Combined utilization of untimed single urine of MCP-1 and TWEAK as a potential indicator for proteinuria in lupus nephritis

A case–control study

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Abstract

The aim of this study was to determine whether combined utilization of untimed single urine monocyte chemoattractant protein 1 (uMCP-1) and tumor necrosis factor (TNF)-like weak inducer of apoptosis (uTWEAK) could serve as a screening test for proteinuria in patients with lupus nephritis (LN).

A case–control study that contained 39 biopsy-proven LN patients, 20 non-LN systemic lupus erythematosus (SLE) patients, and 10 healthy controls (HCs) were carried out. Correlations between uMCP-1, uTWEAK, and traditional clinical markers were analyzed by Spearman correlation test. Diagnostic values of uMCP-1, uTWEAK, and urine albumin/creatinine ratio (uACR) in the assessment of proteinuria were investigated by receiver operating characteristic (ROC) curves.

Biopsy-proven LN patients showed higher levels of uMCP-1 and uTWEAK than non-LN patients. uMCP-1 and uTWEAK were elevated in renal active patients (rSLEDAI $\geq$4). Both uMCP-1 and uTWEAK showed significant correlation with patients' rSLEDAI, 24-hour urine proteinuria (24hr UP), and anti-double-stranded DNA (anti-dsDNA) antibodies. No correlations of these 2 biomarkers between cystatin C (Cys-C), creatinine (Cr), and blood urea nitrogen (BUN) were observed. An algorithm combining the moderate sensitivity of uMCP-1 and high specificity of uTWEAK displayed great specificity and sensitivity for proteinuria screening.

Both uMCP-1 and uTWEAK were positively correlated with the impairments of LN, and the combined utility of untimed single uMCP-1 and uTWEAK might be used as potential predictors for proteinuria in LN.

Abbreviations: α1MG = alpha-1 microglobulin, β2-MG = beta 2-microglobulin, 24-hr UP = 24-hour proteinuria, ACR = albumin/cretinine ratio, ACR = american college of rheumatology, Alb = albumin, ANA = antinuclear antibody, ANOVA = analysis of variance, anti-dsDNA antibodies = anti-double strand DNA antibodies, AUC = area under the ROC curves, BUN = blood urea nitrogen, C3 = complements C3, C4 = complements C4, Cr = creatinine, Cys-C = Cystatin C, ESR = erythrocyte sedimentation rate, GFR = glomerular filtration rate, IgA = immunoglobulin-alpha, IgG = immunoglobulin-gamma, IgM = immunoglobulin-mu, ISN/RPS = international society of nephrology/renal pathology society, LN = lupus nephritis, MCP-1 = monocyte chemoattractant protein 1, PCR = protein/creatinine ratio, ROC = receiver operating characteristic, rSLEDAI = renal systemic lupus erythematosus disease activity index, SLE = systemic lupus erythematosus, TWEAK = tumor necrosis factor-like weak inducer of apoptosis.

Keywords: lupus nephritis, proteinuria, urinary monocyte chemoattractant protein 1 (uMCP-1), urinary TNF-like weak inducer of apoptosis (uTWEAK)

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1. Introduction

Lupus nephritis (LN) occurs in almost 50% of systemic lupus erythematosus (SLE) patients, and viciously affects their prognosis.[1] Proteinuria quantification is essential during the clinical evaluation of patients with glomerulonephritis, as it is among the strongest determinants of renal prognosis.[2,3] The "gold standard" test for proteinuria quantification is 24-hour urine proteinuria (24 hr UP) test.[4] However, due to its inherited flaws, such as cumbersome and inaccuracy for the collection of 24-hours urine, it was replaced by detection of spot urine protein/creatinine ratio (uPCR) and urine albumin/creatinine ratio (uACR) in many guidelines of kidney disease.[5–7] On the contrary, novel cytokines or chemokines have been recently reported to be correlated with LN renal damage.[8–10] However, little has been reported about the role of cytokines or chemokines in the assessment of proteinuria.

Among those new candidates, monocyte chemoattractant protein 1 (MCP-1) is one of the most well studied one in LN. MCP-1 belongs to CC chemokine family that is mainly expressed by activated monocyte/macrophages, T cells, and natural killer cells. It is responsible for the leukocytes' infiltration to the kidney.[11] Previous researches have demonstrated that MCP-1 levels in urine and serum of LN patients correlated well with LN disease activity.[12,13] Administrating antagonist of MCP-1 could ameliorate the initiation and progression of LN in transgenetic mouse model.[14] These researches show that MCP-1 may be a promising biomarker for LN activity assessment as well as a target for LN therapy. However, MCP-1 may not be a specific marker for LN detection, as increased MCP-1 has also been found in diabetic nephropathy, atherosclerosis, etc.[15–18] What is more, it is challenging to achieve both high specificity and sensitivity simply using 1 analyte, due to the heterogeneity of the LN at presentation. Satisfied renal damage assessment may not be achieved by referring to MCP-1 exclusively, but by the combination of other parameters.

In addition, TNF-like weak inducer of apoptosis (TWEAK) that belongs to the TNF receptor superfamily seems like another promising candidate for LN assessment. TWEAK level has been reported to be closely correlated with renal inflammation.[19] TWEAK induces several nephritis-related inflammatory mediators, including Chemokine (C-C motif) ligand 5, Chemokine (C-X-C motif) ligand 10, and Vascular cell adhesion molecule-1, in the inflammatory cascade, which can cause downstream inflammatory response activation and further renal damage progression.[20,21] Several cross-sectional and longitudinal studies have mentioned that urinary TWEAK levels elevate in active LN patients compared with that of remission ones.[13,22] However, the combined utility of untimed uMCP-1 and uTWEAK still needs investigation.

In these regards, we analyzed uMCP-1 and uTWEAK levels in biopsy-proven LN patients, evaluate the combined utility of uMCP-1 and uTWEAK, and compared it with uACR in proteinuria detection.

2. Materials and methods

2.1. Study design

The study was approved by the ethics reviews committees of Xijing Hospital (No. 20110303–6). SLE patients fitting the 1997 updated American College of Rheumatology (ACR) revised criteria for the classification of SLE or 2009 modified ACR criteria[23,24] concomitant with renal impairment were recruited

Table 1

Demographical and histological characteristics of LN patients.

	LN	Non-LN SLE	HC
Number	39	20	10
Female/male	35/4	20/2	10/2
Median age, y	30 (13–51)	45 (16–65)	33 (16–55)
Duration of disease, mo	48 (1–192)	72 (1–300)	–
Body weight, kg	56.22±9.61	58.49±6.41	58.70±8.53
rSLEDAI	4 (0–11)	–	–
SLEDAI	10 (0–24)	6 (1–29)	–
Histological ISN/RPS class			
II	13	–	–
III	4	–	–
III+V	3	–	–
IV	2	–	–
IV+V	2	–	–
V	8	–	–
V+III	3	–	–
V+IV	4	–	–

ISN/RPS = International Society of Nephrology/Renal Pathology Society Classification of LN, LN = lupus nephritis, rSLEDAI = renal Systemic Lupus Erythematosus Disease Activity Index, SLE = systemic lupus erythematosus, SLEDAI = Systemic Lupus Erythematosus Disease Activity Index.

in Department of Clinical Immunology in Xijing Hospital from December 2015 to February 2016. Patients who had active infection, ongoing pregnancy, cancer, or diabetes were excluded. As the cortisone and immunosuppressive agents may cause fluctuation of inflammation mediators, to achieve reproducible results, patients who had already received induction therapy in previous 3 months were also excluded.

A total of 69 subjects, including 39 LN patients (median age: 30 years, range: 13–51 years; gender: 35 females, 4 males) and 20 non-LN SLE patients (median age: 45 years, range: 16–65 years; gender: 18 females, 2 males) and 10 HC (median age: 33 years, range: 16–55 years; gender: 8 females, 2 males) were enrolled in the study (Table 1). Non-LN SLE patients were defined as patients who had SLE but no signs of kidney involvement recently and previously, and LN patients were defined as SLE patients with kidney involvement based on clinical manifestation as well as kidney biopsies. After signing informed consent forms, patients whose 24-hr UP exceed 300 mg/day underwent kidney biopsy surgery to make further confirmation of the existence of LN and classification according to the International Society of Nephrology/Renal Pathology Society Classification (ISN/RPS).[25] The renal SLE disease activity index (rSLEDAI) score was measured according to the sum of scores of 4 components, naming proteinuria, urinary casts, hematuria, and leucocyturia in urine examination.[12] rSLEDAI scores of ≥4 were reckoned as renal active and <4 as inactive.[26]

2.2. Samples collection and examination

All patients' samples were collected before induction therapy. Ten milliliters of untimed single urine samples from patients were collected and centrifuged at 900g to remove the sediment and stored in -40°C for less than 1 month before detecting. All blood samples and corresponding laboratory examinations were collected and carried out under standard protocols. The clinical parameters, including erythrocyte sedimentation rate (ESR), anti-dsDNA antibodies, 24-hr UP, antinuclear antibody (ANA), complement C3 (C3) and complement C4 (C4), anti-C1q antibodies, cystatin C (Cys-C), Creatinine (Cr), blood urea nitrogen (BUN), serum IgG, serum IgM, and serum IgA were

detected. Radioimmunoassays were introduced to measure serum beta-2 microglobulin (sβ2MG), urinary beta-2 microglobulin (uβ2MG), uIgG, urinary albumin (uAlb), and urinary alpha-1 microglobulin (uα1MG).

2.3. Detection of uMCP-1 and uTWEAK

The concentrations of uMCP-1 and uTWEAK were measured by enzyme-linked immunosorbent assay (ELISA), according to the products' protocols (Neobioscience, Shenzhen, China). Briefly, the urinary samples and diluted recombinant human MCP-1 and TWEAK (8 different concentrations ranging from 0 to 1000 pg/mL) were pipetted into antibody pre-coated 96-well plates. Then, plates were incubated at 37°C for 90 minutes. After washing, detection antibodies were added and incubated for another 2 hours. Then, the plates were washed for 5 times before adding TMB. Incubation was conducted at 36°C for 15 minutes. Absorbance was read by Epoch (Biotek, Vermont) at 450 nm within 3 minutes. Variations within and between batch were all <8% for both MCP-1 and TWEAK ELISA kit. Moreover, the minimum detection limits of the kits were 8 pg/mL. The uMCP-1 and uTWEAK levels were corrected to urine creatinine to avoid urine concentration variation, which expressed as picograms per milligram of creatinine (pg/mgCr). Each experiment has been repeated for at least 3 times.

2.4. Statistical analysis

The statistical analyses were conducted by SPSS 19.0 (IBM, New York). Graphs were drawn by GraphPad Prism 5. Enumeration data were presented as mean±SD or median (range). Comparisons among different groups were carried out by Student t test or the analysis of variance (ANOVA) and Bonferroni multiple comparison test. Correlations between other traditional parameters and MCP-1 and TWEAK were carried out by Spearman ranking correlation. As spot uACR was proposed as a preferred method for measuring proteinuria in 2002 K/DOQI guidelines for chronic kidney disease, comparisons of the utility of uMCP-1/uTWEAK and uACR to predict proteinuria were evaluated by the area under the ROC curve (AUC) and Youden index. P value <.05 was considerate significant.

3. Results

3.1. Characterizations of patients

Demographic and pathological characters are summarized in Table 1. According to ISN/RPS classification, the pathological specimens of 39 patients demonstrated that 13 cases were classified into class II nephritis, 4 patients class III, 3 patients class III+V, 2 patients class IV, 2 patients class IV+V, 8 patients class V, 3 patients class V+III, and 4 patients class V+ IV (Table 1).

3.2. Levels of uMCP-1 and uTWEAK in different groups

Both uMCP-1 and uTWEAK significantly elevated in LN patients (219.45±192.08 pg/mgCr and 21.17±19.63) compared with HC (12.34±4.82 pg/mgCr, P <.0001 and 5.94±3.42, P <.05) and non-LN SLE (66.68±65.38 pg/mgCr, P <.0001 and 7.20± 6.84 pg/mgCr, P <.001).

The levels of uMCP-1 and uTWEAK varied in patients with different biopsy classification. The levels of uMCP-1 and uTWEAK were 111.12±58.92 and 11.09±7.78 pg/mgCr, respectively, in class II nephritis patients, 224.86±168.70 and

14.44±12.99 pg/mgCr in class III (including III+V) patients, 229.70±130.04 and 18.36±17.51 pg/mgCr in class IV (including IV+V) patients, 308.07±248.98 and 33.80±23.80 pg/mgCr in class V (including V+III and V+IV) patients. The subgroup analysis of uMCP-1 and uTWEAK in class V and V+III and V+IV LN did not reveal a significant difference (Supplementary Figure 1A and B, http://links.lww.com/MD/C200). ANOVA showed that the overall difference of means of uTWEAK in the different pathological group was significant (P =.009). Post hoc test revealed a significantly higher level of uTWEAK in class V LN (P <.01) and insignificantly higher level of uTWEAK class III LN (P >.05) and IV LN (P >.05) compared with that of class II LN (Fig. 1C). Although no significant difference, levels of uMCP-1 in class V (P <.05), IV (P >.05), and III patients (P >.05) obviously increased than that of class II patients (Fig. 1D). In addition, levels of both uMCP-1 and uTWEAK were significantly elevated in renal active (rSLEDAI ≥4) patients rather than renal inactive (rSLEDAI <4) patients (uMCP-1, P <.01; uTWEAK, P <.01), while elevation of uACR was not significant (P =.083). (Supplementary figure 1C-E, http://links.lww.com/MD/C200)

3.3. Correlations of uMCP-1/uTWEAK and traditional parameters

Spearman correlation tests were conducted to test the corrections of uMCP-1 and uTWEAK with other renal damage related parameters (Table 2). uMCP-1 was significantly correlated with rSLEDAI scores (r_s =0.480, P =.002), 24-hr UP (r_s =0.444, P =.005), uAlb (r_s =0.394, P =.019), C3 (r_s =-0.381, P =.017), anti-dsDNA antibodies (r_s =0.363, P =.023), and C4 (r_s =-0.322, P =.045). uTWEAK was correlated with rSLEDAI scores (r_s = 0.380, P =.017), 24-hr UP (r_s =0.367, P =.021), and anti-dsDNA antibodies (r_s =0.367, P =.021).

Nearly half of the class V patients in our study were accompanied by class III or class IV LN. To further eliminate potential influence of class V+III or class V+IV patients, we also reanalyzed data of other groups and class V patients of pure membranous glomerulonephritis (Supplementary Table 1, http://links.lww.com/MD/C200). The results also showed that both uMCP-1 and uTWEAK were correlated with rSLAEDAI scores (uMCP-1, r_s =0.497, P =.004; uTWEAK, r_s =0.331, P =.044) and 24-hr UP (uMCP-1, r_s =0.435, P =.013; uTWEAK, r_s = 0.411, P =.019).

3.4. Comparisons of uMCP-1, uTWEAK, and uACR in proteinuria prediction

The abilities of uMCP-1, uTWEAK, and uACR to screen proteinuria were evaluated by analyzing ROC curves. Twenty-four hour UP > 0.15 g/day was defined as positive for proteinuria. ROC curves of uMCP-1, uTWEAK, and uACR to predict proteinuria were generated. As shown in Fig. 2, the black dashed, grey dashed line, and grey solid line represented uMCP-1, uTWEAK, and uACR, respectively. The black solid ROC curve represented the combined utility of uTWEAK and uMCP-1 with an algorithm of $Y=0.07^*uMCP\text{-}1+0.22^*uTWEAK\text{-}3.72$. uMCP-1 had an AUC (area under ROC curve) of 0.730 and was moderately sensitive (70.0%) and specific (77.8%) for proteinuria prediction. uTWEAK showed higher specificity (88.9%) than the uMCP-1, but lower sensitivity (36.7%) (Table 3). The combination of uMCP-1 and uTWEAK showed elevated AUC (0.767) with better sensitivity (76.7%) and higher specificity (88.9%), which was of equal specificity but less sensitivity than
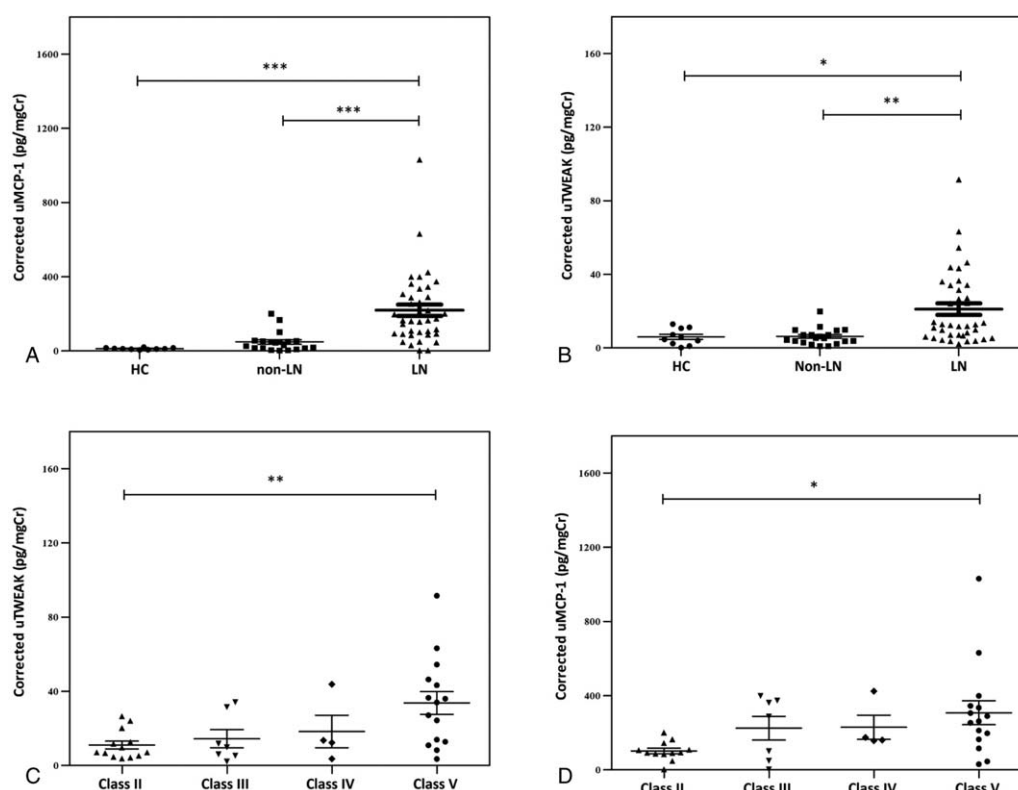
Figure 1. Distribution of uMCP-1 and uTWEAK. (A) uMCP-1 significantly elevated in LN SLE patients compared with those in HC ($P < .0001$) and non-LN SLE patients ($P < .0001$). (B) uTWEAK significantly elevated in LN SLE patients compared with those in HC ($P < .05$) and non-LN patients ($P < .01$). (C) uTWEAK in class V patients were significantly increased, while class III and IV patients were insignificantly increased compared with that of class II patients (class V vs class II, $P < .01$; class VI vs class II, $P > .05$; class III vs class II, $P > .05$). (D) Although not significant, the levels of uMCP-1 in class III, IV, and V patients were detected increasing compared with that of class II patients (class V vs class II, $P < .05$; class IV vs class II, $P > .05$; class III vs class II, $P > .05$). rSLEDAI = renal Systemic Lupus Erythematosus Disease Activity Index; uACR = urine albumin/creatinine ratio; uMCP-1 = urinary monocyte chemoattractant protein-1; uTWEAK = urinary tumor necrosis factor-like inducer of apoptosis.

that of uACR (sensitivity; uACR vs combined model, 76.7% vs 80.0%).

Reanalysis of other groups and class V LN patients of pure membranous glomerulonephritis was also carried out (Supplementary table 2 and Supplementary figure 2, http://links.lww.com/MD/C200). The evaluation ability slightly increased for uMCP-1 (AUC increased from 0.730 to 0.745), uTWEAK (AUC increased from 0.626 to 0.635), and the combined algorithm (AUC increased from 0.767 to 0.792), while those of uACR decreased (from 0.841 to 0.839).

4. Discussion

Protein in the urine not only serves as a reliable marker for SLE renal involvement but also initiates the tubulointerstitial fibrosis and deteriorate glomerular diseases.[27] Measuring and assessing kidney involvement have therefore become vital parts of LN patients' evaluation. In the present study, we revealed that high levels of uMCP-1 and uTWEAK in untimed single urine MCP-1 and TWEAK were correlated with rSLEDAI and abnormal 24-hr UP, and proposed a new model to assess proteinuria.

uMCP-1 and uTWEAK were elevated in LN patients compared with HC and non-LN SLE. Both uMCP-1 and uTWEAK were elevated in LN active patients compared with inactive LN patients and were correlated to rSLEDAI score, which is an indicator of renal activity. These results suggested that uMCP-1 and uTWEAK elevated parallel to the severity of

renal damage, confirming the previous discovery of the tight relationship between these 2 markers and renal damage.[9,22,28]

uMCP-1 demonstrated significant correlation with rSLEDAI scores, 24-hr UP, anti-dsDNA antibodies, C3, and C4, while uTWEAK was correlated with rSLEDAI scores, 24-hr UP, and anti-dsDNA antibodies.[29,30] Although C3, C4, and anti-dsDNA antibodies were more or less correlated with renal damage in LN, we did not put more focus on them as their predictive values show highly inconsistency.[21,31–33] At the same time, other traditional biomarkers, such as Cys-C, Cr, BuN, serum IgG, IgM, IgA, and ESR, etc, had been correlated with neither uMCP-1 nor uTWEAK.

For uMCP-1 and uTWEAK as individual analytes, their specificities and sensitivities were not satisfying (reach near 80%) at the same time. Consequently, it is very difficult to achieve both high specificity and sensitivity simply using 1 analyte, due to the heterogeneity of the LN at presentation.[34] In addition, the present study showed that uTWEAK possessed high specificity to proteinuria, while uMCP-1 showed moderate sensitivity and specificity, indicating that the combined model may gather their advantages and enhance the assessment ability of proteinuria. Such hypothesis was verified by the fact that the combination resulted in the elevation of sensitivity to 76.7% and specificity to 88.9%, suggesting that the combination exceeded single utility of them.

As a matter of fact, albuminuria is sensitive to the measure of proteinuria and that untimed uACR was recommended in the

Table 2

Correlations of uMCP-1, uTWEAK, and other parameters.

	N	Means	uMCP-1 r_s	uMCP-1 Sig. (2-tail)	uTWEAK r_s	uTWEAK Sig. (2-tail)
rSLEDAI	39	4.00	0.480	0.002[†]	0.380	0.017[*]
SLEDAI	32	10.00	0.204	0.262	0.017	0.925
24hr UP, g/d	39	1.49	0.444	0.005[†]	0.342	0.033[*]
Cys-C, mg/L	39	1.34	0.018	0.916	−0.045	0.788
Cr, μmol/L	39	97.85	−0.156	0.342	−0.074	0.656
BuN, mmol/L	39	10.00	0.005	0.974	0.046	0.783
sIgG, mg/dL	39	1541.70	0.020	0.904	−0.160	0.331
sIgA, mg/dL	39	12.09	0.056	0.736	0.173	0.291
sIgM, mg/dL	39	382.01	−0.264	0.104	−0.077	0.641
ESR, mm/h	39	46	0.180	0.273	0.262	0.107
C3, mg/dL	39	12.09	−0.381	0.017[*]	0.095	0.563
C4, mg/dL	39	19.83	−0.322	0.045[*]	0.035	0.830
anti-C1q antibodies	35	19.82	0.313	0.067	0.039	0.824
anti-dsDNA antibodies	39	1:10,000	0.363	0.023[*]	0.367	0.021[*]
ANA	39	1:1280	−0.140	0.396	0.100	0.546
GFR, mL/min	10	81.57	0.188	0.603	−0.139	0.701
sβ2MG, mg/L	34	4.29	0.120	0.500	0.028	0.873
uβ2MG, μg/L	35	446.60	0.237	0.170	−0.008	0.963
uAlb, mg/L	35	17.11	0.394	0.019[*]	0.190	0.273
uIgG, mg/L	34	21.93	0.258	0.140	−0.021	0.904
uα1MG, mg/L	35	6.33	−0.056	0.750	−0.253	0.143
uTWEAK, pg/mgCr	39	21.17	0.359	0.025[*]	–	–

[*] $P < .05$.

[†] $P < .01$.

24hr UP = 24-h urine proteinuria, ANA = antinuclear antibody, anti-C1q antibodies = anti-complement 1q antibodies, anti-dsDNA antibodies = anti-double strand DNA antibodies, BuN = blood urea nitrogen, C3 = complement 3, C4 = complement 4, Cr = creatinine, Cys-C = Cystatin C, ESR = erythrocyte sedimentation rate, GFR = glomerular filtration rate, rSLEDAI = renal Systemic Lupus Erythematosus Disease Activity Index, sβ2MG = serum beta-2 microglobulin, sIgA = serum immunoglobulin-alpha, sIgG = serum immunoglobulin-gamma, sIgM = serum immunoglobulin-Mu, SLEDAI = Systemic Lupus Erythematosus Disease Activity Index, uAlb = urinary albumin, uβ2MG = urinary beta-2 microglobulin, uIgG = urinary immunoglobulin-gamma, uα1MG = urinary alpha-1 microglobulin, uMCP-1 = urinary monocyte chemoattractant protein-1, uTWEAK = urinary tumor necrosis factor-like inducer of apoptosis.
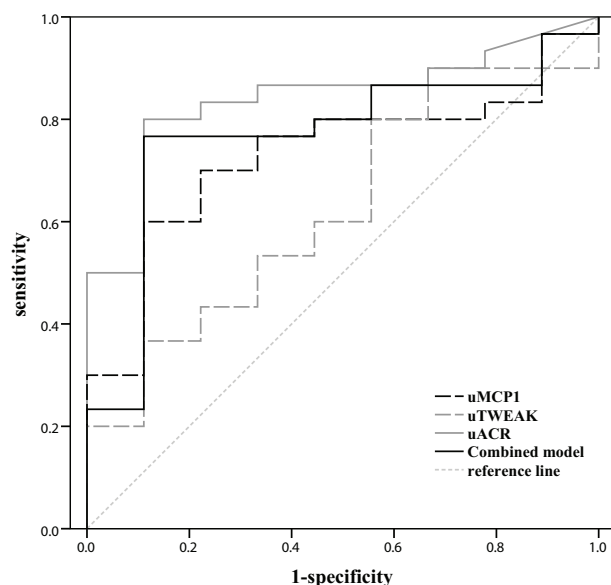

Figure 2. Receiver-operation characteristic curves of uMCP-1, uTWEAK, uACR, and combined model in assessing proteinuria. The black dotted, grey dotted, and grey solid ROC curves represented uMCP-1, uTWEAK, and uACR, respectively. The black solid ROC curve represented the combined model of uTWEAK and uMCP-1. The AUC (area under ROC curves) of the combined model was larger than those of the uMCP or uTWEAK (0.767 vs 0.730 or 0.626). AUC of uACR was larger than other models (0.841). Combined model: $Y = 0.07^* uMCP + 0.22^* uTWEAK - 3.72$. uACR = urine albumin/creatinine ratio; uMCP-1 = urinary monocyte chemoattractant protein-1; uTWEAK = urinary tumor necrosis factor-like inducer of apoptosis.

diagnosing and evaluation of CKD patients.[5] A comparison of uMCP-1, uTWEAK, and uACR was carried out. uACR was much better than the single utilization of uMCP-1 and uTWEAK, but only exceed the combination of them slightly in sensitivity (uACR vs combined model, 80% vs 76.7%). Furthermore, we found that elevation of uACR in renal active (rSLEDAI ≥4) compared with inactive patients (rSLEDAI < 4) was not as significant as those of uMCP-1 and uTWEAK; Both uMCP-1 and uTWEAK were correlated with the rSLEDAI score, a reflection of renal involvement. These results suggested that uMCP-1 and uTWEAK have their advantages in evaluating renal involvement.

The reason for this is that both uMCP-1 and uTWEAK are inflammatory factors, which are associated with not only proteinuria (from the result of our experiment) but also immune-related renal damage,[20] while uACR may not possess such characters. Moreover, the measurements of uACR are mostly based on radioimmunoassay that requires high standard equipment and produces potential radiotoxicity to the technician. On the contrary, detection of uMCP-1 and uTWEAK could be simply carried out by ELISA, which is more money-saving and harmless. Above all, the utility of uMCP-1 and uTWEAK still hold the potential to be a screen test for proteinuria and renal involvement that will benefit both patients and hospital.

In our study, serum counterparts of uMCP-1 and uTWEAK had not been measured and compared. This mainly attributed to the notion that the correlations between serum levels of these parameters and LN activities were weak.[35] Urinary biomarkers, which were either infiltrated through glomerulus or produced

Table 3

Sensitivity and specificity to assess proteinuria with uMCP-1 and uTWEAK.

	AUC (95% CI)	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
uMCP-1	0.730 (0.562–0.898)	151.42	70.0	77.8	0.538
uTWEAK	0.626 (0.427–0.825)	26.95	36.7	88.9	0.256
uACR	0.841 (0.710–0.972)	15.56	80.0	88.9	0.689
Combined model[*]	0.767 (0.596–0.938)	9.64	76.7	88.9	0.656

The AUC of 0.5 is completely random, while 1.0 indicates perfect discrimination. Values between 0.7 and 0.8 are considered acceptable and values greater than 0.8 are considered excellent. According to this principle, both uMCP-1 and uTWEAK were acceptable, whereas their combination was excellent to assess proteinuria. The cut-off value was determined in a way to achieve the highest Youden index (Youden index = Sensitivity + Specificity-1), which represented high diagnostic accuracy. The uMCP-1 presented moderate sensitivity and specificity, while the uTWEAK showed poor sensitivity and excellent specificity. With the combination of 2 methods, a new model that preserved acceptable sensitivity (73.1%) and excellent specificity (92.3%) was acquired.

24-hr UP = 24-hour urine proteinuria, AUC = area under ROC curve, uMCP-1 = urinary monocyte chemoattractant protein-1, uTWEAK = urinary tumor necrosis factor-like inducer of apoptosis.

[*] The combined model was acquired using the following algorithm. $Y = 0.07^* uMCP + 0.22^* uTWEAK - 3.72$.

locally, could discern between renal manifestation and other organs' manifestations more accurately than their serum counterparts.[14,20] Also, Bland–Altman plots were not introduced in our research to analyze the agreement between 24-hr UP and urinary parameters, because they were more suitable to evaluate the agreements among 2 different instruments or 2 measurements techniques rather than 2 different parameters.[36,37] Nevertheless, future researches still need to concentrate more on the confirmation of our results as well as excavation of other potential evaluating biomarkers.

The numbers of class III and class IV patients enrolled in our study seemed small compared with that of class V in the study. The reason for this discrepancy is that renal biopsy in our center is carried out according to proteinuria > 300 mg/day, which are more commonly seen in class V as our results shown. In addition, as for a repeat biopsy in the patients with partial/complete remission was hard to acquire approve from the ethics committee, no patients in remission were recruited in the study. However, the follow-up data of these patients in the future would provide the more details on the utility of uMCP-1 and uTWEAK in patients with LN.

In conclusion, we revealed that both uMCP-1 and uTWEAK were elevated in patients with active LN and were significantly corrected with 24-hr UP. The further statistical model suggested that combined utilization of untimed single uMCP-1 and uTWEAK could serve as screen examination for proteinuria in active LN patients.

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References

[1] Almaani S, Meara A, Rovin BH. Update on lupus nephritis. Clin J Am Soc Nephrol 2017;12:825–35.
[2] Balow JE. Clinical presentation and monitoring of lupus nephritis. Lupus [; Review] 2005;14:25–30. 2005.
[3] Bastian HM, Roseman JM, McGwin GJr, et al. Systemic lupus erythematosus in three ethnic groups. XII. Risk factors for lupus nephritis after diagnosis. Lupus 2002;11:152–60.
[4] Radhakrishnan J, Cattran DC. The KDIGO practice guideline on glomerulonephritis: reading between the (guide)lines|[mdash]| application to the individual patient. Kidney Int 2012;82:840.
[5] National Kidney FoundationK/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39:S1–266.
[6] Medina-Rosas J, Yap KS, Anderson M, et al. Utility of urinary protein-creatinine ratio and protein content in a 24-hour urine collection in systemic lupus erythematosus: a systematic review and meta-analysis. Arthritis Care Res 2016;68:1310–9.
[7] Birmingham DJ, Rovin BH, Shidham G, et al. Spot urine protein/creatinine ratios are unreliable estimates of 24h proteinuria in most systemic lupus erythematosus nephritis flares. Kidney Int 2007;72:865–70.
[8] Mak A, Isenberg DA, Lau CS. Global trends, potential mechanisms and early detection of organ damage in SLE. Nat Rev Rheumatol [Review] 2013;9:301–10.
[9] Misra R, Gupta R. Biomarkers in lupus nephritis. Int J Rheum Dis 2015;18:219–32.
[10] Bagavant H, Deshmukh US, Gaskin F, et al. Lupus glomerulonephritis revisited 2004: autoimmunity and end-organ damage. Scand J Immunol 2004;60:52–63.
[11] Deshmane SL, Kremlev S, Amini S, et al. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009; 29:313–26.
[12] Gupta R, Yadav A, Aggarwal A. Longitudinal assessment of monocyte chemoattractant protein-1 in lupus nephritis as a biomarker of disease activity. Clin Rheumatol 2016;35:2707–14.
[13] Abulaban KM, Song H, Zhang X, et al. Predicting decline of kidney function in lupus nephritis using urine biomarkers. Lupus 2016; 25:1012–8.
[14] Hasegawa H, Kohno M, Sasaki M, et al. Antagonist of monocyte chemoattractant protein 1 ameliorates the initiation and progression of lupus nephritis and renal vasculitis in MRL/lpr mice. Arthritis Rheum 2003;48:2555–66.
[15] Lin J, Kakkar V, Lu X. Impact of MCP-1 in atherosclerosis. Curr Pharm Des 2014;20:4580–8.
[16] Tesch GH. MCP-1/CCL2: a new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. Am J Physiol Renal Physiol 2008;294:F697–701.
[17] Bjorkander S, Heidari-Hamedani G, Bremme K, et al. Peripheral monocyte expression of the chemokine receptors CCR2, CCR5 and CXCR3 is altered at parturition in healthy women and in women with systemic lupus erythematosus. Scand J Immunol 2013;77:200–12.
[18] Kalnins A, Thomas MN, Andrassy M, et al. Spiegelmer inhibition of MCP-1/CCR2-potential as an adjunct immunosuppressive therapy in transplantation. Scand J Immunol 2015;82:102–9.
[19] Schwartz N, Su L, Burkly LC, et al. Urinary TWEAK and the activity of lupus nephritis. J Autoimmun 2006;27:242–50.
[20] Reyes-Thomas J, Blanco I, Putterman C. Urinary biomarkers in lupus nephritis. Clin Rev Allergy Immunol 2011;40:138–50.
[21] Keiserman B, Ronchetti MR, Monticielo OA, et al. Concomitance of IgM and IgG anti-dsDNA antibodies does not appear to associate to active lupus nephritis. Open Rheumatol J 2013;7:101–4.
[22] Schwartz N, Rubinstein T, Burkly LC, et al. Urinary TWEAK as a biomarker of lupus nephritis: a multicenter cohort study. Arthritis Res Ther 2009;11:R143.
[23] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
[24] Cader MZ, Filer A, Hazlehurst J, et al. Performance of the 2010 ACR/EULAR criteria for rheumatoid arthritis: comparison with 1987 ACR criteria in a very early synovitis cohort. Ann Rheum Dis 2011;70:949–55.
[25] Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol 2004;15:241–50.
[26] Rosa RF, Takei K, Araujo NC, et al. Monocyte chemoattractant-1 as a urinary biomarker for the diagnosis of activity of lupus nephritis in Brazilian patients. J Rheumatol 2012;39:1948–54.
[27] Hasegawa T, Suzuki K, Kaneko Y, et al. Proteinuria selectivity index as a prognostic biomarker in lupus nephritis. Lupus 2017;26:656–60.
[28] 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.
[29] Tucci M, Barnes EV, Sobel ES, et al. Strong association of a functional polymorphism in the monocyte chemoattractant protein 1 promoter gene with lupus nephritis. Arthritis Rheum 2004;50:1842–9.
[30] Chan RW, Lai FM, Li EK, et al. Expression of chemokine and fibrosing factor messenger RNA in the urinary sediment of patients with lupus nephritis. Arthritis Rheum 2004;50:2882–90.
[31] Oelzner P, Deliyska B, Funfstuck R, et al. Anti-C1q antibodies and antiendothelial cell antibodies in systemic lupus erythematosus:

relationship with disease activity and renal involvement. Clin Rheumatol 2003;22:271–8.

[32] Moroni G, Radice A, Giammarresi G, et al. Are laboratory tests useful for monitoring the activity of lupus nephritis? A 6-year prospective study in a cohort of 228 patients with lupus nephritis. Ann Rheum Dis 2009; 68:234–7.

[33] Lopez E, Hanna RM, Wilson J. Decreased sensitivity of anti-dsDNA antibody assay observed in a cohort of Hispanic patients with biopsy-proven lupus nephritis. Saudi J Kidney Dis Transplant 2016;27:1270–3.

[34] Wolf BJ, Spainhour JC, Arthur JM, et al. Development of biomarker models to predict outcomes in lupus nephritis. Arthritis Rheumatol (Hoboken, NJ) 2016;68:1955–63.

[35] Li Y, Tucci M, Narain S, et al. Urinary biomarkers in lupus nephritis. Autoimmun Rev 2006;5:383–8.

[36] Bland JM, Altman DG. Measuring agreement in method comparison studies. Stat Methods Med Res 1999;8:135–60.

[37] Carkeet A. Exact parametric confidence intervals for Bland-Altman limits of agreement. Optom Vis Sci 2015;92:e71–80.