2.2. Renal histopathology and efficacy assessments

All patients underwent percutaneous renal biopsy. Each light microscopy specimen included at least 10 glomeruli. HE staining was performed the evaluate the kidney lesions. Renal biopsies were evaluated by light microscopy and were graded according to the ISN/RPS classification for LN and scored for acute index (AI) and chronicity index (CI) [17,23].

Complete remission (CR) was defined as proteinuria < 0.3 g/d, serum ≥ 35 g/l and normal serum creatinine (Scr); non-remission (NR) was defined as proteinuria decreasing 50% from the baseline level, serum ≥ 30 g/L and normal creatinine; and partial remission (PR) was defined as parameters between CR and NR [4,8,9].

2.3. LN severity assessment and patient follow-up

Patients were divided into two groups according to whether the SLE Disease Activity Index (SLEDAI) was > 9. Thirty-seven patients with SLEDAIs > 9 were defined as the active group, and 43 patients with SLEDAIs ≤9 were defined as the inactive group [4,24]. The patients were treated in accordance with the treatment regimen modified from the NIH protocol [4,17,23] for LN. Patients in the severe group were treated with methylprednisolone 0.5 g/day for 3 days as pulsed therapy, and followed by oral prednisolone 0.5 mg/kg/day and infusion cyclophosphamide 0.5 g/m^2 monthly for 6 months as induction therapy. Then the server group followed oral azathioprine 2 mg/kg/day and prednisolone 5˜10 mg/day for maintenance of remission. Patients in the mild group were treated with prednisolone 0.5 mg/kg/day. Patients were followed up regularly every 2 months for a period of 6 months. At each visit, clinical data, blood and urinary samples were collected, and a clinical examination was performed to assess SLE disease severity by the SLEDAI. Sera were stored at −70 °C.

2.4. Laboratory evaluation and serum cytokine estimation

At each visit, routine physical examinations were performed and blood samples were obtained for hematological and biochemical screening. Complement 3 (C3), complement 4 (C4) and 24-h urine protein were detected according to recommended laboratory standard methods.

The serum concentrations of IL-17 and IL-23 were estimated for all study populations at baseline and the active group patients at 6 months of follow-up. IL-17 was quantitatively determined using the Human IL-17 Quantikine HS ELISA Kit (Bio-Techne China Co. Ltd. Catalog HS170) by sandwich enzyme-linked immunosorbent assays (ELISAs). IL-23 was detected by Human IL-23 Quantikine ELISA Kit (catalog D2033B, R&D Systems, Abingdon, UK). The assay procedure was performed according to the manufacturer's instructions. Blood sampling for assessing serum levels of cytokines and other laboratory investigations were performed at the same time as the clinical examination and assessment of SLE disease severity.

2.5. Statistical analysis

Analyses of data were performed using SPSS statistical software for Windows (Version 17, SPSS Inc., USA). The quantitative parametric data are presented as the mean ± standard deviation (SD), and non-parametric data are presented as the median and range. Comparisons clinical and laboratory parameters of the active group, inactive patient group and healthy controls were performed by ANOVA for parametric data and comparisons different response groups in active group patients were performed using Mann-Whitney tests for non-parametric data. The correlations of serum IL-17 and IL-23 concentrations with the SLEDAI or AI score and laboratory parameters of LN patient groups were calculated using Spearman's rank correlation. The validity of IL-17 and IL-23 as predictors of disease severity and response to the immunosuppression therapy was evaluated by receiver operating characteristic (ROC) curves, area under the curve (AUC), cutoff points, sensitivity, and specificity. All tests for significance and the resulting *P* values were two-sided, with significance level of 0.05.

Table 1
Demographic and baseline characteristics of all subjects.

	Active group n = 37	Inactive group n = 43	Healthy controls n = 20	*P*
Age (year)	43.3 ± 11.3	39.9 ± 9.1	37.9 ± 9.5	0.12
Sex (male/female)	11/26	14/29	9/11	0.49
C3 (g/L)	0.47 ± 0.15	0.51 ± 0.23	1.06 ± 0.26	0.010[#]
C4 (g/L)	0.10 ± 0.04	0.13 ± 0.05	0.34 ± 0.10	< 0.001[#]
Albumin (g/L)	27.39 ± 5.32	33.14 ± 5.27	39.65 ± 5.56	< 0.001[#]
Uria protein (g/d)	3.93 ± 0.77	1.83 ± 0.72		< 0.001[※]
Scr (μmol/L)	107.15 ± 35.47	92.40 ± 35.29	59.05 ± 24.58	< 0.001[#]
SLEDAI	12.57 ± 1.78	5.69 ± 2.01		< 0.001[※]
AI	10.63 ± 1.58	6.39 ± 1.78		< 0.001[※]
CI	3.35 ± 0.71	2.35 ± 0.73		< 0.001[※]
LN pathologic class (case)				< 0.05[ζ]
I	0	0		
II	3	8		
III	15	17		
IV	15	16		
V	4	2		
VI	0	0		

Scr: serum creatinine; SLEDAI: systemic lupus erythematosus disease activity index; AI: acute index; CI: chronicity index.
[#] *P* value from ANOVA in three groups.
[※] *P* value from independent *t* tests between the active group and inactive group.
[ζ] *P* value from χ^2 tests between the active group and inactive group.

3. Results

3.1. Demographic, clinical and laboratory characteristics of LN patients are shown in Table 1

Eighty LN patients, including 37 active onset LN patients and 43 inactive patients, and 20 healthy controls were enrolled in this study. The baseline age and gender proportion were not significantly different among the active onset group, inactive group and healthy controls ($P > 0.05$). The levels of C3 and C4 in LN patients were significantly lower than those in healthy controls ($P < 0.05$). Protein in LN patient serum was lower than that in healthy controls, and the Scr in LN patient plasma was higher than that in healthy controls ($P < 0.001$). SLEDAI and 24-hour proteinuria in the active group were significantly higher than those in the inactive group ($P < 0.001$). AI and CI in the active group were higher than those in the inactive group ($P < 0.001$). Pathological types in the active group and inactive group were significantly different ($P < 0.05$). The ratios of type II and type III in the active group were lower than those in the inactive group and healthy controls, and the ratios of type IV and type V in the active group were higher than those in the inactive group and healthy controls.

3.2. Comparison of IL-17 and IL-23 among the active group, inactive group and control group, as shown in Fig. 1

At baseline, the average levels of IL-17 in the active group, inactive group and healthy control group were (9.08 ± 1.39) pg/ml, (6.82 ± 1.41) pg/ml and (6.13 ± 1.00) pg/ml, respectively. The levels were significantly different among the groups ($P < 0.001$). IL-17 in the active group was higher than that in the inactive group and healthy control group ($P < 0.001$). IL-17 was not significantly different between the inactive group and healthy control group ($P = 0.059$). The levels of IL-23 in the three groups were significantly different ($P < 0.001$). The average IL-23 values in the three groups were (175.79 ± 26.61) pg/ml, (133.72 ± 21.79) pg/ml and (114.62 ± 19.70) pg/ml, respectively. IL-23 in the active group was higher than that in the inactive group and healthy control group ($P < 0.001$). IL-23 in the inactive group was also higher than that in the healthy control group ($P = 0.004$).

3.3. The changes in IL-17 and IL-23 after therapy in the active group are shown in Fig. 2

Thirty-seven active LN patients accepted the therapy and were followed up for 6 months. After therapy, 19 patients showed a CR, 13 patients showed a PR and 5 patients showed NR in the active group. After 6 months of therapy, IL-17 was (7.32 ± 2.17) pg/ml and IL-23 was (118.92 ± 51.55) pg/ml in the active group. Compared with the baseline, IL-17 was significantly decreased ($P < 0.001$), and IL-23 was also decreased significantly after therapy ($P < 0.001$). The median values of IL-17 in CR, PR and NR patients were 9.16 pg/ml, 9.93 pg/ml and 10.43 pg/ml. IL-17 was not significantly different among the CR, PR and NR patients at baseline ($P = 0.119$). At baseline, the IL-23 median value in CR patients was 168.6 pg/ml, the IL-23 median value in PR patients was 191.3 pg/ml, and the IL-23 median value in NR patients was 182.7 pg/ml. IL-23 was significantly different among the CR, PR and NR patients at baseline ($P = 0.0014$). At baseline, IL-23 in the CR patients was lower than that in the PR patients ($P = 0.0015$) and the NR patients ($P = 0.013$). However, IL-23 in the PR patients and the NR patients was not significantly different ($P = 0.922$) at baseline.

3.4. IL-17 and IL-23 in relation to clinical characteristics and pathological indexes

At baseline, IL-17 was negatively correlated with C3 ($r = -0.44$, $P = 0.002$; Fig. 3a), and there was no significant difference between IL-23 and C3 ($P > 0.05$). IL-17 and IL-23 were not correlated with C4 ($P > 0.05$). IL-17 was correlated with SLEDAI ($r = 0.63$, $P < 0.001$; Fig. 3b). IL-23 was correlated with SLEDAI ($r = 0.056$, $P < 0.001$; Fig. 3c). IL-17 was correlated with AI ($r = 0.52$, $P < 0.001$; Fig. 4a). IL-23 was also correlated with AI ($r = 0.41$, $P < 0.01$; Fig. 4b).

3.5. The predictive values of IL-17 and IL-23 for evaluation of LN activity, AI and outcome of active LN treatment

The AUC of the ROC curve of IL-17, as a biomarker to predict the activity of LN (SLEDAI > 9), was 0.91 ($P < 0.001$; Fig. 5a). As a biomarker to predict the activity of LN (SLEDAI > 9), IL-23 had an AUC of 0.78 ($P < 0.01$; Fig. 5b). The AUC of IL-17, as a marker to predict AI > 8, was 0.81 ($P < 0.01$; Fig. 5c). The ROC curve analysis showed that IL-23 could not be used to predict the AI > 8 ($P > 0.05$). ROC curve analysis of IL-23 to predict the outcome of therapy for

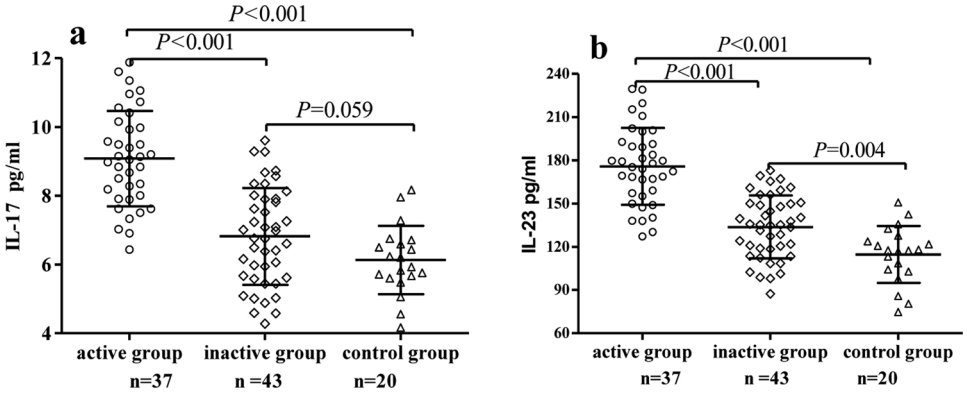

Fig. 1. Comparison of baseline serum IL-17 and IL-23 in all study groups. The baseline level of IL-17 in patients with active LN was higher than that in patients with inactive LN or controls ($P < 0.001$), and it was not significantly different between patients with inactive LN and controls ($P = 0.059$); Fig. 1a. IL-23 in patients with active LN was higher than that in patients with inactive LN ($P < 0.001$), and it was higher in patients with inactive LN than controls ($P = 0.004$); Fig. 1b.

induction of remission of active LN showed that the AUC of IL-23 was 0.87 ($P < 0.001$; Fig. 5d). The ROC curve analysis showed that IL-17 could not be used to predict the outcome of therapy for induction of remission in active LN patients ($P > 0.05$).

4. Discussion

Cytokine dysregulation plays a very important role in the pathogenesis of LN. A specific cytokine may be used to assess the disease activity and severity to replace renal biopsy [1,4,8,9]. As a third subset of CD4 + T effector cells, Th17 cells have been examined for their role in the Th17 axis, especially IL-17 and IL-23 in the pathogenesis of LN [5,7,10,11,13]. To assess the role of IL-17 and IL-23 in the pathogenesis of LN, we detected these molecules as biomarkers to assess LN activity and predictors of LN remission and treatment outcomes in patients with active LN in this study.

As double negative (DN) T-cells, which are a major source of IL-17, showed increased infiltration of the kidneys, the IL-17 in LN patients increased [25,26]. Previous studies have shown that an increase in the number of IL-17-producing cells in peripheral blood or in kidneys led to increased concentrations of IL-17 in the peripheral blood of LN patients [8,9,13,27]. Recent evidence has shown that enforced expression of IL-17 could enhance the severity of LN, while blockade of IL-17 decreased the severity of LN [26,28]. Similar findings were also shown in this study. The levels of IL-17 were significantly different in active LN patients, inactive patients and healthy controls in this study, which agreed with previous results [8,9,27]. The level of IL-17 in patients with active LN was higher than that in patients with inactive LN or controls, and it was not significantly different between patients with inactive LN and controls in this study. IL-17 was positively correlated with the AI scored by renal histopathology. The results of this study and previous studies indicate that IL-17 is involved in the inflammatory process in LN and may be a biomarker for assessing LN disease exacerbations.

The main source of IL-23 is antigen-presenting cells, and IL-23 induces expansion of Th17 cells and is necessary for their proliferation and maintenance [5,6,9,16]. IL-23 accounts for the main aspects of human or murine lupus, including the expansion of DN T cells, decreased IL-2, and increased IL-17 production [29]. IL-23 induced IL-17 and limited IL-2 production, whereas T follicular helper and DN T cells significantly expanded [29]. The level of IL-23 increased in LN patients

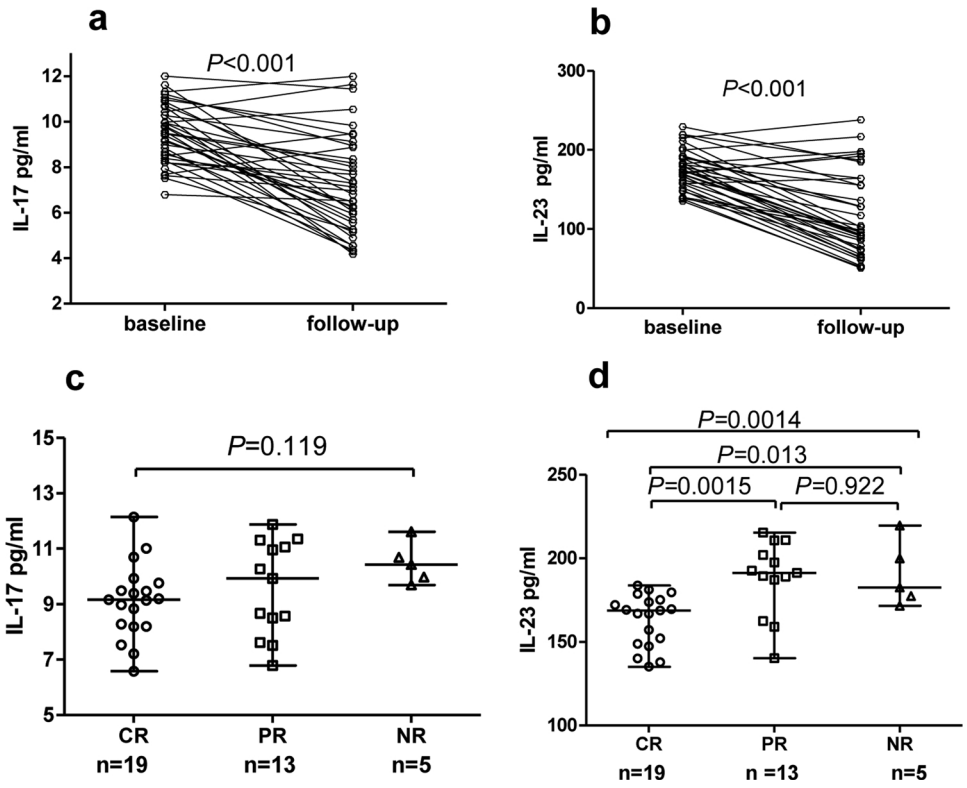

Fig. 2. The changes in serum IL-17 and IL-23 in all study groups during treatment. The IL-17 levels decreased significantly in patients with active LN who accepted treatment ($P < 0.001$); Fig. 2a. IL-23 decreased significantly in patients with active LN who accepted treatment ($P < 0.001$); Fig. 2b. In patients with active LN who accepted treatment, the baseline levels of IL-17 among the CR, PR and NR groups were not significantly different ($P = 0.119$); Fig. 2c. In patients with active LN who accepted treatment, the baseline levels of IL-23 among the CR, PR and NR groups were significantly different ($P = 0.0014$). IL-23 in the CR group was lower than that in the PR group ($P = 0.0015$) and the NR group ($P = 0.013$). IL-23 was not significantly different between the PR and NR groups ($P = 0.922$); Fig. 2d.

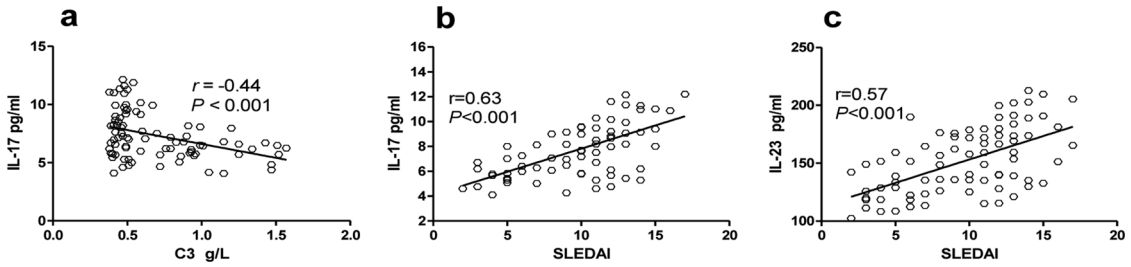

Fig. 3. Investigation of the correlation between serum IL-17 and IL-23 and clinical characteristics. IL-17 was negatively correlated with C3 (r = -0.44, $P < 0.001$; Fig. 3a). IL-17 was correlated with SLEDAI ($r = 0.63$, $P < 0.001$; Fig. 3b). IL-23 was correlated with SLEDAI ($r = 0.057$, $P < 0.001$; Fig. 3c).

vs. healthy controls [6,9]. A significant difference in LN patients and healthy controls was also shown in this study. IL-23 in the active LN group was higher than that in the inactive LN group and the control group. IL-23 in patients with inactive LN was higher than that in the controls. These results not only agreed with the findings of a previous study [6,9] but also provided the subgroup differences. IL-23 was positively correlated with the AI scored by renal histopathology. The data in this study and previous evidence suggested that IL-23, as a non-invasive method, may be used to assess the exacerbations of LN patients.

IL-17 and IL-23 decreased significantly in active LN patients who accepted therapy [8,9]. In this study, IL-17 and IL-23 decreased dramatically after accomplishment of therapy in active LN patients, and levels of IL-23 in CR patients were significantly lower than those in PR or NR patients. These results were supported by previous evidence [8,9]. The level of IL-17 in the CR group was lower than that in the PR group, and the level of IL-17 in the PR group was also lower than that in the NR group, but these differences were not significant in this study. This emphasized by the observation that the patients with higher baseline IL-17 also had higher IL-23, and a majority of these patients showed NR to treatment [9]. Consistent with previous studies [8,9], IL-17 was negatively correlated with C3 in this study, and there was no significant difference between IL-23 and C3 and between IL-17 or IL-23 and C4. This discrepancy may be due to multiple factors, such as the small sample size in previous studies, the heterogeneity of LN [30], differences in sensitivity and specificity of the ELISAs, different effects of immunosuppressive medications [31], and more localized production of IL-17 in the affected tissues than in the plasma [9].

The elevated serum concentrations of IL-17 and IL-23 in LN patients were correlated positively with SLEDAI, denoting a significant correlation with LN disease exacerbations, which is consistent with previously reported data [8,9,28,29]. Although ROC curve analysis of IL-17 and IL-23 showed that both cytokines can act as biomarkers of disease activity, the AUC of IL-17 was larger than that of IL-23. IL-17 may be used for earlier detection of disease exacerbation. IL-17 can also act as a biomarker to evaluate the AI instead of renal biopsy because IL-17 was correlated with the AI and the AUC of IL-17 for prediction of AI > 8 reached 0.81. Moreover, ROC curve analysis of IL-17 and IL-23 proved that IL-23 can act as a sensitive and specific biomarker to predict the outcome of active LN therapy, which agreed with previous evidence [9,16,29]. However, in this study, IL-17 was not shown to have predictive value for the outcome of active LN therapy, which was consistent with the findings of a previous study [31]. IL-23 did not have sufficient sensitivity and specificity for prediction of AI > 8.

5. Conclusions

The results of this study clearly suggest that the IL-23/IL-17 axis has a major role in the pathogenesis of LN and indicate that both cytokines can be useful as biomarkers for renal disease activity. IL-17 can be used as a biomarker replacing renal biopsy to assess the pathogenic AI in some LN patients. IL-23 can be useful as a biomarker to predict the response to LN immunosuppressive treatment with a high sensitivity and specificity. Whether monitoring of IL-17/ IL-23 could be helpful in early detection of LN flares, in replacing renal biopsy to assess the pathogenic AI or as predictors of long-term prognosis needs to be further studied in a larger cohort of LN patients with a prolonged follow-up period to be confirmed.

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Conflict of interests

The authors declare that they do not have any financial or nonfinancial competing interests.

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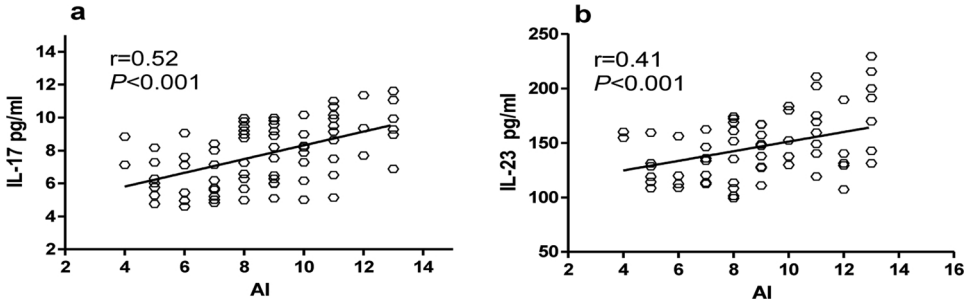

Fig. 4. The correlation between serum IL-17 and IL-23 and AI. Fig. 4a. IL-17 was correlated with AI ($r = 0.52$, $P < 0.001$). Fig. 4b. IL-23 was correlated with AI ($r = 0.41$, $P < 0.001$).

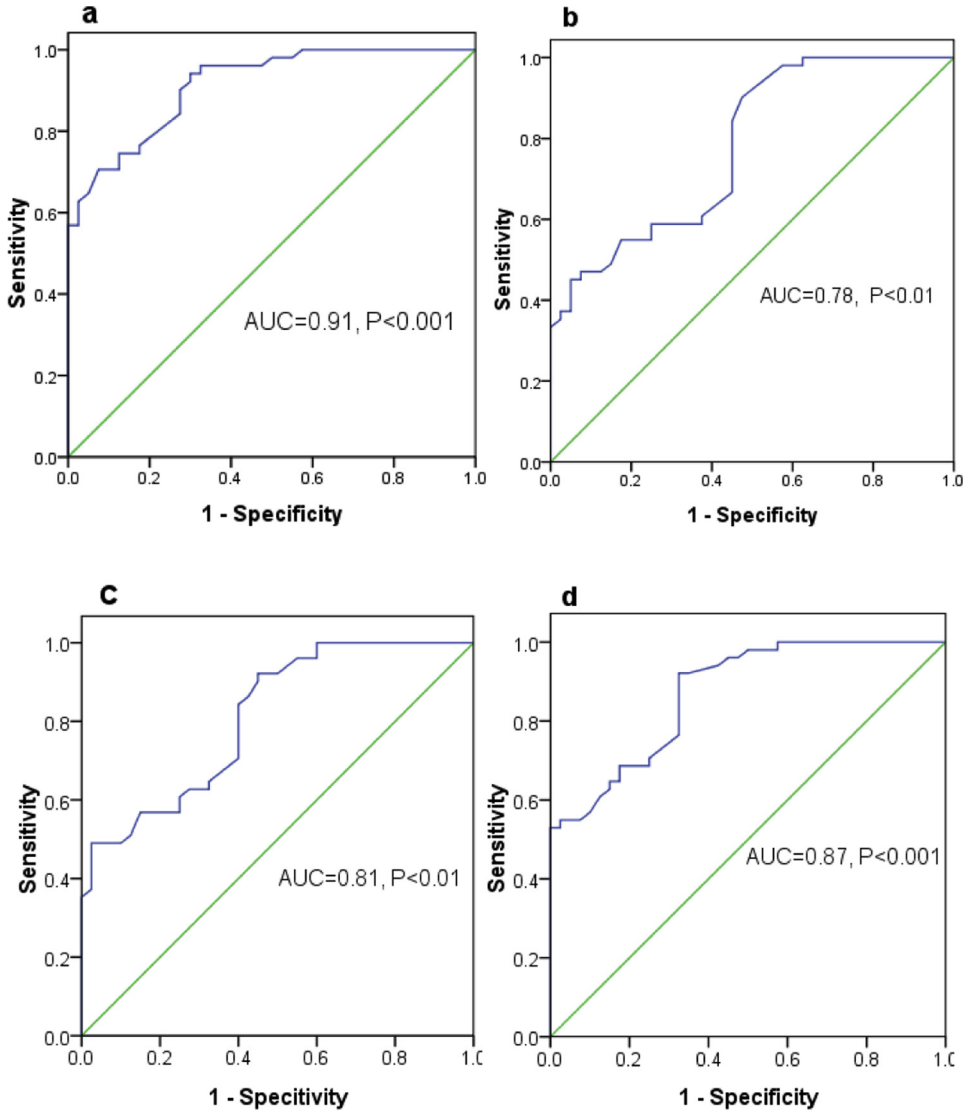

Fig. 5. Receiver operating characteristic (ROC) curve of IL-17 and IL-23 for predicting LN disease activity, AI and therapeutic effect. The horizontal axis is 1-specificity, and the vertical axis is sensitivity. Fig. 5a and Fig. 5b show the values of IL-17 and IL-23 for prediction of LN disease activity, respectively. IL-17 was a biomarker for prediction of AI > 8; Fig. 5c. IL-23 was a biomarker for prediction of active LN therapeutic effect; Fig. 5d.

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