Urinary MCP-1 and TWEAK as non-invasive markers of disease activity and treatment response in patients with lupus nephritis in South Africa

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Abstract

Background: Treatment of patients with lupus nephritis (LN) requires judicious use of immunosuppression. Novel biomarkers may be useful for monitoring disease activity and treatment response. We assessed the utility of urinary monocyte chemoattractant protein-1 (uMCP-1) and urinary tumour necrosis factor-like weak inducer of apoptosis (uTWEAK) for disease activity and treatment response monitoring in South Africans with LN. **Methods:** We recruited consenting patients with active LN confirmed on kidney biopsy. Urinary levels of MCP-1 and TWEAK were assayed at baseline and after completion of induction therapy using ELISA methods. We also collected relevant demographic, clinical and biochemical data for patients included in this study. **Results:** The mean age of patients in this study was 29.8 ± 10.7 years, 60% were patients of mixed ancestry, 70% had proliferative LN and mean spot urine proteinuria at baseline was 0.37 (0.18-0.59) g/mmolCr. At completion of induction therapy, the level of uMCP-1 had reduced to 314.5 (IQR: 197.0 – 622) pg/mgCr from a baseline of 1092.7 (IQR: 578.6-1848) pg/mgCr ($P=0.06$) while uTWEAK had reduced to 36.0 (IQR: 17.0-88.0) pg/mgCr from 159.0 (IQR: 88.5-295.5) pg/mgCr ($P=0.03$). For patients reaching early complete or partial remission, both biomarkers had significantly declined in their urine: uMCP-1 ($p=0.018$) and uTWEAK ($p=0.015$). There was no reduction of both biomarkers in patients not achieving remission and no association between uMCP-1 or uTWEAK with renal histological features. **Conclusion:** Our study shows that uMCP-1 and uTWEAK are elevated in patients with active LN, correlated with the remission status (response to treatment) at the end of induction therapy and can therefore be useful for monitoring disease activity and treatment response.

Background

Lupus nephritis (LN) complicates up to 60% of cases of systemic lupus erythematosus and is associated with increased morbidity and mortality.[1-3] Treatment of patients with LN requires judicious use of immunosuppression to reduce further complications associated with treatment.[4] Reduced use of immunosuppression is possible if disease flare or response to ongoing treatment can be identified early and tapered or halted. However, kidney biopsy remains the gold standard for diagnosis, assessing flares and guiding treatment and is often unavailable in many developing countries.[5-7] Moreover, the decision to biopsy is often dictated by conventional clinical parameters like an active urinary sediment, elevated serum creatinine, low serum complement levels and raised autoimmune antibody levels, all of which may not be accurate.[8-10] Renal biopsy is invasive with risk of major or minor complications and may be unavailable in resource poor countries.[5]

When measured in the urine, novel biomarkers such as monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-related weak inducer of apoptosis (TWEAK) may be useful for monitoring disease activity, and assessing response to therapy as they are secreted within the kidney during inflammation. [11-13] MCP-1 is a chemokine responsible for monocyte and T-lymphocyte recruitment during the acute and chronic phases of inflammation, [14] while TWEAK is a pro-inflammatory cytokine produced mainly by innate immune cells resulting in glomerular and tubular injury.[15] Our study aim was to assess the

value of uMCP-1 and uTWEAK as markers of disease activity and treatment response in South Africans with biopsy proven LN.

Methods

Study population and study design

This was a prospective observational study conducted at the renal clinic at Groote Schuur Hospital in Cape Town. The study was approved by the University of Cape Town human research ethics committee (HREC #332/2017) following which we enrolled consenting adult patients (≥ 18 years) diagnosed with biopsy proven active LN from June 2017 to December 2018. Patients received induction treatment using high dose corticosteroids with either oral mycophenolate mofetil (1 gram twice daily) or monthly pulsed cyclophosphamide using the protocol of the National Institute of Health (NIH).[16] A standardized data abstraction sheet to obtain socio-demographic and clinical information was administered at baseline and at end of induction therapy. Physical examinations and collection of biological specimens were performed, including serum, plasma and urine.

Clinical and laboratory measurements

We used the systemic lupus erythematosus disease activity index 2000 (SLEDAI-2K) score[17] to assess disease activity and the systemic lupus collaborating clinics / American college of rheumatology damage index (SLICC/ACR Damage Index) [18] to assess irreversible impairment. Standard of care blood tests were performed at the hospital's accredited laboratory and included full blood count (FBC), serum creatinine, anti-nuclear antibodies (ANA), anti-Smith antibodies (anti-Sm), anti-double stranded deoxyribonucleic acid antibodies (anti-dsDNA) and serum complement (C3 and C4) at each visit. Estimation of glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) equation.[19] A fresh mid-stream urine sample was used to assess urine protein to creatinine ratio (UPCR) at the laboratory and for the qualitative analysis of protein and blood (dipstick) in the clinic. Aliquots of urine were centrifuged to remove urinary sediment and stored frozen at -80 degrees Celsius until batched analysis for MCP-1 and TWEAK. Histological findings for the renal biopsies were reported using the International Society of Nephrology/Renal Pathology Society classification of 2003.[20] Activity and chronicity indices were assessed using previously described methods. [21] Proliferative LN was defined as histological classes III, IV, or if mixed with class V and interstitial fibrosis was reported as present or absent, irrespective of degree, if reported by the pathologist.

Measurement of Urinary MCP-1

Urine MCP-1 was measured using the Human MCP-1 Quantikine ELISA Kit by R&D Systems. Briefly, monoclonal antibody specific for human MCP-1 had been pre-coated onto a microplate. Standards, controls and samples were prepared, and 200 uL of each was pipetted into the wells in duplicate. The microplate was covered and left to incubate on a microplate shaker at room temperature for two hours. MCP-1 present in the sample bound the immobilized antibody. After washing away any unbound

substances three times, an enzyme-linked polyclonal antibody specific for human MCP-1 was added to the wells and incubated for one hour. Following three washes a substrate solution was added to the wells and incubated for 30 minutes protected from light. Colour development was stopped, and the intensity of the colour was measured using a spectrophotometer to determine the optical density of each well at 450 nm, with wavelength correction at 540 nm.

Measurement of Urinary TWEAK

Urine TWEAK was measured using the TWEAK Human Instant ELISA Kit by eBioscience (Thermo Fisher Scientific). Briefly, an anti-human TWEAK antibody had been pre-coated onto a microplate, with a number of the reagents already present in the wells in a lyophilised pellet. The appropriate volume of distilled water was first added to each well, followed by 50 μ L of standard, control or sample, in duplicate. The microplate was covered and incubated on a microplate shaker at room temperature for 3 hours, followed by six washes. Substrate solution was then added, and the colour development was monitored using the plate reader set to 620 nm. The substrate reaction was stopped as soon as Standard 1 reached an OD of 0.9–0.95. The absorbance of each microwell was measured at 450 nm as the primary wavelength and wavelength correction at 630 nm.

Determining the Concentration of uMCP-1 and uTWEAK

Colour development in both ELISAs occur in proportion to the concentration of analyte in the sample. The standard curves prepared during each run were used to determine the concentration of the other samples and were derived from an average of the duplicates performed. Samples with analyte concentrations above the measuring range of the kit were repeated in dilution to obtain an absolute value in picograms per millilitre (pg/mL). Both MCP-1 and TWEAK results were also mathematically standardised to the urine creatinine concentration present in the respective sample to account for variations in urine concentration, as per convention. These results were denoted “corrected” and expressed as picograms per milligram of creatinine (pg/mgCr). In this study we provide the results for both absolute and corrected concentrations of the respective biomarkers.

Definition of remission status

Complete remission was defined as return of serum creatinine to previous baseline, plus a decline in the UPCR to < 0.05 g/mmol. Partial remission was defined as stabilization ($\pm 25\%$), or improvement of serum creatinine, but not to normal, plus a $\geq 50\%$ decrease in UPCR from start of induction therapy. Alternatively, if there was nephrotic-range proteinuria then remission required a $\geq 50\%$ reduction in UPCR, and a UPCR < 0.300 g/mmol. Patients not meeting the above criteria were considered to not be in remission.[22]

Statistical Analysis

Statistical analysis was undertaken using Stata 15.1 (Stata Corp. Texas, USA). Data was assessed for normality using the Shapiro-Wilk test. Continuous variables were reported as means $\pm$ standard deviation if normally distributed and median (with interquartile ranges) if not normally distributed. Categorical variables were reported as frequencies (or percentages). The Spearman rank correlation was used to

determine the degree of linear relationship between continuous variables. The Student t-test or its non-parametric equivalent, the Wilcoxon rank-sum test was used to compare means between two groups. The Wilcoxon sign rank test was used for comparing the pre and post treatment values of the corrected uMCP1 and uTWEAK levels. A p-value < 0.05 was interpreted as statistically significant.

Results

Demographic, clinical and biochemical features at baseline

Only 20 patients with active LN were willing to give consent to be followed up and were enrolled into the study. Most of the patients (70%) were females and the mean age at biopsy was 29.8 ± 10.7 years. There were 12 (60%) patients of mixed ancestry, while 7 (35%) were black Africans and 1 (5%) Caucasian (Table 1). There were 70% with proliferative LN (class III, IV, III + V and IV + V), and the activity and chronicity indexes were 4 (inter-quartile range [IQR]: 3–9) and 2 (1–4) respectively. The median and IQR for systolic and diastolic blood pressure were 132.5 mmHg (120.5–140.0) mmHg and 75.0 mmHg (70.0–87.0) mmHg, respectively. The median serum creatinine was 104 (56.5–153.5) $\mu\text{mol/L}$ with an estimated glomerular filtration of 64.7 (41.6–118.8) ml/min/1.73 m^2 and UPCR was 0.37 (0.18–0.59) g/mmol . Corrected uMCP-1 and uTWEAK median values were 1093.0 (576.5–2013.5) pg/mgCr and 159.0 (88.5–295.5) pg/mgCr , respectively. Treatment approach was mainly with a combination of high dose steroids and monthly pulse intravenous cyclophosphamide (55%) (Table 1).

Table 1
Demographic, clinical and biochemical features at baseline

Variable	Median (n = 20)
Age, years	29.8 ± 10.7 [‡]
Gender, female (%)	70
Ethnicity (%)	
o Black	35
o Coloured	60
o White	5
Body mass index, kg/m ²	22.5 (20.7–25.6)
Systolic blood pressure, mmHg	132.5 (120.5–140.0)
Diastolic blood pressure, mmHg	75.0 (70.0–87.0)
SLEDAI	17 (13–26)
SLICC	1.5 (1–2)
Biopsy features (%)	
o Proliferative	70
o Non-proliferative	30
o Activity index	4 (3–9)
o Chronicity index	2 (1–4)
o Interstitial Fibrosis	50 ^ψ
Treatment (%)	
o CS	25
o CS + CYC	55
o CS + MMF	20
Haemoglobin, g/dL	9.25 (8.15–10.0)

Key: [‡] mean, SLEDAI: Systemic lupus erythematosus disease activity index, SLICC: CS: corticosteroids
CYC: cyclophosphamide, MMF: mycophenolate mofetil, eGFR: estimated glomerular filtration rate,
UPCR: urine protein creatinine ratio, MCP-1: monocyte chemoattractant- 1, TWEAK: tumor necrosis
factor-like weak inducer of apoptosis

Variable	Median (n = 20)
Platelets, x 10 ⁹ /L	228.0 (148.5–278)
Serum creatinine, umol/L	104 (56.5–153.5)
Estimate glomerular filtration rate, mL/min/1.73 m ²	64.7 (41.6–118.8)
UPCR, g/mmol creatinine	0.37 (0.18–0.59)
C3, g/L	0.47 (0.34–0.68)
C4, g/L	0.09 (0.05–0.14)
Anti-dsDNA Antibodies, IU/ml	247.5 (66.5–379)
Anti-sm Antibodies, U/ml	1.7 (0.6–42.7)
Urine biomarkers	
o Urine MCP-1, pg/mL	1440.1 (683.5–2729.5)
o Corrected Urine MCP-1, pg/mg creatinine	1092.7 (578.6–1884.0)
o Urine TWEAK, pg/mL	209.0 (117.0–312.0)
o Corrected Urine TWEAK, pg/mg creatinine	159.0 (88.5–295.5)
Key: ¯ mean, SLEDAI: Systemic lupus erythematosus disease activity index, SLICC: CS: corticosteroids CYC: cyclophosphamide, MMF: mycophenolate mofetil, eGFR: estimated glomerular filtration rate, UPCR: urine protein creatinine ratio, MCP-1: monocyte chemoattractant- 1, TWEAK: tumor necrosis factor-like weak inducer of apoptosis	

Correlation of uMCP-1 and uTWEAK with clinical and histologic features

Table 2 provides a summary of the correlations between levels of uMCP-1 and uTWEAK with renal histological features. Urinary MCP-1 and uTWEAK did not correlate with biopsy activity index ($\rho = 0.05$, $p = 0.85$ and $\rho = 0.13$, $p = 0.61$, respectively). Furthermore, there was no correlation with chronicity index ($\rho = 0.32$, $p = 0.19$ for uMCP-1, and $\rho = 0.28$, $p = 0.28$ for uTWEAK) and both biomarkers did not correlate with presence of interstitial fibrosis on biopsy. Clinical and biochemical features, including serum C3 and C4, anti-dsDNA as well as SLEDAI and SLICC scores did not correlate with levels of both biomarkers (Figs. 1 and 2).

Table 2
Correlation of U-MCP-1 and U-TWEAK with histologic features

Histologic feature	Corrected uMCP-1, pg/mg creatinine		Corrected uTWEAK, pg/mg creatinine	
	rho	p - value	rho	p - value
Activity index	0.05	0.85	0.13	0.61
Chronicity index	0.32	0.19	0.28	0.28
Fibrosis	-	0.29	0.20	0.44
Proliferative LN	1395.4 (531.3–2162.3)	0.41	159.0 (100.0–277.0)	0.87
Non-proliferative LN	882.1 (621.1–1348.0)		190.5 (75.0–301.0)	

Comparison of baseline and post-induction clinical and biochemical parameters

At the 6-month follow-up, there was a significant decline in the SLEDAI-2K score, UPCR and anti-dsDNA (p value = 0.002, p = 0.006 and p < 0.001, respectively) while, C3 and C4, haemoglobin and platelets improved (Table 3). Overall, the reduction in level of uMCP-1 at 6 months (from baseline) was not significantly different (p = 0.06); however, the reduction in level of uTWEAK was significant (p = 0.03) (Table 3).

Table 3
Comparison of baseline and post-induction clinical and biochemical parameters

Variable	Visit 1 (Month 0)	Visit 2 (Month 6)	p – value
SLEDAI	17 (13–26)	4 (0–10)	0.002
rSLEDAI	8 (8;12)	4 (0;4)	
UPCR, g/mmol creatinine	0.37 (0.12–0.86)	0.06 (0.001–0.52)	< 0.001
Complement C3, g/L	0.465 (0.33–0.68)	1.00 (0.80–1.18)	0.001
Complement C4, g/L	0.09 (0.04–0.14)	0.21 (0.14–0.23)	0.001
Anti-Sm antibodies, U/ml	3.3 (0.9–42.7)	1.6 (0.7–9.5)	
Anti- Double stranded Antibodies, IU/ml	247.5 (66.5–379)	23 (12.0–109)	0.006
Haemoglobin, g/dL	9.25 (8.15–10.0)	11.4 (11–12.1)	0.002
Platelets, x 10 ⁹ /L	228 (148.5–278.0)	276.0 (241.0–314.0)	0.006
Serum Creatinine, umol/L	104.0 (39.0–1104.0)	90.5 (51–192)	0.272
eGFR, mL/min/1.73 m ²	64.7 (41.6–118.8)	92.3 (53.6–114.5)	0.04
Urinary MCP-1, pg/mg creatinine			
• All	1092.7 (578.6–1848.0)	314.5 (197.0–622.0)	0.06
• CR/PR	1012.5 (529–2013.5)	298 (226–351)	0.018
• NR	594 (453–735)	1845.5 (727–2964)	0.654
Urinary TWEAK, pg/mg creatinine			
• All	159.0 (88.5–295.5)	36.0 (17.0–88.0)	0.03
• CR/PR	142 (88.5–297)	36 (12.5–76.5)	0.015
• NR	126 (18–234)	166.5 (162–171)	0.65

Remission status at the end of induction phase of treatment

Following induction therapy, 85% of patients attained partial or complete remission. For patients achieving early remission, the median values for uMCP-1 decreased from 1012.5(529–2013.5) pg/mgCr at baseline, to 298 [226–351] pg/mgCr at 6 months, ($p = 0.0186$). For patients not achieving remission, there was no reduction in the level of uMCP-1 from baseline (594[453–735]) pg/mgCr to 6 months (1846 [729–2964]) pg/mgCr, ($p = 0.654$). Similarly, uTWEAK values reduced from 142 (88.5–297) pg/mgCr to 36 (12.5–76.5) pg/mgCr ($p = 0.015$) in patients who reached remission while the values were not

significantly changed over the same period of time for patients not achieving remission (126 [18–234] pg/mgCr at baseline and 166.5 [162–171] pg/mgCr ($p = 0.65$) at 6 months) (Table 3).

Discussion

Diagnosis and assessment of flares as well as treatment response in LN remains an area of unmet need in patients with LN.[8] Kidney biopsy is the gold standard for diagnosis and assessment of LN flares to guide treatment despite its invasiveness and associated complications.[6, 7] Furthermore, kidney biopsy as a diagnostic test is not readily available in all centres, especially in developing countries[5] and some conventional clinical and laboratory parameters used for making decisions on timing of kidney biopsy may lack specificity and sensitivity.[23] There is therefore need to use more accurate and less invasive techniques other than kidney biopsy, serum creatinine, complements, urine protein and sediment in assessing disease activity and treatment response in LN. Although some studies have shown the usefulness of urinary biomarkers in diagnosis of disease activity in patients with LN,[11, 24, 25] we are unaware of any studies from Africa and whether these biomarkers could be useful in our population.

The main finding of this study is the correlation between both biomarkers and disease activity in patients with LN in our population. This suggests that these biomarkers could be useful for monitoring disease activity in patients with LN and for assessing response to induction treatment to determine if use of immunosuppression should be continued, tapered or stopped. It also suggests that these biomarkers can be useful for disease stratification and prognosis in patients. This is of relevance in resource poor countries where access to kidney biopsy is often unavailable. As kidney biopsy remains the most reliable way of “nailing down” the diagnosis of LN, easily available and non-invasive methods that are equally reliable needs to be sourced for such populations.

The pathogenesis of LN is known to involve a complex process, leading to glomerular autoantibody deposition, activation of complement and macrophages and production of pro-inflammatory cytokines and chemokines.[26] Several studies have assessed for non-invasive ways of making a diagnosis or predicting increased disease activity in patients with LN.[11, 25, 27, 28] Most of these studies have relied on the activated inflammatory milieu in the kidney during an active state of the disease for production of cytokines or increased loss of epithelial cells into the urine. TWEAK belongs to the TNF superfamily of structurally related cytokines and its gene is expressed in multiple kidney cells including mesangial, glomerular and tubular cells of the kidney.[15] During active inflammation, TWEAK binds to its receptor Fn14 (fibroblast growth factor-inducible 14) on cell surfaces signalling through the NF- κ B pathway to stimulate a wide array of other cytokines, chemokines and cell adhesion molecules including MCP-1.[29] This explains the rise in both uTWEAK and uMCP-1 during intra-renal inflammation.

Observations of increased levels of uTWEAK and uMCP-1 have been documented in other studies with some showing correlation with histological features on the kidney biopsy. One study from China reported a higher concentration of both biomarkers in the urine of patients with active LN compared to those with non-active disease ($P < 0.01$) and also showed significant correlation between both biomarkers and

kidney biopsy activity index ($P < 0.01$) but not with chronicity index of the biopsies ($P > 0.05$) [11] Similar findings have been reported by other studies. [12]

Although our study did not demonstrate correlation between the biomarkers and renal activity scores, we think this may be related to the low sample size of our study given that other clinical and biochemical markers of activity were elevated at baseline and declined following treatment. This could suggest that these biomarkers can be useful for monitoring renal disease flares which are more aggressive and often more frequent in non-Caucasian populations.[30] The usefulness of these biomarkers for monitoring flare of LN have been demonstrated in some studies [31, 32] while others have also shown their benefit with monitoring response to treatment.[25] A study has suggested that combining both uMCP-1 and uTWEAK might be useful as potential predictors for proteinuria in LN.[33] In a prospective multicentre study, uTWEAK was used, at month 3 of induction therapy, to predict complete response to treatment with a sensitivity of 70% and specificity of 63%.[25] When they combined uTWEAK and level of urine protein excretion at month 3, this significantly improved the predictive performance for complete response at 6 months (ROC-AUC 0.83, $p < 0.001$).[25]

The strength of this study is the ability to demonstrate that non-invasive methods can be used to detect disease activity in patients with LN in an African setting. If this can be further developed for routine clinical use, it will be of benefit in determining the treatment needs and prognosis of patients with LN. Limitations of our study include the size of our sample as well as our study design in which a repeat biopsy was not performed to clearly demonstrate reduced inflammation / activity of disease in the kidney. A repeat kidney biopsy could not be done due to the invasiveness of the procedure in the face of reducing amounts of daily urine protein excretion. Also, our study was not designed to assess the specificity and sensitivity of these biomarkers in predicting disease activity or treatment response which could be useful in making a preferred choice between both. Despite these limitations, we believe our study results demonstrate the usefulness of using non-invasive techniques in evaluating patients with LN in resource limited countries.

Conclusion

Our study has shown that uMCP-1 and uTWEAK are significantly elevated in South African patients with active LN and correlated with the remission status at the end of induction phase of treatment. These findings support their use for disease activity and treatment response monitoring.

Declarations

Ethics approval and consent to participate:

Ethics approval was obtained from the University of Cape Town Human Research Ethics Committee (HREC #332/2017). All participating patients provided signed consent form.

Consent for publication

Not Applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

MWM, JAR, FO, DJ and IGO conceived the study. MWM, JAR, CD and IGO were involved in data collection, MWM, JAR, FO, UE, and IGO were involved in data analysis, writing up and interpretation, all authors were involved in revision of the manuscript for intellectual content. All authors approved the final version of the manuscript.

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Authors' information (optional)

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Figures

Figure 1

Correlation of urinary MCP-1 with biochemical parameters and disease activity and chronicity scores.

Figure 2

Correlation of urinary TWEAK with biochemical parameters and disease activity and chronicity scores.