Monitoring of serum TWEAK levels guides glucocorticoid dosages in the treatment of systemic lupus erythematosus

Jingyun Chen

The Second Affiliated Hospital of Xi'an Jiaotong University

Huixia Wang

The Second Affiliated Hospital of Xi'an Jiaotong University

Yi Wu

Xi'an Jiaotong University School of Basic Medical Sciences

Xiaokang Wu

The Second Affiliated Hospital of Xi'an Jiaotong University

Li Wang

The Second Affiliated Hospital of Xi'an Jiaotong University

Tong Xiao

The Second Affiliated Hospital of Xi'an Jiaotong University

Fei Tan

The Second Affiliated Hospital of Xi'an Jiaotong University

Mai Luo

The Second Affiliated Hospital of Xi'an Jiaotong University

Shengxiang Xiao

The Second Affiliated Hospital of Xi'an Jiaotong University

Yumin Xia (✉ xiayumin1202@163.com)

The Second Affiliated Hospital of Xi'an Jiaotong University <https://orcid.org/0000-0002-3493-7198>

Research article

Keywords: TWEAK; SLE; SLEDAI; Glucocorticoid; Lupus nephritis; Biomarker

Posted Date: June 20th, 2019

DOI: <https://doi.org/10.21203/rs.2.10476/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background Accurate assessment of systemic lupus erythematosus (SLE) disease activity is critical. Currently existing indices or measures for assessment are either expensive, intricate, or inaccurate. The novel indices with higher sensitivity and specificity have become one of the aims of the investigators. This study was designed to explore the relationship between serum tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and systemic lupus erythematosus disease activity index (SLEDAI) as well as its role in guiding glucocorticoid dosages in the treatment of SLE. Of 59 patients with SLE, 20 patients with subacute cutaneous lupus erythematosus (SCLE), 13 patients with discoid lupus erythematosus (DLE) and 32 healthy volunteers, soluble TWEAK level was determined in both serum and urine. Monomeric C-reactive protein, antinuclear antibody, interleukin 6, complements, erythrocyte sedimentation rate, and white blood cells were measured in serum samples. Moreover, SLEDAI-2K was used for evaluating the disease. Finally, methylprednisolone was administrated orally to SLE patients with the doses depending on serum TWEAK levels. **Results** We found that serum TWEAK levels are higher in patients with SLE (383.0 ± 45.37 ng/ml, $p < 0.001$ for both) or SCLE (129.1 ± 25.73 ng/ml, $p < 0.05$ for both) than in patients with DLE (78.38 ± 22.85 ng/ml) or in healthy controls (78.38 ± 22.85 ng/ml). Also, serum TWEAK levels correlate positively with SLEDAI-2K in patients with SLE ($r^2 = 0.101$, $p < 0.001$), whereas urine TWEAK levels reflect renal damage in patients with lupus nephritis. Moreover, serum TWEAK levels had a higher correlation coefficient with SLEDAI-2K scores compared with the other serum parameters. Furthermore, TWEAK-based glucocorticoid therapy is associated with lower SLEDAI-2K scores and fewer flares in patients with SLE. **Conclusions** Serum TWEAK is a useful biomarker reflecting SLE disease, and monitoring of serum TWEAK levels can improve the outcomes of glucocorticoid therapy for patients with SLE.

Background

Systemic lupus erythematosus (SLE) is a severe autoimmune disease. Currently, systemic administration of glucocorticoids is one of the common therapies for patients with SLE in China. The disease flares are commonly observed in SLE patients undergoing various pharmacotherapies, including glucocorticoid administration. Accurate assessment of lupus flares is critical but problematic in clinical trials. Until now, different indices or measures have been developed for the assessment of SLE disease activity [1-3]. These indices are divided into two categories: the global score systems and individual organ/system assessment scales [4]. The former category includes the European Consensus Lupus Activity Measurements, Systemic Lupus Activity Measure, and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), which provide an overall measure of SLE disease activity. The latter category assesses disease activity in single organs, such as the British Isles Lupus Assessment Group Index. Additionally, the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index score can measure chronic damage in SLE and has a prognostic value in clinical trials [4]. SLEDAI-2K is an updated version of SLEDAI, and has been considered among the global scoring systems to have the highest predictability of treatment change [3,4]. However, most indices should be applied based on the

number of collected clinical manifestations and laboratory results, which may be both time consuming and costly. Moreover, the global score systems and individual organ/system assessment scales may be insufficient in predicting important regional damage such as lupus nephritis (LN) and may be affected by systemic inflammation, respectively. Therefore, the novel indices with higher sensitivity and specificity have become one of the aims of the investigators.

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a proinflammatory cytokine mainly synthesized by macrophages and monocytes [5]. TWEAK acts by binding to its receptor fibroblast growth factor-inducible 14 (Fn14), subsequently inducing the production of downstream cytokines such as interferon gamma-induced protein 10 (IP-10), monocyte chemotactic protein-1 (MCP-1), and regulated upon activation normal T cell expressed and secreted (RANTES) [6]. TWEAK/Fn14 activation participates in inflammatory responses in injured tissue [7-9]. Actually, TWEAK/Fn14 signaling is prominent in various damage in patients with SLE, including cutaneous lupus erythematosus [10,11], LN [12,13], neuropsychiatric disease [14,15], and vasculitis [16]. Soluble TWEAK exhibits higher levels in sera of patients with SLE and in urine of patients with LN [16]. Recently, we found that TWEAK/Fn14 activation damages the glomerular filtration barrier and increases filtration of anti-dsDNA IgG in MRL/lpr lupus-prone mice, which is suggested to participate in the pathogenesis of LN [13,17]. Moreover, inhibition of the TWEAK/Fn14 pathway can ameliorate SCLE as well as LN or nephrotoxic serum nephritis in murine models [11-13]. Obviously, the serum or urine levels of TWEAK correlate closely with tissue inflammation and damage in SLE [10-11,13-16].

Recent studies had demonstrated that elevation of TWEAK levels in peripheral blood mononuclear cells is correlated with the disease activity in patients with SLE [18]. The serum levels of TWEAK correlate positively with SLEDAI-2K scores and LN in patients with SLE [19]. However, TWEAK levels are only slightly elevated in the cerebrospinal fluid in SLE patients compared with non-autoimmune controls, and serum TWEAK levels do not vary with neuropsychiatric disease activity [20]. Furthermore, urinary TWEAK levels are significantly correlated with the activity index of LN in patients [21]. This study was designed to investigate the relationship between TWEAK levels and other biomarkers in patients with SLE, as well as the role of serum and urine TWEAK levels in guiding glucocorticoid dosages in the treatment of SLE.

Methods

Patients

There were 59 patients with SLE (age range: 8-73 years; gender: 54 females, 5 males), 20 patients with SCLE (age range: 10-74 years; gender: 16 females, 4 males), 13 patients with discoid lupus

erythematosus (DLE) (age range: 13-55 years; gender: 7 females, 6 males) recruited in this study, wherein 32 healthy donors were the controls (age range: 16-52 years; gender: 26 females, 6 males). The demographic characteristics of the patients and healthy donors are presented in Table 3. There were 39 LN patients and 20 non-LN SLE patients in all SLE patients. Non-LN SLE patients were defined as patients who had SLE but no signs of kidney involvement recently and previously. LN patients were cooperatively diagnosed by nephrologists as SLE patients with kidney involvement based on clinical manifestation and kidney biopsies. Patients whose proteinuria levels exceeded 300mg/day or have urinary casts, hematuria, and leucocyturia in urine examination were suggested to seek care from a nephrologist to have kidney biopsy surgery to make further confirmation of the existence of LN and classification. Among LN patients enrolled in this study, only 11 patients performed renal biopsy (Table 1). Serum and urine samples were collected and then detected for relevant parameters. Patients with SLE were divided randomly into non-monitoring (n = 20) and TWEAK-monitoring (n = 25) groups. Methylprednisolone tablets (Pfizer, Italia) were administered orally according to the serum levels of TWEAK in the TWEAK-monitoring group. The methylprednisolone doses were less than 0.2 mg/kg/day, 0.2 to 0.4 mg/kg/day and 0.4 to 0.8 mg/kg/day when serum TWEAK levels were less than 150 ng/ml, 150 to 500 ng/ml, 500 to 1000 ng/ml respectively. Patients need to be hospitalized when the serum TWEAK level is greater than 1000 ng/ml. In the non-monitoring group, the methylprednisolone doses are determined according to the assessment of patients. The demographic characteristics of the non-monitoring and TWEAK-monitoring groups patients are presented in Table 4. This clinical trial was randomized, non-placebo controlled, and unblinded. These treatments lasted for 3 to 12 months. Serum and urine samples were collected before (month 0) and after (months 1 to 12) treatment. An online SLEDAI-2K calculator (<http://www.s2k-ri-50.com>) was used for evaluating the disease.²²

Enzyme-linked immunosorbent assay

The TWEAK levels in sera or urine were quantitated with a sandwich enzyme-linked immunosorbent assay (ELISA) method. Briefly, the capture antibody (Cat # DY1090, R&D Systems Inc., Minneapolis, MN, USA) was coated to 96 well microplates, followed by blocking with 2% bovine serum albumin in phosphate buffer saline. The serum or urine samples (1:100 diluted) and streptavidin-horseradish peroxidase conjugated detection antibody (Cat # DY1090, R&D Systems Inc.) were added to plates in order. Finally, substrate (H_2O_2 and tetramethylbenzidine mixture) and stop (2N sulfuric acid) solutions were added accordingly. The optical densities were determined using a microplate reader set to 450 nm (Thermo Fisher Scientific, Waltham, MA, USA).

Monomeric C-reactive protein (mCRP) was also measured by a modified ELISA method.²³ Human mCRP, mouse anti-CRP IgG, peroxidase-labeled secondary antibody, and 3,3',5,5'-tetramethylbenzidine substrate

were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-dsDNA ELISA was performed as previously described.¹⁷ Anti-nuclear antibody (ANA) was detected by an indirect immunofluorescence kit that has Hep-2 slides as substrate (Oumeng Inc., Beijing, China). Urine albumin was determined by using a sandwich ELISA kit (Elabscience Inc., Wuhan, China).

Determination of interleukin 6, complements, and erythrocyte sedimentation rate

Interleukin (IL) 6 was determined by using a chemiluminescent immunoassay kit (Siemens, Marburg, Germany). C3 and C4 were determined by using scattering turbidimetric assay kits (Siemens). Erythrocyte sedimentation rate (ESR) was determined by using an automatic ESR machine (Vital Diagnostics, Forli, Italy). Protocols were provided by the manufacturers.

Statistical analysis

The STATA 10.0 software package (StataCorp, College Station, TX) was used for the analysis of experimental data. Data were expressed as the means $\pm$ standard error of the mean. For comparison of more than two groups, analysis of variance was used, followed by the Bonferroni test or the Student *t* test that compared two groups for statistical difference. The chi-square test was used for the comparison of genders. Linear regression was used to analyze the relationship between two parameters. Differences were considered significant at $p < 0.05$.

Results

Serum TWEAK levels correlates positively with SLEDAI-2K in patients with systemic lupus erythematosus

Serum TWEAK levels were determined in patients and in healthy controls. It showed that the patients with SLE (383.0 ± 45.37 ng/ml) or SCLE (129.1 ± 25.73 ng/ml) had higher TWEAK levels than patients with DLE (78.38 ± 22.85 ng/ml) or healthy controls (49.28 ± 6.04 ng/ml) ($p < 0.05$) (Fig. 1A). The SLE patients also had higher TWEAK levels than patients with SCLE ($p < 0.05$), whereas there was no significant difference between patients with DLE and healthy controls ($p > 0.05$) (see Fig. 1A). Also, LN patients (548.73 ± 77.31 ng/ml) had higher TWEAK levels than patients with non-LN SLE (346.15 ± 44.81 ng/ml) ($p < 0.001$) (Table. 1). No difference of serum TWEAK levels between proliferative and membranous

nephritis was noted (612.49 ± 42.50 ng/ml vs. 577.16 ± 91.28 ng/ml) ($p > 0.05$). The serum TWEAK levels correlated positively with the SLEDAI-2K scores in LN patients ($r^2 = 0.339$, $p < 0.001$) (see Fig. 2A).

By Spearman Rho test, the correlations between the serum parameters and SLEDAI-2K were analyzed in patients with SLE (Table 2). It showed that TWEAK, mCRP, ANA and ESR had positive correlations with SLEDAI-2K ($p < 0.05$). Moreover, TWEAK levels had the highest correlation coefficient among these parameters. The other parameters including IL-6, C3, C4, anti-dsDNA, and white blood cell had no significant correlations with SLEDAI-2K score ($p > 0.05$) (Table 2).

The relationships between these parameters (TWEAK, mCRP, ANA, and ESR) and SLEDAI-2K were further analyzed by linear regression. The TWEAK levels correlated positively with the SLEDAI-2K scores in patients ($r^2 = 0.101$, $p < 0.001$) (see Fig. 1B). Similarly, other parameters including mCRP ($r^2 = 0.137$, $p = 0.005$), ANA ($r^2 = 0.037$, $p = 0.020$), and ESR ($r^2 = 0.079$, $p < 0.001$) had positive correlations with the SLEDAI-2K scores (see Fig. 1C to E).

Urine TWEAK levels reflect renal damage in patients with lupus nephritis

Urine TWEAK levels were determined in patients and healthy controls, revealing that the patients with SLE (226.72 ± 40.71 ng/ml) had higher TWEAK levels than patients with DLE (55.50 ± 13.74 ng/ml) or healthy controls (54.97 ± 16.94 ng/ml) ($p < 0.05$), whereas there was no significant differences between patients with other different types (SCLE or DLE) and healthy controls ($p > 0.05$) (Table. 3, Fig. 3A). No difference in urine TWEAK levels between proliferative and membranous nephritis was noted (249.03 ± 41.87 ng/ml vs. 221.66 ± 37.42 ng/ml) ($p > 0.05$). The relationship between urine TWEAK and SLEDAI-2K levels was analyzed in patients with LN and in patients without LN, respectively. It showed that urine TWEAK levels had a positive correlation with SLEDAI-2K scores in these two subpopulations ($r^2 = 0.222$, $p < 0.001$ in LN patients and $r^2 = 0.074$, $p = 0.028$ in non-LN SLE patients) (see Fig. 2 B and C).

By the Spearman Rho test, the urine TWEAK levels correlated positively with urine albumin levels in patients with LN (correlation coefficient = 0.703, $p < 0.001$). Similarly, the serum titer of anti-dsDNA IgG had a positive correlation with the urine albumin levels (correlation coefficient = 0.321, $p = 0.024$). The relationships between these two parameters (urine TWEAK and serum anti-dsDNA IgG) and SLEDAI-2K were further analyzed by linear regression. It showed that urine TWEAK ($r^2 = 0.360$, $p = 0.030$) and serum

anti-dsDNA IgG ($r^2 = 0.296$, $p = 0.036$) exhibited a positive correlation with proteinuria levels (see Fig. 3B and C).

TWEAK-based glucocorticoid therapy is associated with fewer lupus flares

To further explore the guidance role of serum TWEAK, methylprednisolone was administered orally to SLE patients with the doses depending on the TWEAK levels. We observed clinical manifestation such as fever, oral ulcer, erythema, arthralgia, alopecia, neuropsychiatry, and LN (proteinuria, urinary casts, hematuria, and leucocyturia). During the sixth to twelfth months, the TWEAK-monitoring group had lower SLEDAI-2K scores as compared with the non-monitoring group (see Fig. 4A). Accordingly, the average doses of glucocorticoid were lower in the TWEAK-monitoring group (Fig. 4B). Moreover, the TWEAK-monitoring group had fewer flares of certain manifestations during months 10 to 12, including cutaneous erythema and arthralgia (see Fig. 4C).

Discussion

In this study we demonstrated that serum TWEAK levels are higher in patients with SLE or SCLE as compared with patients with DLE or healthy controls. Among several serum parameters (TWEAK, mCRP, ANA, ESR, dsDNA antibodies and complement levels), TWEAK levels had the highest correlation coefficient with SLEDAI-2K scores. Moreover, urine TWEAK levels have a positive correlation with SLEDAI-2K scores and correlate positively with urine protein in patients with LN. Furthermore, serum TWEAK-based glucocorticoid therapy leads to lower SLEDAI-2K scores, lower glucocorticoid doses, and fewer lupus flares in patients with SLE.

It was previously demonstrated that the serum TWEAK level in SLE patients is significantly higher compared with healthy donors, and fluctuates with the MCP-1 and IP-10 levels in sera, whereas the patients with SCLE or DLE were not discussed [19]. We found that TWEAK, as well as downstream proinflammatory cytokines including RANTES, MCP-1, and IP-10, is also highly expressed in skin lesions of CLE patients [10]. Our results are consistent with these previous findings. However, other cytokines such as RANTES, MCP-1, and IP-10 also correlate with virus infection and non-inflammatory disease including hypertension [22,23]. DLE is preferably a chronic disease restricted to skin. Only few patients with DLE may develop other organ injuries or systemic manifestations, even after long-term observation [24]. Therefore, serum TWEAK may be suitable for monitoring patients with SLE or SCLE.

SLEDAI-2K is increasingly used as a global scoring system for patients with SLE, and has the highest predictability of treatment change [3]. Our data revealed that TWEAK levels had a higher correlation coefficient with SLEDAI-2K scores than other serum parameters such as ANA, ESR and mCRP. The serum titer of ANA is slowly altered with the fluctuation of SLE disease because ANA reflects many subtypes of autoantibodies that target nuclear antigens. ESR, CRP, dsDNA antibodies and complement levels are the frequently used traditional indicators in the diagnosis and assessment of SLE and other rheumatic diseases. However, clinicians need to frequently distinguish lupus flares and infectious conditions, and the significance of ESR and CRP is different [25]. It indicates that serum TWEAK level is a novel biomarker in the assessment of SLE disease.

In this study, we found that urinary TWEAK levels are comparable between the subgroups of lupus patients and healthy donors. It was reported that urinary TWEAK levels correlate positively with the activity of LN [21]. We observed similar changes in urinary TWEAK levels in this study. Urinary TWEAK level fluctuates with proteinuria level in patients with LN. Serum positivity of anti-dsDNA IgG is one of the characteristics of patients with LN [26]. The serum titer of anti-dsDNA IgG also has a positive correlation with the proteinuria. However, urine TWEAK levels had a higher correlation coefficient with proteinuria levels than serum anti-dsDNA IgG. So, urine TWEAK level is more sensitive than serum anti-dsDNA IgG in monitoring LN.

Glucocorticoids constitute the basis of SLE therapy. On the contrary, steroids are responsible for many of the most severe comorbidities that threaten patients with lupus and may be a key factor contributing to infection, which is the common cause of death for patients with lupus [27]. In this study, methylprednisolone was administered orally to patients with SLE with the doses depending on serum TWEAK levels. We found that the average doses of glucocorticoid were lower in the TWEAK-monitoring group. Moreover, the TWEAK-monitoring group had lower SLEDAI-2K scores and fewer flares of certain manifestations. A flare or relapse of SLE can be defined as an increase in disease activity requiring more-intensive treatment [28]. These results strongly suggest that monitoring serum TWEAK levels may improve the outcomes of glucocorticoid therapy for patients with SLE.

A limitation in this study was that the clinical trial was randomized but not placebo-controlled and unblinded. Also, the number of patients in each group was as few as 20. Only 11 enrolled patients had renal biopsies. Hence, the comparison in the classes of LN was infeasible. This may somehow affect the final results, and needs further clarification in large-scale and double-blinded clinical trials.

Conclusions

In conclusion, serum TWEAK levels are higher in patients with SLE or SCLE as compared with patients with DLE or healthy controls. Serum TWEAK levels correlate positively with SLEDAI-2K in patients with SLE, whereas urine TWEAK levels reflect renal damage in patients with LN. More importantly, TWEAK-based glucocorticoid therapy is associated with fewer flares in patients with SLE.

Abbreviations

SLE: Systemic lupus erythematosus **SLEDAI:** Systemic Lupus Erythematosus Disease Activity Index
SCLE: subacute cutaneous lupus erythematosus **DLE:** discoid lupus erythematosus **LN:** lupus nephritis
TWEAK: serum tumor necrosis factor-like weak inducer of apoptosis **Fn14:** fibroblast growth factor-inducible 14 **IP-10:** interferon gamma-induced protein 10 **MCP-1:** monocyte chemotactic protein-1
RANTES: regulated upon activation normal T cell expressed and secreted **ELISA:** enzyme-linked immunosorbent assay **mCRP:** Monomeric C-reactive protein **ANA:** Anti-nuclear antibody **IL 6:** Interleukin 6
ESR: Erythrocyte sedimentation rate **SLEDAI-2K:** systemic lupus erythematosus disease activity index-2K
WBC: white blood cell

Declarations

Ethics approval and consent to participate

The experimental protocols were established following the Declaration of Helsinki and approved by the Research Ethics Committee of the hospital. Written informed consent was obtained from all patients, include the parents of each child.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Open Access

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was partially supported by the National Natural Science Foundation of China (Grants No.81874241 and No.81472876).

Authors' contributions

JC carried out evaluated patients, the experiments, and analyzed, and interpreted the data, HW carried out parts of evaluated patients, analyzed, and interpreted the data, YW contributed to the parts of design of the study, XW and LM carried out parts of evaluated patients and compiled data., TX, FT, ML and SX interpreted the data and discussed the results, which are vital for the formation of conception. YX contributed to the conception and design of the study, the analysis, and interpretation of the data, and drafting and revising the manuscript. All authors have read and approved the final manuscript.

Acknowledgements

Not applicable.

References

- 1 Castrejon I, Tani C, Jolly M, Huang A, Mosca M .Indices to assess patients with systemic lupus erythematosus in clinical trials, long-term observational studies, and clinical care. Clin Exp Rheumatol. 2014; 85: S-85-95.
- 2 Miles A, Pope Je. A comparison of rheumatoid arthritis and systemic lupus erythematosus trial design: a commentary on ways to improve the number of positive trials in SLE. Clin Exp Rheumatol. 2015; 33: 671-680.
- 3 Fatemi A, Raeisi A, Sayedbonakdar Z, Smiley A. Sensitivity analyses of four systemic lupus erythematosus disease activity indices in predicting the treatment changes in consecutive visits: a longitudinal study. Clin Rheumatol. 2018; 37: 955-962.
- 4 Romero-Diaz J, Isenberg D, Ramsey-Goldman R. Measures of adult systemic lupus erythematosus: updated version of British Isles Lupus Assessment Group (BILAG 2004), European Consensus Lupus Activity Measurements (ECLAM), Systemic Lupus Activity Measure, Revised (SLAM-R), Systemic Lupus Activity Questionnaire for Population Studies (SLAQ), Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). Arthritis Care Res (Hoboken). 2011; 63: S37-S46.
- 5 Liu Y, Peng L, Li L, Liu C, Hu X, Xiao S et al. TWEAK/Fn14 activation contributes to the pathogenesis of bullous pemphigoid. J Invest Dermatol. 2017; 137: 1512-1522.
- 6 Cheng H, Zhan N, Ding D, Liu X, Zou X, Li K et al. HPV type 16 infection switches keratinocytes from apoptotic to proliferative fate under TWEAK/Fn14 interaction. J Invest Dermatol. 2015; 135: 2427-2436.
- 7 Cheng H, Xu M, Liu X, Zou X, Zhan N, Xia Y. TWEAK/Fn14 activation induces keratinocyte proliferation under psoriatic inflammation. Exp Dermatol. 2016; 25: 32-37.
- 8 Hu G, Zeng W, Xia Y. TWEAK/Fn14 signaling in tumors. Tumor Biol. 2017; 39: 1010428317714624.
- 9 Liu Q, Xiao S, Xia Y. TWEAK/Fn14 activation participates in skin inflammation. Mediators Inflamm. 2017; 2017: 6746870.
- 10 Liu Y, Xu M, Min X, Wu K, Zhang T, Li K et al. TWEAK/Fn14 activation participates in Ro52-mediated photosensitization in cutaneous lupus erythematosus. Front Immunol. 2017; 8: 651.
- 11 Doerner J, Wen J, Xia Y, Paz KB, Schairer D, Wu L et al. TWEAK/Fn14 signaling involvement in the pathogenesis of cutaneous disease in the MRL/lpr model of spontaneous lupus. J Invest Dermatol. 2015; 135: 1986-1995.

- 12 Xia Y, S Campbell R, Broder A, Herlitz L, Abadi M, Wu P et al. Inhibition of the TWEAK/Fn14 pathway attenuates renal disease in nephrotoxic serum nephritis. *Clin. Immunol.* 2012; 145: 108-121.
- 13 Xia Y, Herlitz Lc, Gindea S, Wen J, Pawar RD, Misharin A et al. Deficiency of fibroblast growth factor-inducible 14 (Fn14) preserves the filtration barrier and ameliorates lupus nephritis. *J Am Soc Nephrol.* 2015; 26: 1053-1070.
- 14 Wen J, Xia Y, Stock A, Michaelson JS, Burkly LC, Gulinello M et al. Neuropsychiatric disease in murine lupus is dependent on the TWEAK/Fn14 pathway. *J Autoimmun.* 2013; 43: 44-54.
- 15 Wen J, Doerner J, Weidenheim K, Xia Y, Stock A, Michaelson JS et al. TNF-like weak inducer of apoptosis promotes blood brain barrier disruption and increases neuronal cell death in MRL/lpr mice. *J Autoimmun.* 2015; 60: 40-50.
- 16 Xu Wd, Zhao Y, Liu Y. Role of the TWEAK/Fn14 pathway in autoimmune diseases. *Immunol Res.* 2016; 64: 44-50.
- 17 Xia Y, Pawar Rd, Nakouzi As, Herlitz L, Broder A, Liu K et al. The constant region contributes to the antigenic specificity and renal pathogenicity of murine anti-DNA antibodies. *J Autoimmun.* 2012; 9: 398-411.
- 18 Liu Zc, Zhou Ql, Li Xz, Yang JH, Ao X, Veeraragoo P et al. Elevation of human tumor necrosis factor-like weak inducer of apoptosis in peripheral blood mononuclear cells is correlated with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Cytokine.* 2011; 53: 295-300.
- 19 Choe Jy, Kim Sk. Serum TWEAK as a biomarker for disease activity of systemic lupus erythematosus. *Inflamm Res.* 2016; 65: 479-488.
- 20 Fragoso-Loyo H, Atisha-Fregoso Y, Nuñez-Alvarez Ca, Llorente L. Utility of TWEAK to assess neuropsychiatric disease activity in systemic lupus erythematosus. *Lupus.* 2016; 25: 364-369.
- 21 Xuejing Z, Jiazhen T, Jun L, Xiangqing X, Shuguang Y, Fuyou L. Urinary TWEAK level as a marker of lupus nephritis activity in 46 cases. *J Biomed Biotechnol.* 2012; 2012: 359647.
- 22 Sládková T, Kostolanský F. The role of cytokines in the immune response to influenza A virus infection. *Acta Virol.* 2006; 50: 151-162.
- 23 Martynowicz H, Janus A, Nowacki D, Mazur G. The role of chemokines in hypertension. *Adv Clin Exp Med.* 2014; 23: 319-325.
- 24 Zhang Yp, Wu J, Han Yf, Shi Zr, Wang L. Pathogenesis of cutaneous lupus erythema associated with and without systemic lupus erythema. *Autoimmun Rev.* 2017; 16: 735-742.

- 25 Dima A, Opris D, Jurcut C, Baicus C. Is there still a place for erythrocyte sedimentation rate and C-reactive protein in systemic lupus erythematosus? *Lupus*. 2016; 25: 1173-1179.
- 26 Zhang Y, Yang J, Jiang S, Fang C, Xiong L, Cheng H et al. The lupus-derived anti-double-stranded DNA IgG contributes to myofibroblast-like phenotype in mesangial cells. *J Clin Immunol*. 2012; 32: 1270-1278.
- 27 Ugarte A, Danza A, Ruiz-Irastorza G. Glucocorticoids and antimalarials in systemic lupus erythematosus: an update and future directions. *Curr Opin Rheumatol*. 2018; 30: 482-489.
- 28 Gordon C, Jayne D, Pusey C, Adu D, Amoura Z, Aringer M et al. European consensus statement on the terminology used in the management of lupus glomerulonephritis. *Lupus*. 2009; 18: 257-263.

Tables

Table 1. Demographic characteristics of patients with SLE

	LN (n = 39)	Non-LN SLE (n = 20)	<i>p</i> value
Gender (female/male)	36/3	18/2	0.049
Age (year)	34.97 ± 2.29	35.90 ± 3.34	0.817
Age of onset (year)	32.14 ± 2.26	34.72 ± 3.30	0.515
Disease duration (year)	2.84 ± 0.65	1.24 ± 0.34	0.104
Serum TWEAK(ng/mL)	548.73 ± 77.31	346.15 ± 44.81	< 0.001
Urine TWEAK(ng/mL)	165.97 ± 37.63	83.96 ± 77.89	< 0.001
Renal histology (type II)	2	-	-
Renal histology (type III)	2	-	-
Renal histology(type III + IV)	1	-	-
Renal histology (type IV)	3	-	-
Renal histology (type V)	3	-	-

LN, Lupus nephritis; SLE, subacute lupus erythematosus; TWEAK, tumor necrosis factor-like weak inducer of apoptosis.

¹Chi-square test was used for comparison of genders.

²Mann-Whitney U test was used for comparison of age, onset age, disease duration, and TWEAK levels.

Table 2. The correlations between the serum parameters and SLEDAI-2K

	TWEAK	mCRP	ANA	ESR	IL-6	C3	C4	Anti-dsDNA	WBC
Correlation coefficient	0.603	0.289	0.227	0.327	0.106	-0.167	-0.144	0.247	-0.161
<i>p</i> value	< 0.001	0.005	0.038	< 0.001	0.556	0.070	0.109	0.061	0.081

ANA, Antinuclear antibody; ESR, erythrocyte sedimentation rate; IL-6, interleukin 6; mCRP, monomeric C-reactive protein; SLEDAI-2K, systemic lupus erythematosus disease activity index-2K; TWEAK, tumor necrosis factor-like weak inducer of apoptosis; WBC, white blood cell.

¹Spearman Rho test (two-tailed) was used for the analysis of correlations.

Table 3. Demographic characteristics of patients with SLE and healthy donors

	SLE (n = 59)	SCLE (n = 20)	DLE (n = 13)	Healthy (n = 32)	<i>p</i> Value
Gender (female/male)	54/5	16/4	7/6	26/6	0.018
Age (year)	35.29 ± 1.87	35.45 ± 3.24	41.18 ± 4.41	33.00 ± 1.84	0.519
Age of onset (year)	33.02 ± 1.86	33.28 ± 2.90	39.38 ± 4.37	-	0.380
Disease duration (year)	2.31 ± 0.46	2.17 ± 0.86	1.80 ± 0.49	-	0.903
Serum TWEAK (ng/mL)	383.00 ± 45.37	129.10 ± 25.73	78.38 ± 22.85	49.28 ± 6.04	< 0.001
Urine TWEAK (ng/mL)	226.72 ± 40.71	110.11 ± 24.01	55.50 ± 13.74	54.97 ± 16.94	< 0.05
Total WBC (10 ¹² /L)	6.98 ± 2.99	6.86 ± 2.59	6.52 ± 1.41	-	0.773
Platelet count (10 ⁹ /L)	200.90 ± 63.93	188.31 ± 39.97	190.22 ± 72.19	-	0.102
ESR (mm/h)	22.34 ± 5.61	14.91 ± 4.77	15.44 ± 3.66	-	0.386
C3 (U/mL)	79.01 ± 23.13	98.43 ± 23.14	109.42 ± 24.43	-	< 0.001
C4 (U/mL)	13.15 ± 1.33	16.61 ± 1.46	21.16 ± 0.72	-	0.057
ANA titer (10 ⁻³ /L)	2.92 ± 0.42	2.50 ± 0.62	4.42 ± 1.10	-	0.227

ANA, Antinuclear antibody; DLE, discoid lupus erythematosus; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; mCRP, monomeric C-reactive protein; SCLE, subacute cutaneous lupus erythematosus; SLE,

subacute lupus erythematosus; TWEAK, tumor necrosis factor-like weak inducer of apoptosis; WBC, white blood cell.

¹Chi-square test was used for comparison of genders. There was no significant difference between any two groups ($p > 0.05$) except SLE versus DLE ($p = 0.003$).

²Analysis of variance was used for the comparison of age, onset age, disease duration, and laboratory parameters.

³The SLE group had higher C3 levels than the SCLE and DLE groups ($p < 0.001$).

Table 4. Demographic characteristics of patients in non-monitoring and TWEAK-monitoring groups

	Non-monitoring group (n = 20)	TWEAK-monitoring group (n = 25)	<i>p</i> Value
Gender (female/male)	18/2	22/3	0.832
Age (year)	35.14 ± 19.22	31.71 ± 16.58	0.621
Age of onset (year)	31.60 ± 15.89	33.31 ± 19.66	0.270
Disease duration (year)	1.95 ± 3.07	1.88 ± 2.53	0.503
Serum TWEAK (ng/mL)	367.5 ± 37.84	331.2 ± 32.39	0.674
Urine TWEAK (ng/mL)	195.69 ± 15.63	207.32 ± 19.46	0.563
Total WBC ($10^{12}/L$)	6.109 ± 3.127	6.425 ± 1.998	0.927
Platelet count ($10^9/L$)	206.7 ± 72.88	198.5 ± 69.30	0.350
ESR (mm/h)	20.91 ± 23.66	21.73 ± 20.65	0.581
C3 (u/mL)	79.62 ± 25.94	76.87 ± 21.99	0.782
C4 (u/mL)	15.97 ± 21.62	16.12 ± 22.35	0.596
ANA titer ($10^{-3}/L$)	2.39 ± 3.81	2.57 ± 3.73	0.661

WBC, white blood cell; ESR, erythrocyte sedimentation rate; TWEAK, tumor necrosis factor-like weak inducer of apoptosis; ANA, Antinuclear antibody.

¹Chi-square test was used for comparison of genders. There was no significant difference between two groups.

²Mann-Whitney U test was used for comparison of age, onset age and disease duration. There was no significant difference between two groups.

³ Mann-Whitney U test was used for the comparison of clinical parameters.

Figures

Figure 1

Correlations between serum parameters and systemic lupus erythematosus disease activity index-2K (SLEDAI-2K) scores in patients with lupus erythematosus. (A) Serum tumor necrosis factor-like weak inducer of apoptosis (TWEAK) levels were determined in patients and in healthy controls. (B to E) The correlation between serum levels of TWEAK (B), monomeric C-reactive protein (mCRP) (C), ANA (D), or erythrocyte sedimentation rate (ESR) (E), and SLEDAI-2K scores was analyzed in patients with SLE. Number of samples: patients with SLE, 59; patients with subacute cutaneous lupus erythematosus (SCLE), 20; patients with discoid lupus erythematosus (DLE), 13; healthy controls, 32.

Figure 2

The correlations between TWEAK (serum and urine) and SLEDAI-2K scores in LN patients and non-LN SLE patients. (a and b) serum and urine TWEAK levels were determined in LN patients. (c) Urine TWEAK levels were determined in non-LN SLE patients. Number of samples: patients with LN, 39; patients with non-LN SLE, 20.

Figure 3

Correlations between urine tumor necrosis factor-like weak inducer of apoptosis (TWEAK) (or serum anti-dsDNA IgG) and urine protein in patients with lupus erythematosus. (A) Urine TWEAK levels were determined in both patients and healthy controls. (B and C) The correlation between urine levels of TWEAK (B) or serum anti-dsDNA IgG (C) and urine protein was analyzed in patients with lupus nephritis (LN). Number of samples: patients with SLE, 59; patients with subacute cutaneous lupus erythematosus

(SCLE), 20; patients with discoid lupus erythematosus (DLE), 13; patients with LN, 39; healthy controls, 32.

Figure 4

Comparison in systemic lupus erythematosus disease activity index (SLEDAI)-2K, glucocorticoid doses, and lupus flares between systemic lupus erythematosus (SLE) patients receiving different therapies. Methylprednisolone (MP) was administrated to patients with SLE with the doses depending on (monitoring) or independent of (non-monitoring) the tumor necrosis factor-like weak inducer of apoptosis (TWEAK) levels. (A) SLEDAI-2K scores were recorded in the two groups during months 0 to 12. (B) Daily doses of MP were calculated in the two groups during months 0 to 12. (C) Flares of SLE-related

manifestations were recorded in the two groups during months 10 to 12. There were 25 patients in the TWEAK-monitoring and 20 patients in the non-monitoring groups, respectively.