



Serum TWEAK as a biomarker for disease activity of systemic lupus erythematosus

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Abstract

Objective This study aimed to identify serum and urine biomarkers that correlate with disease activity in female patients with systemic lupus erythematosus (SLE).

Methods Serum and urine levels of interferon γ -induced protein-10 (IP-10), TNF-like weak inducer of apoptosis (TWEAK), and monocyte chemoattractant protein-1 (MCP-1) of 70 patients with SLE and 61 healthy controls were measured with enzyme-linked immunosorbent assays.

Results The serum and urine levels of TWEAK, IP-10, and MCP-1 in both high and low SLE disease activity index (SLEDAI) groups of SLE patients were markedly higher compared with those of healthy controls. Moreover, the serum TWEAK level was positively correlated with the serum IP and serum MCP-1 levels ($p < 0.001$ for both). The serum TWEAK levels, SLEDAI scores, and urine protein/creatinine levels were significantly different between the SLE group with non-renal involvement and with renal involvement ($p = 0.034$, $p < 0.001$, and $p < 0.001$, respectively). The serum TWEAK level was a crucial determinant for a high SLEDAI score and renal involvement ($p = 0.044$ and $p = 0.046$, respectively).

Conclusion Serum TWEAK might be a serologic biomarker candidate that reflects disease activity and renal involvement in patients with SLE.

Keywords Systemic lupus erythematosus · TWEAK · IP-10 · MCP-1 · Biomarker · Kidney

Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by diverse and heterogeneous clinical manifestations, ranging from subtle symptoms to life-threatening organ failure [1, 2]. The crucial features of SLE are related to immune dysregulation and include production of autoantibodies, activation of complement, and immune complex deposition in target tissues or organs [3]. Although considerable advances have been made in understanding the complex clinical features and unpredictable progression of this disease, the pathogenesis of SLE has not yet been clearly defined.

For several decades, investigations on SLE have focused on achieving early diagnosis, monitoring disease activity and severity, and developing potent therapeutics that can be used in clinical practice. More recently, physicians or investigators have turned to biomarkers, which would ideally correlate with disease pathogenesis and/or clinical features and could be easily assessed in routine practice [4–6]. Biomarkers related to disease susceptibility, diagnosis, and organ involvement in SLE have been found to be associated with the presence of antibodies against dsDNA, C1q, and monocyte chemoattractant protein-1 (MCP-1) [6]. Recently, multiple cytokines and chemokines, including interferon γ -induced protein-10 (IP-10) [7–9], TNF-like weak inducer of apoptosis (TWEAK) [10, 11], and MCP-1

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[7, 9, 12] have been suggested as potent indicators for disease activity and/or renal involvement in SLE. However, no reliable and specific biomarkers for SLE have yet been developed and widely validated.

Few data are available regarding whether potential serum and urine biomarkers such as IP-10, TWEAK, and MCP-1 correlate with disease activity and/or renal disease status. Therefore, the aim of this study was to determine whether the levels of IP-10, TWEAK, and MCP-1, three potential serum and urine biomarker candidates for SLE, correlated with SLEDAI score and/or renal involvement in Korean females with SLE.

Subjects and methods

Subjects

This study enrolled 70 female with SLE (ranged from 20 to 64 years), each of whom met at least 4 of the 1982 revised American College of Rheumatology criteria for the diagnosis of SLE, from our outpatient Rheumatology clinic [13] and 61 healthy female controls (ranged from 22 to 62 years). Patients concomitantly having other autoimmune rheumatic diseases such as rheumatoid arthritis, Sjögren syndrome, systemic sclerosis, inflammatory myositis, and mixed connective tissue disease at the enrollment of this study were excluded. Patients who additionally used more than 0.5 mg/kg of prednisolone, oral/intravenous cyclophosphamide, azathioprine, or mycophenolate mofetil before 2 months of enrollment were also excluded from this study. All enrolled participants gave informed consent. The protocol of this study was approved by the Institutional Review Board/Ethics Committee of the Catholic University Medical Center.

Clinical data collection

Basic characteristics including age and disease duration were verified by interview with each patient and review of medical records. Data of laboratory tests such as whole blood count, platelet, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), serum creatinine, erythrocyte sediment rate (ESR), C-reactive protein (CRP), spot urine protein/creatinine ratio, anti-dsDNA titers, and serum complements (C3 and C4) levels were measured at the time of enrollment.

Lupus activity status was classified on the basis of SLE disease activity index (SLEDAI) scores: no activity (SLEDAI = 0), mild activity (SLEDAI = 1–5), moderate activity (SLEDAI = 6–10), high activity (SLEDAI = 11–19), and very high activity (SLEDAI \geq 20) [14]. According to disease activity based on SLEDAI-K

scores [15], two groups were arbitrarily allocated following as low (SLEDAI \leq 5) and high (SLEDAI \geq 6) groups [16].

The presence of renal involvement in SLE was determined by renal SLEDAI score of SLEDAI-2 K consisting of hematuria, proteinuria, pyuria, and urinary casts. Renal SLEDAI scores range from 0 (non-active renal disease) to a maximum score of 16. Renal involvement was classified into two groups following as non-renal involvement (score = 0) and renal involvement (scores \geq 1) at the time of enrollment of this study on the basis of their renal SLEDAI scores.

Measurement of IP-10, MCP-1, and TWEAK levels

Serum and spot urine samples from each patient were collected at the time of enrollment in the study and were stored at -70°C refrigerator after centrifuge until analysis. IP-10, MCP-1, and TWEAK levels at each sample were measured by enzyme linked immunosorbent assay (ELISA) kit according to assay instructions (R&D Systems, Minneapolis, MN, USA). 96-well plates were coated with 100 μl per well of captured antibody was incubated overnight at room temperature. Plates were then washed twice in wash buffer (0.05 % Tween 20 in phosphate buffered saline (PBS)) and blocked with 300 μl per well of 1 % bovine serum albumin in PBS for 1 h at room temperature. After washing, 100 μl of samples was added to each well and incubated for 2 h at room temperature. The plates were washed and detection antibodies were added with incubated for 2 h at room temperature. IP-10, MCP-1, and TWEAK levels were developed with Streptavidin-HRP (1:200 diluted) and substrate solutions. The absorbance values of the samples were read at 450 nm in a microplate reader.

Statistical analysis

Data were described as mean \pm standard deviation (SD) for continuous variables and number (%) for categorical variables. The distribution of data was verified by normality test using Kolmogorov–Smirnov and Shapiro–Wilk tests. From here, we applied non-parametric analysis in this study. The statistical difference between two groups (controls versus SLE or renal versus non-renal involvement) was performed using Mann–Whitney *U* test. Correlation between biomarkers and clinical parameters with sequential variables was simply assessed by Spearman's correlation analysis. Multivariate regression analysis was applied to identify association of clinical and laboratory factors for serum and urine biomarkers. Multivariate logistic regression analysis was used to determine the risk factor for disease activity status such as high SLEDAI or

renal involvement. Receiver operating characteristic (ROC) curve analysis and difference between area under the curve (AUC) was performed to assess the predictive value of cytokines for renal involvement. All statistical analyses were performed using SPSS (version 18.0, Inc, Chicago, IL, USA). Statistical significance was considered less than 0.05 of p value.

Results

Baseline characteristics

The baseline characteristics of the patients with SLE are illustrated in Table 1. This study included 70 female patients with SLE (mean age, 40.4 ± 11.2 years) and 61 female healthy controls (43.6 ± 9.1). The mean age was not significantly different between the two groups ($p > 0.05$). The mean disease duration of SLE was 6.4 years (SD 4.4).

Comparison of biomarkers between healthy controls and lupus patients

The serum IP-10, TWEAK, and MCP-1 levels in patients with SLE were significantly higher than those in control patients (142.4 ± 64.2 vs. 309.5 ± 222.7 pg/ml for IP-10, 156.7 ± 101.3 vs. 716.4 ± 618.1 pg/ml for TWEAK, and 124.8 ± 54.7 vs. 409.3 ± 294.6 pg/ml for MCP-1; $p < 0.001$ for all), as shown in Supplemental Figure 1. In addition, the urine levels of IP-10, TWEAK, and MCP-1 were also significantly different between healthy controls and patients with SLE (20.8 ± 19.6 vs. 125.5 ± 152.1 pg/ml for IP-10, 23.0 ± 29.51 vs. 175.5 ± 306.9 pg/ml for TWEAK, and 21.7 ± 41.3 vs. 108.3 ± 222.9 pg/ml for MCP-1, $p < 0.001$ for all).

According to disease activity of SLEDAI score, 33 patients for high SLEDAI and 37 for low SLEDAI group was classified. As shown in Fig. 1, both SLE patients with both low and high SLEDAI activity scores showed elevated levels of all biomarkers compared with healthy controls.

Table 1 Baseline characteristics of enrolled subjects ($n = 70$)

Parameters	Values
Age (years)	40.4 ± 11.2
Disease duration (year)	6.4 ± 4.4
White blood count ($10^3/\mu\text{L}$)	5.2 ± 1.9
Platelet ($10^3/\mu\text{L}$)	213.2 ± 75.4
AST (IU/L)	24.7 ± 15.3
ALT (IU/L)	23.7 ± 18.6
Blood urea nitrogen (mg/dL)	13.4 ± 5.6
Creatinine (mg/dL)	0.8 ± 0.8
ESR (mm/h)	21.7 ± 15.7
CRP (mg/L)	2.1 ± 3.5
Urine protein/creatinine (mg/mg)	9.4 ± 68.0
Anti-dsDNA (IU/mL)	53.9 ± 102.6
C3 (mg/dL)	89.8 ± 44.4
C4 (mg/dL)	19.1 ± 15.6
SLEDAI	5.7 ± 5.0
Renal SLEDAI	2.7 ± 3.8
Extrarenal SLEDAI	3.0 ± 2.2
Current medications, n (%)	
Corticosteroid	51 (72.9)
Hydroxychloroquine	53 (75.7)
Cyclophosphamide	4 (5.7)
Mycophenolate mofetil	5 (7.1)
Azathioprine	10 (14.3)
Methotrexate	4 (5.7)
Cyclosporine	1 (1.4)

Data were described as mean \pm standard deviation for continuous variables and number (%) for categorical variables

AST aspartate aminotransferase, ALT alanine aminotransferase, SLEDAI systemic lupus erythematosus disease activity index, ESR erythrocyte sediment rate, CRP C-reactive protein, C complement

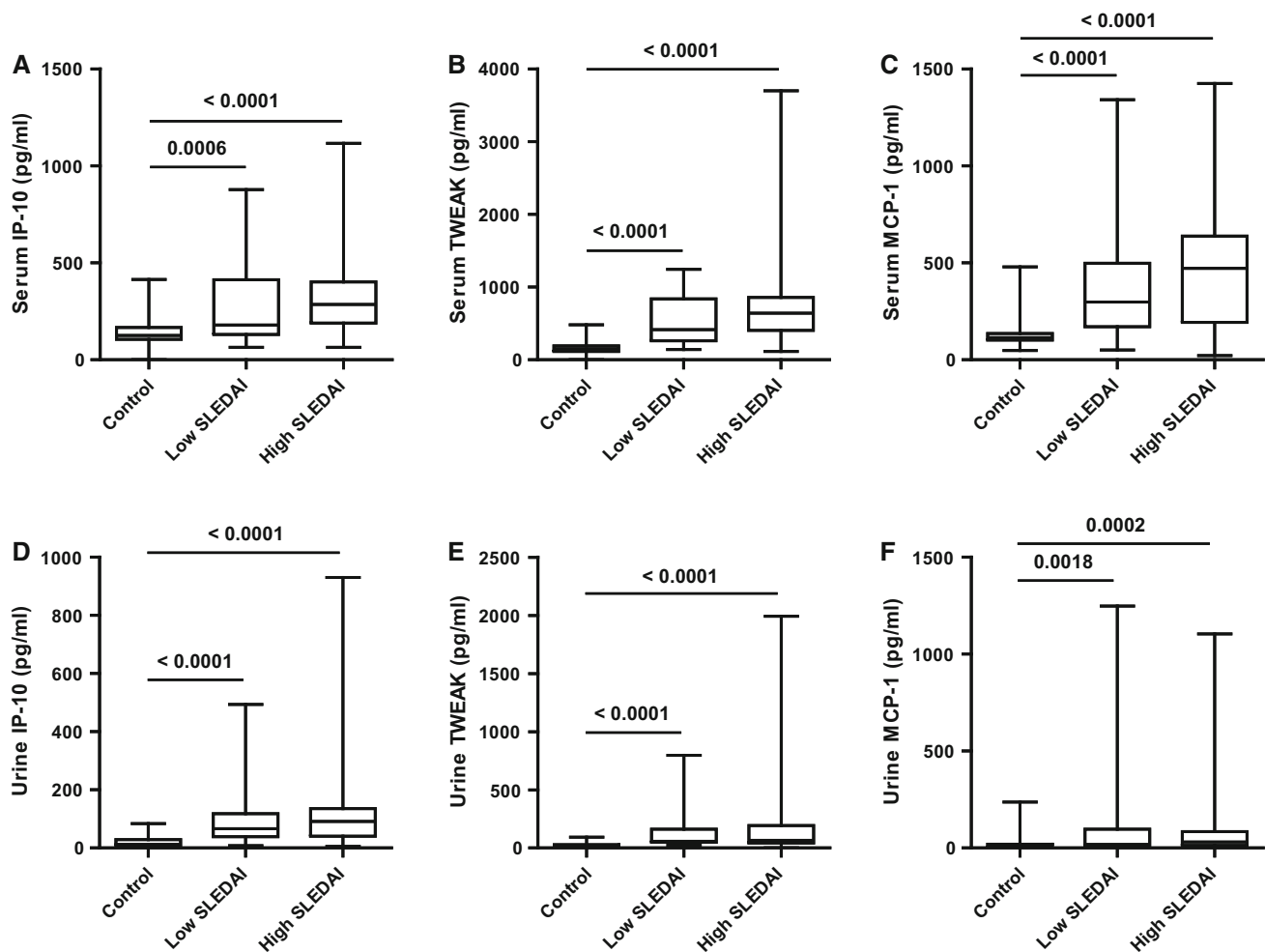


Fig. 1 Comparison for the differences of biomarkers between controls and SLE patients. *IP-10* interferon γ -induced protein-10, *TWEAK* TNF-like weak inducer of apoptosis, *MCP-1* monocyte chemotactic protein-1, *SLE* systemic lupus erythematosus

However, no differences regarding any of the biomarkers were observed between low and high SLEDAI activity groups in SLE patients (data not shown). In addition, levels of all biomarkers were not significantly different between patients with taking and without taking each medication in SLE patients (data not shown).

Correlation of biomarkers with clinical parameters

Significant correlations of biomarkers with clinical parameters were identified by calculating the Spearman's correlation coefficients (Table 2). The serum level of IP-10 was negatively correlated with disease duration and the C3 level ($r = -0.341$, $p = 0.004$ and $r = -0.274$, $p = 0.022$, respectively) and positively correlated with the level of ESR and the titer of anti-dsDNA antibodies ($r = 0.383$, $p = 0.001$ and $r = 0.309$, $p = 0.009$, respectively). Moreover, the serum TWEAK level was negatively correlated with disease duration ($r = -0.273$, $p = 0.022$),

whereas positive correlations were observed between the serum TWEAK level and the ESR level, the urine protein/creatinine level, and the renal SLEDAI score ($p = 0.001$, $p = 0.010$, and $p = 0.014$, respectively). Furthermore, the serum MCP-1 level was significantly correlated with the urine protein/creatinine level, the SLEDAI score, and the renal SLEDAI score ($r = 0.328$, $p = 0.006$; $r = -0.337$, $p = 0.004$; $r = 0.287$, $p = 0.016$; and $r = 0.258$, $p = 0.031$, respectively). Additionally, the serum MCP-1 level was negatively correlated with the C3 level ($r = -0.337$, $p = 0.004$). Interestingly, none of the three urinary biomarkers was significantly correlated with any clinical parameter.

Next, multivariate regression analysis was used to identify associations between biomarkers and clinical parameters. This analysis revealed that the serum TWEAK level was positively associated with the serum IP-10 and serum MCP-1 levels (Table 3). Similarly, the urinary TWEAK level was also found to be associated with the

Table 2 Correlation between biomarkers and clinical parameters

Parameters	Serum IP-10	Urine IP-10	Serum TWEAK	Urine TWEAK	Serum MCP-1	Urine MCP-1
Age	$r = -0.191$, $p = 0.112$	$r = 0.051$, $p = 0.676$	$r = -0.090$, $p = 0.457$	$r = 0.114$, $p = 0.347$	$r = -0.186$, $p = 0.123$	$r = 0.042$, $p = 0.730$
Disease duration	$r = -0.341$, $p = 0.004$	$r = -0.148$, $p = 0.222$	$r = -0.273$, $p = 0.022$	$r = -0.221$, $p = 0.066$	$r = -0.192$, $p = 0.112$	$r = -0.043$, $p = 0.722$
ESR	$r = 0.383$, $p = 0.001$	$r = 0.178$, $p = 0.146$	$r = 0.402$, $p = 0.001$	$r = 0.117$, $p = 0.340$	$r = 0.218$, $p = 0.074$	$r = 0.123$, $p = 0.319$
CRP	$r = 0.169$, $p = 0.164$	$r = 0.097$, $p = 0.430$	$r = 0.139$, $p = 0.255$	$r = 0.210$, $p = 0.083$	$r = 0.047$, $p = 0.701$	$r = 0.056$, $p = 0.646$
Anti-dsDNA	$r = 0.309$, $p = 0.009$	$r = 0.080$, $p = 0.510$	$r = 0.217$, $p = 0.071$	$r = 0.117$, $p = 0.143$	$r = 0.183$, $p = 0.129$	$r = 0.023$, $p = 0.850$
Urine protein/ creatinine	$r = 0.185$, $p = 0.126$	$r = 0.003$, $p = 0.982$	$r = 0.306$, $p = 0.010$	$r = 0.042$, $p = 0.732$	$r = 0.328$, $p = 0.006$	$r = 0.010$, $p = 0.935$
C3	$r = -0.274$, $p = 0.022$	$r = -0.087$, $p = 0.474$	$r = -0.185$, $p = 0.125$	$r = 0.009$, $p = 0.939$	$r = -0.337$, $p = 0.004$	$r = -0.032$, $p = 0.795$
C4	$r = -0.204$, $p = 0.090$	$r = -0.072$, $p = 0.552$	$r = -0.072$, $p = 0.068$	$r = -0.128$, $p = 0.290$	$r = -0.226$, $p = 0.060$	$r = -0.180$, $p = 0.136$
Blood urea nitrogen	$r = -0.209$, $p = 0.083$	$r = -0.209$, $p = 0.083$	$r = -0.225$, $p = 0.061$	$r = -0.128$, $p = 0.290$	$r = -0.178$, $p = 0.140$	$r = 0.088$, $p = 0.468$
Creatinine	$r = 0.052$, $p = 0.666$	$r = -0.018$, $p = 0.881$	$r = -0.120$, $p = 0.321$	$r = -0.161$, $p = 0.184$	$r = -0.099$, $p = 0.417$	$r = -0.054$, $p = 0.655$
SLEDAI	$r = 0.222$, $p = 0.065$	$r = 0.105$, $p = 0.368$	$r = 0.224$, $p = 0.062$	$r = 0.090$, $p = 0.458$	$r = 0.287$, $p = 0.016$	$r = 0.075$, $p = 0.537$
Renal SLEDAI	$r = 0.210$, $p = 0.081$	$r = 0.030$, $p = 0.806$	$r = 0.292$, $p = 0.014$	$r = -0.016$, $p = 0.897$	$r = 0.258$, $p = 0.031$	$r = -0.024$, $p = 0.844$

Data analysis was performed by using spearman's correlation analysis

IP-10 interferon γ -induced protein-10, *TWEAK* TNF-like weak inducer of apoptosis, *MCP-1* monocyte chemoattractant protein-1, *SLEDAI* systemic lupus erythematosus disease activity index, *ESR* erythrocyte sediment rate, *CRP* C-reactive protein, *C* complement

urinary IP-10 and MCP-1 levels. However, the IP-10 level was not associated with the MCP-1 level in either the serum or the urine. In addition, the serum IP-10 level was shown to be determined by the levels of ESR and C4 ($p = 0.034$ and $p = 0.003$, respectively). Specific parameters were identified as determinants for each biomarker, such as anti-dsDNA antibody titer, C4 level, SLEDAI score, and renal SLEDAI score for the serum TWEAK level ($p < 0.001$, $p < 0.001$, $p = 0.009$, and $p = 0.008$, respectively) and anti-dsDNA antibody titer for the urine TWEAK level ($p = 0.046$). Additionally, the serum MCP-1 level was found to be determined by the anti-dsDNA antibody titer and the C4 level ($p < 0.001$ and $p = 0.029$, respectively).

Comparison of clinical parameters according to renal involvement

Patients were classified into two different groups, non-renal ($n = 38$) and renal ($n = 32$), according to renal involvement status of SLE. We noted significance differences of IP-10, TWEAK, and MCP-1 in both serum and urine between controls and SLE with/without renal involvement

(Supplementary Table 1). No differences were also observed between SLE patients with and without renal involvement regarding serum IP-10 level, urinary IP-10 level, urinary TWEAK level, serum MCP-1 level, or urinary MCP-1 level (Supplementary Table 1). However, the serum TWEAK level, SLEDAI score, and urine protein/creatinine level were all significantly higher in patients with renal involvement compared with patients with non-renal involvement ($p = 0.034$, $p < 0.001$, and $p < 0.001$, respectively) (Fig. 2). Among SLE patients enrolled in this study, only 14 patients performed renal biopsy. The results showed eight patients for proliferative nephritis and six patients for membranous nephritis. No difference of serum TWEAK level between proliferative and membranous nephritis was noted (1141.9 ± 1277.9 vs. 959.4 ± 647.3 pg/ml, $p > 0.05$).

We also performed ROC curve analysis to measure the value of cytokines for predicting renal involvement of SLE (Fig. 3). For renal involvement, the AUC was 0.648 for serum TWEAK (95 % CI 0.52–0.78, $p = 0.034$), 0.470 for urine TWEAK (95 % CI 0.33–0.61, $p = 0.663$), 0.595 for serum IP-10 (95 % CI 0.46–0.73, $p = 0.175$), 0.519 for urine IP-10 (95 % CI 0.38–0.66, $p = 0.786$), 0.617 for serum MCP-1 (95 % CI 0.48–0.75, $p = 0.094$),

Table 3 Determination of parameters associated with serum and urine biomarkers

Parameters	Serum IP-10		Urine IP-10		Serum TWEAK		Urine TWEAK		Serum MCP-1		Urine MCP-1	
	<i>B</i>	<i>p</i>	<i>B</i>	<i>p</i>	<i>B</i>	<i>p</i>	<i>B</i>	<i>p</i>	<i>B</i>	<i>p</i>	<i>B</i>	<i>p</i>
Age	-1.722	0.270	-0.936	0.528	2.152	0.479	2.644	0.203	-1.019	0.675	-0.256	0.870
Disease duration	0.460	0.902	-1.332	0.705	-1.452	0.841	-3.275	0.509	-0.760	0.895	5.587	0.128
Serum IP-10			-0.244	0.071	1.002	<0.001	0.127	0.510	0.300	0.177	0.051	0.725
Urine IP-10	-0.272	0.071			0.497	0.090	0.417	0.037	-0.110	0.643	0.227	0.134
serum TWEAK	0.266	<0.001	0.118	0.090			-0.178	0.069	0.452	<0.001	0.044	0.552
urine TWEAK	0.071	0.510	0.210	0.037	-0.377	0.069			0.213	0.202	0.554	<0.001
serum MCP-1	0.125	0.177	-0.041	0.643	0.711	<0.001	0.158	0.202			-0.081	0.382
urine MCP-1	0.051	0.725	0.204	0.134	0.167	0.552	0.991	<0.001	-0.196	0.382		
ESR	2.732	0.034	1.986	0.106	-0.222	0.931	-0.069	0.969	-1.256	0.537	0.154	0.907
CRP	4.973	0.489	9.903	0.142	-1.080	0.938	2.480	0.797	-0.984	0.930	-4.249	0.554
Urine Prot/Cr	-0.051	0.873	-0.254	0.404	-0.227	0.717	-0.131	0.761	0.140	0.779	0.067	0.835
Anti ds-DNA	-0.460	0.143	-0.48	0.106	3.308	<0.001	0.830	0.046	-1.723	<0.001	-0.292	0.355
C3	-0.275	0.526	-0.228	0.579	0.549	0.515	-0.009	0.988	-0.286	0.671	0.009	0.983
C4	4.190	0.003	2.191	0.116	-13.881	<0.001	-3.048	0.121	4.915	0.029	0.671	0.652
SLEDAI (initial)	15.687	0.113	-1.152	0.903	-48.998	0.009	0.071	0.996	28.558	0.061	3.277	0.743
Renal SLEDAI	-23.383	0.063	3.325	0.784	63.454	0.008	0.060	0.997	-30.544	0.119	-8.197	0.521

Data analysis was performed by using multivariate regression analysis

IP-10 interferon γ -induced protein-10, *TWEAK* TNF-like weak inducer of apoptosis, *MCP-1* monocyte chemoattractant protein-1, *SLEDAI* systemic lupus erythematosus disease activity index, *ESR* erythrocyte sediment rate, *CRP* C-reactive protein, *C* complement

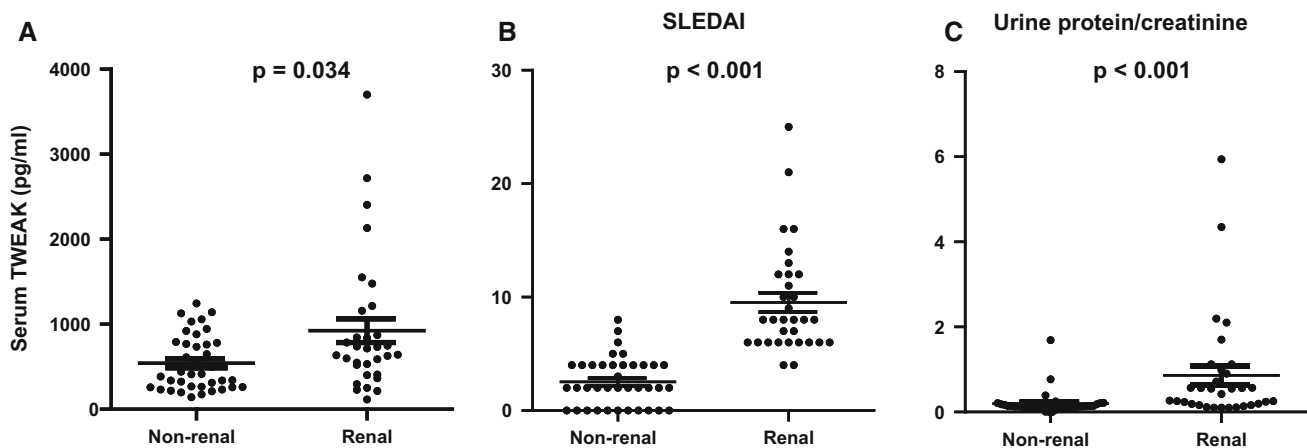


Fig. 2 Comparison for the differences of each parameter between renal and non-renal involvement. *TWEAK* TNF-like weak inducer of apoptosis, *SLEDAI* systemic lupus erythematosus disease activity

and 0.465 for urine MCP-1 (95 % CI 0.33–0.60, $p = 0.616$). Based on the results of ROC analysis, a serum TWEAK level of 395.0 pg/mL yielded the highest Youden's index value for detection of renal involvement in SLE at a sensitivity of 81.3 % and specificity of 47.5 %, and overall accuracy rate of 62.8 %. A false positive rate of 52.6 %, a false negative rate of 18.8 % were noted at this level of serum TWEAK.

index. p values for differences between non-renal and renal involvements were calculated using Mann–Whitney U test

Determination of risk factors for disease activity and renal involvement

We next used logistic regression analysis to identify the risk factors associated with high disease activity and renal involvement (Table 4). This analysis showed that the serum TWEAK level was consistently a crucial determinant for both high disease activity and renal involvement

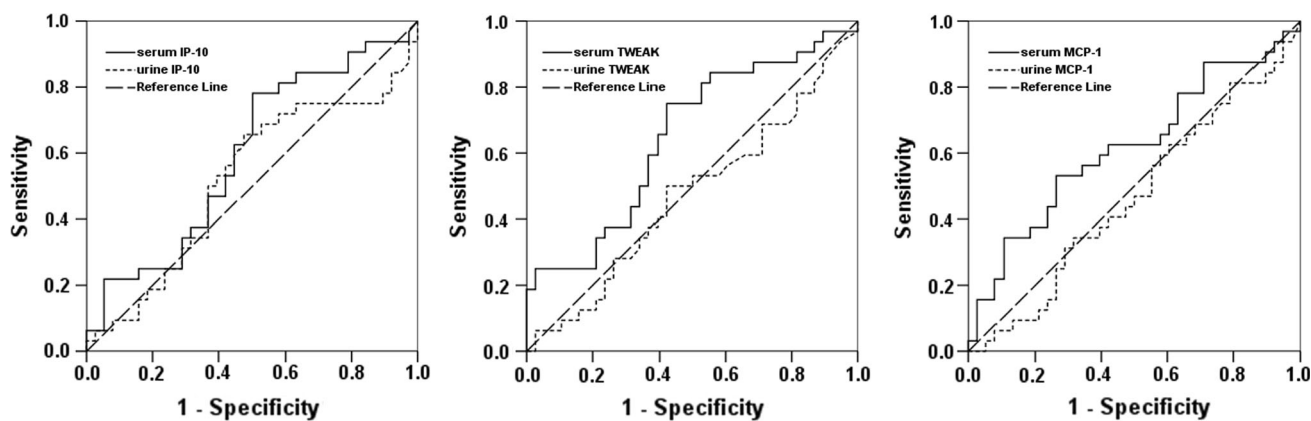


Fig. 3 Receiver operating characteristic curve analysis of association between cytokine levels and renal involvement in SLE patients. *IP-10* interferon γ -induced protein-10, *TWEAK* TNF-like weak inducer of apoptosis, *MCP-1* monocyte chemotactic protein-1

Table 4 Determination for risk factors to high disease activity based on SLEDAI and renal involvement

	High SLEDAI			Renal involvement		
	<i>p</i> values*	OR	95 % CIs for OR	<i>p</i> values*	OR	95 % CIs for OR
Age	0.284	1.059	0.954–1.175	0.752	0.985	0.900–1.079
Disease duration	0.528	0.935	0.758–1.153	0.392	0.914	0.743–1.123
Serum IP-10	0.099	0.991	0.979–1.002	0.091	0.991	0.981–1.001
Urine IP-10	0.581	1.003	0.992–1.014	0.437	1.005	0.993–1.017
Serum TWEAK	0.044	1.006	1.000–1.011	0.046	1.005	1.000–1.009
Urine TWEAK	0.210	1.006	0.997–1.015	0.579	1.003	0.993–1.012
Serum MCP-1	0.247	0.997	0.992–1.002	0.541	0.999	0.994–1.003
Urine MCP-1	0.133	0.987	0.970–1.004	0.204	0.990	0.975–1.005
Urine protein/creatinine	0.068	1.101	0.993–1.220	0.087	1.070	0.990–1.156
Anti ds-DNA	0.059	1.622	0.982–2.678	0.269	1.208	0.864–1.687
C3	0.109	5.875	0.675–51.122	0.062	5.908	0.912–38.265
C4	0.425	1.012	0.983–1.043	0.581	1.007	0.983–1.031
ESR	0.049	0.896	0.804–0.999	0.204	0.957	0.894–1.024
CRP	0.564	1.037	0.916–1.174	0.510	1.037	0.931–1.156

IP-10 interferon γ -induced protein-10, *TWEAK* TNF-like weak inducer of apoptosis, *MCP-1* monocyte chemotactic protein-1, *SLEDAI* systemic lupus erythematosus disease activity index, *ESR* erythrocyte sediment rate, *CRP* C-reactive protein, *C* complement, *OR* odd ratio, *CIs* confidence intervals

p values indicate differences between controls and low SLEDAI or high SLEAI using Mann–Whitney *U* test

* Statistical analysis was performed by multivariate logistic regression analysis

($p = 0.044$, $OR = 1.006$, 95 % CI 1.000–1.011 and $p = 0.046$, $OR = 1.005$, 95 % CI 1.000–1.009, respectively). In addition, patients with a lower ESR level tended to have a higher SLEDAI score ($p = 0.049$, $OR = 0.896$, 95 % CI 0.804–0.999).

Discussion

SLE is a chronic autoimmune disease that is characterized by activation of immune complexes/complement, abundant autoreactive B cells and T cells, and aberrant expression of

cytokines and chemokines. Although these characteristics are known to lead to the overproduction of autoantibodies and end-organ damage, the precise mechanisms of SLE pathogenesis remain unclear. Recent investigations have focused on the roles of chemokines and cytokines in the regulation of lupus disease activity and specific organ involvement [17]. Among the molecules putatively associated with SLE, evidence has accumulated that cytokines and chemokines such as IP-10, TWEAK, and MCP-1 are potent mediators of disease activity and organ involvement, particularly for the kidney [7–12]. In the present study, we found that the serum TWEAK level, but not the urinary

TWEAK level, reflected both the disease activity and renal involvement status of SLE. On the other hand, neither the urinary nor the serum levels of IP-10 or MCP-1 reflected these factors.

TWEAK is a member of the TNF ligand superfamily and is also a multifunctional soluble cytokine. TWEAK mRNA and protein have mainly been detected in endothelial cells, activated monocytes and T cells, macrophages, and dendritic cells [18]. The multiple biological effects of TWEAK are mediated by the binding to its cognate receptor Fn14 and include cell death, apoptosis, inflammation, angiogenesis, and cell proliferation. Regarding the proinflammatory effects of TWEAK, incubation with TWEAK on human dermal fibroblasts has known to employ to trigger induction of chemokines, cytokines, and matrix metalloproteinase (MMP) such as prostaglandin E2, MMP-1, interleukin-8 (IL-8), regulated on activation, normal T cell expressed and secreted (RANTES), and IP-10, then which were significantly attenuated by anti-TWEAK monoclonal antibody [19]. Experiments for the role of TWEAK in renal injury have shown potent evidence that TWEAK prominently stimulated MCP-1, RANTES, and IP-10 in primary murine glomerular mesangial cells exposed to pro-inflammatory stimuli such as TNF- α and IL-1 β [20]. Similar to results obtained in renal and skin tissues, TWEAK was shown to induce the human macrophage-like cell line THP-1 to secrete inflammatory molecules related to atherosclerosis, including IL-6, MCP-1, IL-8, and IFN- γ [21]. Therefore, TWEAK is an established key player in the pathogenesis of inflammatory diseases.

Considering the potent proinflammatory effects of TWEAK, this cytokine has been implicated in the pathogenesis of various inflammatory rheumatic diseases such as collagen-induced arthritis [22, 23] and human SLE [10, 11]. Schwartz et al. demonstrated that the urinary TWEAK level might be a useful biomarker in lupus nephritis (LN), since the urinary TWEAK level was significantly correlated with the renal SLEDAI score ($r = 0.405$, $p < 0.001$) [10]. Another study confirmed the potential usefulness of the urinary TWEAK level for distinguishing between LN and non-LN patients with SLE and also for predicting the development of LN (odds ratio = 7.36) [11]. However, the serum TWEAK level did not correlate with either the presence of LN or the degree of LN activity. Consistent with previous studies [10, 11], we also observed significantly increased levels of urinary and serum TWEAK in patients with SLE (both high and low SLEDAI score groups) compared with controls. Interestingly, the serum TWEAK level was found to be a potential biomarker for disease activity and renal involvement, whereas the urinary TWEAK level was not. This conclusion is supported by the findings that the serum TWEAK level was closely

correlated with the anti-dsDNA antibody titers, the level of C4, and the renal SLEDAI score, all of which are known markers of SLE. Future prospective studies with larger populations should be performed to further study the differential abilities of serum versus urinary TWEAK levels to act as biomarkers for SLE.

Chemokines have also been found to play a central role in the resolution and progression of inflammatory processes in diverse diseases [24, 25]. Considering the pathogenesis of SLE, diverse chemokines including IP-10, MCP-1, and IL-8 have been implicated in disease activity and/or specific organ damage [7–9, 12]. IP-10/CXCL10 belongs to the CXCL subfamily of chemokines, which are secreted from diverse cell types including CD4⁺ lymphocytes, CD8⁺ lymphocytes, natural killer cells, endothelial cells, and fibroblasts in an IFN- γ -dependent manner. The binding of this chemokine to its unique receptor CXCL3 has been shown to lead to leukocyte recruitment, angiogenesis, and integrin activation [26]. The finding that IP-10 is highly expressed by infiltrating CD4⁺ and CD8⁺ cells in lupus with cutaneous involvement suggests that IP-10 plays a crucial role in the recruitment of inflammatory cells, thus driving SLE pathogenesis [27, 28]. Interestingly, CXCR3-expressing T cells were shown to infiltrate into the tubulointerstitium in patients with SLE and to be closely associated with the degree of renal dysfunction [29]. In addition, the serum level of IP-10 has been shown to be significantly increased in SLE and also to be closely correlated with disease activity [7, 9]. In particular, the urinary IP-10 level has been noted as a potent indicator of renal disease progression and renal flare [9]. We also observed significantly increased serum and urine IP-10 levels in patients with SLE compared with controls, in addition to close relationships between these biomarkers and traditional activity markers including ESR and C4. However, multivariate regression analysis did not find any evidence that the serum or urine IP-10 level can be used as an indicator of disease activity or renal involvement. Although accumulating evidence has suggested that IP-10 is abundantly present in the serum and tissues of patients with SLE, more research is needed to identify the precise role of IP-10.

MCP-1, also known as chemokine ligand 2 (CCL2), belongs to the CC chemokine family. This chemokine is mainly secreted from endothelial cells, tubular epithelial cells, and mesangial cells. Both human and animal studies have implicated MCP-1 in the inflammatory response of SLE, especially in lupus nephritis [7, 9, 12, 30, 31]. For instance, renal MCP mRNA expression has been shown to be significantly increased in the glomeruli of NZB/W lupus-prone mice, whereas MCP expression is downregulated when mice are treated with cyclophosphamide up to 12 months of age [30]. Moreover, MCP-1-deficient MRL-

Fas(lpr) mice exhibit reduced macrophage and T cell recruitment, whereas MCP-1-intact MRL-Fas(lpr) mice show enhanced macrophage infiltration and accumulation of T cells in renal tissue in proportion to MCP-1 expression [31]. In clinical studies, the serum and urine MCP-1 levels have been shown to be significantly increased in patients with SLE, either with or without LN, compared with healthy controls. This finding indicates that MCP-1 might be a useful biomarker for lupus disease activity [7, 9, 12]. Furthermore, the urinary MCP-1 level was found to reflect and to predict renal flare and response to treatment for LN [12]. In contrast to these findings, Narumi et al. demonstrated that the serum MCP-1 level was less associated than conventional markers with lupus activity, including anti-DNA antibody titer ($p = 0.012$) and C3 level ($r = 0.17$) [7]. We also observed elevated serum and urinary MCP-1 levels in patients with SLE, although neither the serum nor the urinary MCP-1 level was different between patients with high and low SLEDAI scores. Based on results from previous studies [7, 9, 12, 30, 31], we conclude that MCP-1 is a crucial player in the regulation of inflammation in SLE. However, the precise role of MCP-1 in disease activity and/or organ damage should be clarified to fully understand SLE pathogenesis.

This study demonstrated no significant differences of cytokine levels between patients with high and low SLEDAI (Fig. 1, data not shown). The disparity of clinical significance of serum TWEAK level might be explained by an apparent reason. This study arbitrarily divided SLEDAI scores into two groups such as high and low SLEDAI [16]. Because the SLEDAI is a global index that was developed a clinical index for the assessment of over all disease activity [15], more detailed classification for clinical disease activity based on SLEDAI might be needed. However, serum TWEAK level was found to be associated with high SLEDAI in the assessment of multivariate logistic regression analysis (Table 4). Considering reasonable classification of disease activity, confounding factors related with cytokine levels were not adjusted at simple comparison of cytokine levels between two groups. Therefore, multivariate logistic regression analysis after adjusting covariates seems to be more reliable for determination for risk factor to high SLEDAI in SLE patients.

Here, we have to consider normal value range of TWEAK level in serum and urine to discriminate between physiologic and pathologic conditions in humans. Establishment of threshold for abnormal TWEAK levels seems not to be easy. Bertin et al. reviewed that normal mean values of serum TWEAK in humans might be determined by variable factors such as diverse kinds of ELISA kits, heterogeneous study population, and sample storage quality [32]. Serum TWEAK level at a investigational study

[33] used same ELISA kit with our study seems to show significant difference in compared to our result. It suggests that normal values of TWEAK in same biological fluids might be dependent on variable conditions, including age, sex, and ethnic groups. Therefore, further researches to enhance the utility in routine clinical practice should be needed.

The present study did have some weaknesses. First, this study was a cross-sectional study. Serum and urinary biomarkers for SLE are required to identify disease activity, monitor treatment response, and predict prognosis. Prospective observations have revealed that urine TWEAK, MCP-1, and IP-10 levels accurately reflect increased renal disease activity before flaring occurs [9, 10, 12]. Therefore, our finding that the serum TWEAK level is a more promising biomarker than IP-10 or MCP-1 requires additional validation. Second, renal disease activity was classified into two groups, renal versus non-renal involvement, without taking into account renal pathological evaluation. Thus, the present study may have incorporated significant heterogeneity with respect to renal involvement activity and severity. Based on the WHO classification system for renal biopsy, the urine MCP-1 levels in LN were shown to be different between class III + IV and class V [12]. Thus, further studies are needed to identify the influence of biomarkers on the activity and chronicity of renal inflammation. Third, this study enrolled so small population of patients with SLE to fully explain for our results, in addition to the lack of histological data. More LN patients with histological evidence should be assessed to clearly clarify the potential of serum TWEAK level to reflect disease activity and/or renal damage. Considering diversity of clinical phenotypes and/or genetic background of SLE in variable ethnic population [1, 2], additional studies with different ethnic populations including male patients are needed. However, bearing in mind this shortcoming, this is the first study to investigate clinical associations of these three potential biomarkers with SLE, which is a strength of this study.

In conclusion, this study provides evidence that the serum TWEAK level, but not the MCP-1 or IP-10 level, is a potentially useful biomarker for measuring disease activity and renal involvement in SLE. Further studies are needed to clarify the ability of the serum TWEAK level to reflect disease activity and predict renal involvement in clinical practice.

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Compliance with ethical standards

Conflict of interest None.

References

- D'Cruz DP, Khamashta MA, Hughes GR. Systemic lupus erythematosus. *Lancet*. 2007;369:587–96.
- Reeves GE. Update on the immunology, diagnosis and management of systemic lupus erythematosus. *Intern Med J*. 2004;34:338–47.
- Cook HT, Botto M. Mechanisms of disease: the complement system and the pathogenesis of systemic lupus erythematosus. *Nat Clin Pract Rheumatol*. 2006;2:330–7.
- Li Y, Tucci M, Narain S, Barnes EV, Sobel ES, Segal MS, Richards HB. Urinary biomarkers in lupus nephritis. *Autoimmun Rev*. 2006;5:383–8.
- Schwartz N, Michaelson JS, Putterman C. Lipocalin-2, TWEAK, and other cytokines as urinary biomarkers for lupus nephritis. *Ann N Y Acad Sci*. 2007;1109:265–74.
- Liu CC, Ahearn JM. The search for lupus biomarkers. *Best Pract Res Clin Rheumatol*. 2009;23:507–23.
- Narumi S, Takeuchi T, Kobayashi Y, Konishi K. Serum levels of IFN-inducible protein-10 relating to the activity of systemic lupus erythematosus. *Cytokine*. 2000;12:1561–5.
- Avihingsanon Y, Phumesin P, Benjachat T, Akkasilpa S, Kitikowit V, Praditpornsilpa K, Wongpiyabavorn J, Eiam-Ong S, Hemachudha T, Tungsanga K, Hirankarn N. Measurement of urinary chemokine and growth factor messenger RNAs: a noninvasive monitoring in lupus nephritis. *Kidney Int*. 2006;69:747–53.
- Abujam B, Cheekatla S, Aggarwal A. Urinary CXCL-10/IP-10 and MCP-1 as markers to assess activity of lupus nephritis. *Lupus*. 2013;22:614–23.
- Schwartz N, Su L, Burkly LC, Mackay M, Aranow C, Kollaros M, Michaelson JS, Rovin B, Putterman C. Urinary TWEAK and the activity of lupus nephritis. *J Autoimmun*. 2006;27:242–50.
- Schwartz N, Rubinstein T, Burkly LC, Collins CE, Blanco I, Su L, Hojaili B, Mackay M, Aranow C, Stohl W, Rovin BH, Michaelson JS, Putterman C. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. *Arthritis Res Ther*. 2009;11:R143.
- Rovin BH, Song H, Birmingham DJ, Hebert LA, Yu CY, Nagaraja HN. Urine chemokines as biomarkers of human systemic lupus erythematosus activity. *J Am Soc Nephrol*. 2005;16:467–73.
- Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. 1982;25:1271–7.
- Cook RJ, Gladman DD, Pericak D, Urowitz MB. Prediction of short term mortality in systemic lupus erythematosus with time dependent measures of disease activity. *J Rheumatol*. 2000;27:1892–5.
- Gladman DD, Ibanez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol*. 2002;29:288–91.
- Choe JY, Park SH, Kim SK. Urine β 2-microglobulin is associated with clinical disease activity and renal involvement in female patients with systemic lupus erythematosus. *Lupus*. 2014;23:1486–93.
- Davis LS, Hutcheson J, Mohan C. The role of cytokines in the pathogenesis and treatment of systemic lupus erythematosus. *J Interferon Cytokine Res*. 2011;31:781–9.
- Wiley SR, Winkles JA. TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TweakR/Fn14 receptor. *Cytokine Growth Factor Rev*. 2003;14:241–9.
- Chicheportiche Y, Chicheportiche R, Sizing I, Thompson J, Benjamin CB, Ambrose C, Dayer JM. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. *Arthritis Res*. 2002;4:126–33.
- Campbell S, Burkly LC, Gao HX, Berman JW, Su L, Browning B, Zheng T, Schiffer L, Michaelson JS, Putterman C. Proinflammatory effects of TWEAK/Fn14 interactions in glomerular mesangial cells. *J Immunol*. 2006;176:1889–98.
- Kim SH, Kang YJ, Kim WJ, Woo DK, Lee Y, Kim DI, Park YB, Kwon BS, Park JE, Lee WH. TWEAK can induce pro-inflammatory cytokines and matrix metalloproteinase-9 in macrophages. *Circ J*. 2004;68:396–9.
- Kamata K, Kamijo S, Nakajima A, Koyanagi A, Kurosawa H, Yagita H, Okumura K. Involvement of TNF-like weak inducer of apoptosis in the pathogenesis of collagen-induced arthritis. *J Immunol*. 2006;177:6433–9.
- Perper SJ, Browning B, Burkly LC, Weng S, Gao C, Giza K, Su L, Tarilonte L, Crowell T, Rajman L, Runkel L, Scott M, Atkins GJ, Findlay DM, Zheng TS, Hess H. TWEAK is a novel arthritogenic mediator. *J Immunol*. 2006;177:2610–20.
- Segerer S, Nelson PJ, Schlöndorff D. Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiology and therapeutic studies. *J Am Soc Nephrol*. 2000;11:152–76.
- Vielhauer V, Anders HJ, Schlöndorff D. Chemokines and chemokine receptors as therapeutic targets in lupus nephritis. *Semin Nephrol*. 2007;27:81–97.
- Lacotte S, Brun S, Muller S, Dumortier H. CXCR3, inflammation, and autoimmune diseases. *Ann N Y Acad Sci*. 2009;1173:310–7.
- Flier J, Boorsma DM, van Beek PJ, Nieboer C, Stoof TJ, Willenze R, Tensen CP. Differential expression of CXCR3 targeting chemokines CXCL10, CXCL9, and CXCL11 in different types of skin inflammation. *J Pathol*. 2001;194:398–405.
- Wenzel J, Wörenkämper E, Freutel S, Henze S, Haller O, Bieber T, Tüting T. Enhanced type I interferon signalling promotes Th1-biased inflammation in cutaneous lupus erythematosus. *J Pathol*. 2005;205:435–42.
- Segerer S, Banas B, Wörnle M, Schmid H, Cohen CD, Kretzler M, Mack M, Kiss E, Nelson PJ, Schlöndorff D, Gröne HJ. CXCR3 is involved in tubulointerstitial injury in human glomerulonephritis. *Am J Pathol*. 2004;164:635–49.
- Zoja C, Liu XH, Donadelli R, Abbate M, Testa D, Corna D, Taraboletti G, Vecchi A, Dong QG, Rollins BJ, Bertani T, Remuzzi G. Renal expression of monocyte chemoattractant protein-1 in lupus autoimmune mice. *J Am Soc Nephrol*. 1997;8:720–9.
- Tesch GH, Maifert S, Schwarting A, Rollins BJ, Kelley VR. Monocyte chemoattractant protein 1-dependent leukocytic infiltrates are responsible for autoimmune disease in MRL-Fas(lpr) mice. *J Exp Med*. 1999;190:1813–24.
- Bertin D, Stephan D, Khrestchatsky M, Desplat-Jégo S. Is TWEAK a biomarker for autoimmune/chronic inflammatory diseases? *Front Immunol*. 2013;4:489.
- Lammens A, Baehner M, Kohnert U, Niewoehner J, von Proff L, Schraeml M, Lammens K, Hopfner KP. Crystal structure of human TWEAK in complex with the Fab fragment of a neutralizing antibody reveals insights into receptor binding. *PLoS One*. 2013;8:e62697.