CORRELATION OF URINARY MCP-1 AND TWEAK WITH RENAL HISTOLOGY AND EARLY RESPONSE TO THERAPY IN NEWLY BIOPSIED PATIENTS WITH LUPUS NEPHRITIS IN CAPE TOWN, SOUTH AFRICA

By

Mothusi Walter Moloi

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Supervisor: **Prof. Ikechi Okpechi**

Division Nephrology and Hypertension

Groote Schuur Hospital and

Department of Medicine

University of Cape Town

Co-investigator: **Dr. Jody Alan Rusch**

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DECLARATION

I, MOTHUSI WALTER MOLOI, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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- My siblings for their constant encouragement.

TABLES AND FIGURES

LIST OF ABBREVIATIONS

ANA	Autoimmune nuclear antibodies
Anti-dsDNA	Anti- double stranded deoxynucleic acid
BMC	Biomedical Central
C3	Complement C3
C4	Complement C4
CKD	Chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
cMCP-1	Corrected monocyte chemoattractant protein-1
cTWEAK	Corrected tumor necrosis factor-like weak inducer of apoptosis

CYC	cyclophosphamide
DNA	Deoxynucleic acid
ELISA	Enzyme-linked immunosorbent assay
GSH	Groote Schuur Hospital
HREC	Human Research Ethics Committee
IC	Immune complexes
IQR	Interquartile range
LN	Lupus nephritis
MCP-1	Monocyte chemoattractant protein-1
MMF	Mycophenolate mofetil
SD	Standard deviation
SLE	Systemic lupus erythematosus
SLEDAI	Systemic lupus erythematosus (SLE) Disease Activity Index
SLICC	Systemic Lupus International Collaborating Clinics
TWEAK	Tumor necrosis factor-like weak inducer of apoptosis
UCT	University of Cape Town
U-MCP-1	Urinary monocyte chemoattractant protein-1
UPCR	Urine protein creatinine ratio
U-TWEAK	Urinary tumor necrosis factor-like weak inducer of apoptosis

ABSTRACT

Background:

There is need for judicious use of immunosuppression in patients with active lupus nephritis (LN), however this is guided by renal biopsy which is invasive and not freely available in most centres. Novel urinary biomarkers such as monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-like weak inducer of apoptosis (TWEAK) are secreted in the kidney and may be useful for predicting histological class, monitoring flares and assessing response to therapy. We assessed the utility of urinary MCP-1 (uMCP-1) and TWEAK (uTWEAK) in predicting renal histological findings, disease flares and treatment response 6 months following initiation of treatment for LN in newly biopsied patients.

Methods:

We recruited consenting patients with active LN confirmed on kidney biopsy. Relevant baseline demographic, biochemical and histological information was collected from the patients. ELISA methods were used to assess uMCP-1 and uTWEAK at baseline and at 6 months after completion of induction therapy.

Results:

There were 14 females and 6 male patients with a mean age of 29.8 ± 10.7 years, 60% were of mixed ancestry, 70% had proliferative LN. There was no association between uMCP-1 and uTWEAK and histological features (LN class, activity index, chronicity index and interstitial fibrosis). At 6 months, 6 patients were lost to follow-up and of the remaining 14, 12 (85%) attained remission (partial remission (n = 7) or complete remission (n = 5)). Both biomarkers were elevated in patients with active disease and significantly declined amongst those attaining

remission, p = 0.018 and p = 0.015 respectively. However, for those not attaining remission, no association was found for both biomarkers (p >0.05).

Conclusion:

Our study did not show correlation between uMCP-1 and uTWEAK with histological features of LN. However, both biomarkers were elevated in patients with active disease and correlated with the remission status at the end of induction phase of treatment.

Keywords:

Lupus nephritis, urinary biomarkers, activity index, chronicity index, monocyte chemoattractant protein-1, tumour necrosis factor-like weak inducer of apoptosis

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CHAPTER ONE: PUBLICATION-READY MANUSCRIPT

CHAPTER 1: INTRODUCTION

1.1 CONTEXT

1.1.1 Background

Systemic lupus erythematous (SLE) is an archetypical multisystemic autoimmune disorder affecting potentially all body organs and characterised by episodes of flares and remission.(1) The disease has a wide geographical variation in incidence and prevalence, highest in North America and lowest in Africa. It affects mainly females irrespective of the population studied and commonest in those of African and Asian ancestry relative to Caucasians.(2) The disease manifests due to loss of self-tolerance to nuclear autoantigens leading to production of autoantibodies, activation of autoreactive T cells, and secretion of cytokines all culminating in complement activation and organ dysfunction(3,4). The exact cause of SLE remains unknown, however it is postulated that genetic, hormonal and environmental factors are central to the etiopathogenesis of the disease.(5) Lupus nephritis (LN) is the biggest contributor to morbidity and mortality in patients with SLE.

The kidney is affected in up to 60% of patients with SLE. In a Cape Town study, the prevalence of biopsy proven lupus nephritis was 39% amongst patients with glomerulopathies and renal survival rates at five years were lower at 63% relative to 98% in the Chinese study.(6,7) These findings agree with data from North America showing poorer prognosis amongst people of African ancestry with LN(8). Genome-wide association studies have highlighted the role of genetic variants predisposing patients to development of significant renal damage during the systemic autoimmune state of SLE.(9) Contrary to the simplistic traditional believe that renal disease is triggered by deposition of circulating immune complexes (IC) in the kidney, IC are now thought to also form in situ in response to intrarenal autoantigens either in the mesangial, endothelial cells and/ or glomerular basement membrane. Once formed, the IC activate the complement cascade leading to damage of adjacent cells.(10) Renal

manifestation and disease prognosis are primarily determined by the primary structures affected within the kidney and this has informed the disease classification.

In 2003, the International Society of Nephrology together and the Renal Pathology Society reclassified lupus nephritis into six classes based on the kidney biopsy findings.(11) In this classification, class I and II reflect mesangial disease with low risk of progression to chronic kidney disease (CKD), classes III and IV reflect endothelial-proliferative disease with higher risk of progression to CKD while class V disease involves mainly the glomerular basement membrane and class VI signify advanced kidney disease. These variations in disease severity and prognosis within the different LN classes thus warrants different treatment approaches for individual cases. Despite recent advances in diagnosis and treatment of LN, up to 25% of patients develop end stage kidney failure after 10 years of diagnosis.[6]

The gold standard for diagnosis of LN remains renal biopsy which unfortunately is not readily available in most centres where the disease burden is high. The procedure is invasive and has complications which include death in 0.02%-0.1% of patients.(12) Clinical parameters like creatinine clearance, proteinuria, urine sediments, anti-double stranded deoxyribonucleic acid antibodies and serum complements remain cornerstone for diagnosis and evaluation of remission in most centres. However, lack the sensitivity and specificity to aid diagnosis, monitor of disease activity and remission. The development of biomarkers that can correlate with disease activity and treatment response will be of great value in aiding early diagnosis and monitoring of treatment response especially in resource poor setting where renal biopsies are not easily available.

A biomarker refers to a biologic, biochemical, or molecular event that can be assayed qualitatively and quantitatively by laboratory techniques. The levels of biomarkers should correlate with disease pathogenesis or activity in different organ systems. An ideal biomarker for lupus nephritis should possess the following properties: (i)good correlation with renal activity as reflected by the degree of proteinuria and urine sediments, (ii)sensitive to change so that it can be used for serial monitoring of disease activity in the kidneys and defining treatment response and clinical remission, (iii) ability to

predict renal activity/flares before an obvious change in conventional clinical parameters occurs so that early treatment/preventive strategies can be considered, (iv) specific to nephritis among patients with SLE, and (v) specific to SLE for aiding early diagnosis of lupus nephritis. In addition, a useful biomarker should be easy to assay, simple to interpret, and readily available in most laboratories with a reasonable cost.(13) Traditionally biomarkers have been measured in serum, however, urine has since been identified as desirable biospecimen since it can be collected recurrently and non-invasively.(14)

The use of urine as a biomarker offers many advantages which include: non-invasive nature of its collection, volumes of collection, stable protein content of urine and solubility of urinary peptides making them easy to analyse. Of the total protein filtered in urine in good health, 70% originates in the urinary track while only 30% is filtered from plasma thus making urine ideal for biomarker study. The presence of biomarkers in urine may thus reflect inflammation within the kidney or filtration from blood into urine(15). This led to the development of variable biomarkers for difference cancers and many studies are ongoing on biomarkers for different diseases affecting the kidney like LN.

A study by Wolf et al on the utility of various urine biomarkers to determine their ability to predict treatment response identified chemokines, cytokines, and markers of cellular damage to be predictive.(16) El-shehaby et al also studied the role of urinary biomarkers in patients with SLE and reported a positive correlation amongst those with renal involvement and urinary TNF-like weak inducer of apoptosis (TWEAK), osteoprotegerin (OPG) and monocyte chemoattractant protein-1 (MCP-1) with reasonable sensitivity, specificity, and predictive values to detect lupus nephritis.(17)

1.1.2 Urinary monocyte chemoattractant protein-1 (uMCP-1)

MCP-1 is a β-chemokine responsible for monocyte and T-lymphocyte recruitment during the acute and chronic phases of inflammation. It is produced by renal mesangial, endothelial, tubular, and monocytic cells in response to the deposition of immune complexes and inflammatory mediators in

the kidney. It is involved in mediating inflammation and injury in LN by playing an important role in the recruitment of monocytes and lymphocytes and enhancement of endothelial and leucocyte adhesiveness.(18) This chemokine is increased in active LN but is not an independent predictor for LN activity and has therefore been proposed to have utility as an adjunctive marker when the clinical diagnosis of LN especially early relapse remains uncertain.(19) A study by Alharazy et al prospectively looked at levels of uMCP-1 and found levels to be significantly higher in those with active disease especially during disease flares in patients with LN relative to those with inactive disease.

Urine MCP-1 level also correlated with proteinuria and renal SLE disease activity index-2K(20), however, it was not an independent predictor of disease activity.(19) The results on renal histopathological correlation have been conflicting. Taha et al in an Egyptian study of 22 active and 20 inactive patients with LN found a positive correlation between uMCP-1 and activity index on biopsy and no correlation with histological class nor chronicity index. Another study however by Torabinejad et al in India reported a positive correlation with activity index and histological class of lupus. Nonetheless, studies agree on the lack of correlation of this biomarker with chronicity index on biopsy suggesting lack of utility of this biomarker in assessing chronic renal damage.(21,22)

1.1.3 Urinary tumor necrosis factor-like weak inducer of apoptosis (uTWEAK)

The TNF-related weak inducer of apoptosis (TWEAK) is a pro-inflammatory cytokine produced mainly by innate immune cells, such as monocytes, dendritic cells and natural killer cells. Within the kidneys, sources of TWEAK include infiltrating monocytes and T lymphocytes, tubular epithelial cells and mesangial cells where it fine tunes renal response to injury.(23) At physiological state, TWEAK is postulated to facilitate tissue repair and regeneration following acute kidney injury, but in the setting of chronic inflammation, this process is dysregulated with expression of pathogenic TWEAK thus promoting inflammation and cell death.(24) Stimulation of mesangial cells and podocytes by TWEAK induces a potent proinflammatory response. It also leads to enhanced vascular permeability leading to increased extravasation of IgG and subsequent glomerular IgG culminating in increased immune

complex mediated activation of effector cells.(25) A study by Xuejing et al in China demonstrated that the urinary levels of TWEAK correlate with activity indexes of LN but not chronicity index making this cytokine a potential biomarker in this patient population.(26)

1.1.4 Hypothesis

Urinary biomarkers (uMCP -1 and uTWEAK) will be elevated in newly biopsied patients with active LN and those with disease flares and significantly correlate with kidney biopsy findings and remission status.

1.1.5 Rationale of the Research

There is need for judicious use of immunosuppression in patients with active lupus nephritis (LN) and this is guided by how well patients respond to treatment. Currently, treatment response is monitored using markers such as urine protein-creatinine ratio (UPCR) and serum creatinine which are not accurate in assessing inflammation within the kidney. Novel biomarkers such as uMCP-1 and uTWEAK which are secreted within the kidney in the setting of inflammation may be more useful in diagnosing and monitoring disease activity within the kidneys. We assessed the utility of uMCP-1 and uTWEAT at diagnosis and 6 months following initial treatment of LN in newly biopsied patients. Our specific objectives were:

1. To assess correlation between histology of lupus nephritis (activity index, chronicity index and histological class) and levels of uMCP-1 and uTWEAK at biopsy.

2. To identify if levels of uMCP-1 and uTWEAK at time of biopsy can be used as a surrogate for early responders to therapy at the end of 6 months of induction therapy

1.1.6 Research Setting

This study was carried out at the Department of Medicine, Division of Nephrology and Hypertension, Groote Schuur Hospital (GSH), Cape Town, South Africa. GSH is government-funded, adult, provincial

academic tertiary hospital that serve the central health district of the Cape Town Metro region. The catchment population of the facility encompasses a diverse socioeconomic and cultural group. This was a prospective observational study of consecutive participants with active biopsy proven lupus nephritis diagnosed at GSH and followed up at the same hospital.

1.2 ETHICAL CONSIDERATIONS

The ethical issues taken into consideration in this study relate to collection of blood and urine samples, gaining access to protected participants' information contained within medical records, and the maintenance of confidentiality of such information obtained for the conduct of this study. The main study had already received ethical approval from the Human Research Ethics Committee (HREC) of the University of Cape Town (HREC Ref # 402/2014) and this sub-study also received the ethical approval from HREC of UCT (HREC Ref: 332/2017).

1.3 AUTHOR GUIDELINES FOR THE NEPHROLOGY DIALYSIS TRANSPLANTATION JOURNAL

Author guidelines for article submission to the Biomedical Central (BMC) Nephrology journal (in their exact words) are attached in appendix 1.

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CHAPTER TWO: PUBLICATION-READY MANUSCRIPT

Title:

Correlation of Urinary MCP-1 and TWEAK with renal histology and early response to therapy in newly biopsied patients with lupus nephritis in Cape Town, South Africa

Mothusi Walter Moloi[1, 2], Jody A. Rusch[3], Fierdoz Omar[3], King J[3]*, Udeme Ekrikpo[1,2,4], Collet Dandara[5] Ikechi G. Okpechi[1,2]

[1]Division of Nephrology and Hypertension University of Cape Town, Cape Town, South Africa

[2]Kidney and Hypertension Research Unit, University of Cape Town Cape Town, South Africa

[3]Division of Chemical Pathology, University of Cape Town and Groote Schuur Hospital, National Health Laboratory Services, Cape Town, South Africa

[4]Department of Medicine, University of Uyo, Uyo, Nigeria

[5] Division of Human Genetics, University of Cape Town, South Africa.

*(Deceased)

Abstract

Background:

There is need for judicious use of immunosuppression in patients with active lupus nephritis (LN), however this is guided by renal biopsy which is invasive and not freely available in most centres. Novel urinary biomarkers such as monocyte chemoattractant protein-1 (MCP-1) and tumour necrosis factor-like weak inducer of apoptosis (TWEAK) are secreted in the kidney and may be useful for predicting histological class, monitoring flares and assessing response to therapy. We assessed the utility of urinary MCP-1 (uMCP-1) and TWEAK (uTWEAK) in predicting renal histological findings, disease flares and treatment response 6 months following treatment for LN in newly biopsied patients.

Methods:

We recruited consenting patients with active LN confirmed on kidney biopsy. Relevant baseline demographic, biochemical and histological information was collected from the patients. ELISA methods were used to assess uMCP-1 and uTWEAK at baseline and at 6 months after completion of induction therapy.

Results:

There were 14 females and 6 male patients with a mean age of 29.8 ± 10.7 years, 60% were of mixed ancestry, 70% had proliferative LN. There was no association between uMCP-1 and uTWEAK with histological features. Patients lost to follow-up were 6 and 14 had 6 month follow-up, 12 (85%) attained remission (partial remission (n = 7) or complete remission (n = 5)). Urinary MCP-1 values at baseline and 6 months were 1012.5 (529 - 2013.5) pg/mgCr and 298 (226 - 351) pg/mgCr (p = 0.0186) for those attaining remission, 594 (453 - 735) pg/mgCr and 1846 (729 - 2964) pg/mgCr (p = 0.654) for non-remitters, respectively. In addition,

uTWEAK values at baseline and 6 months we 142 (88.5 - 297) pg/mgCr and 36 (12.5 – 76.5) pg/mgCr (p = 0.015) for those attaining remission, 126 (18 - 234) pg/mgCr and 166.5 (162 - 171) pg/mgCr (p = 0.65) for non-remitters.

Conclusion:

Our study did not show correlation between uMCP-1 and uTWEAK with histological features of LN. However, both biomarkers were elevated in patients with active disease and correlated with the remission status at the end of induction phase of treatment.

Keywords:

Lupus nephritis, urinary biomarkers, activity index, chronicity index, monocyte chemoattractant protein-1, tumour necrosis factor-like weak inducer of apoptosis

(WORD COUNT – 338)

Background

Lupus nephritis (LN) complicates up to 60% of cases of systemic lupus erythematosus and is associated with increased morbidity and mortality.(1) There is need for judicious use of immunosuppression in patients with active LN to halt renal damage and improve outcomes. This may be aided by improved diagnosis and evaluation of renal flares in this population. Renal biopsy remains the gold standard method for making a diagnosis, assessing flares and guiding treatment in LN.(2) The decision to biopsy is often directed by the presence of active urinary sediments, elevated serum creatinine, low serum complement levels and raised autoimmune antibody levels.(3) Renal biopsy carries with it complications such as haemorrhage or in some instances death and is not readily available in many places where the disease burden is high.(4,5) Markers such as urine protein-to-creatinine ratio (UPCR), serum creatinine and complement (C3 and C4) are not reliable in assessing lupus disease activity as they lack sensitivity and specificity.(6)

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 in predicting renal biopsy findings and disease flares, monitoring disease activity, and assessing response to therapy because they are secreted within the kidney.(7–9)

MCP-1 is a chemokine responsible for monocyte and T-lymphocyte recruitment during the acute and chronic phases of inflammation. It is produced by renal mesangial, endothelial, tubular and monocytic cells in response to the deposition of immune complexes and inflammatory mediators in the kidney. Recent showed that is increased in active LN but not to be an independent predictor of activity.(10) It has therefore been proposed that MCP-1

may have utility as an adjunctive marker when the clinical diagnosis of active LN, especially early relapse, remains uncertain.(11)

TWEAK is a pro-inflammatory cytokine produced mainly by innate immune cells, such as monocytes, dendritic cells and natural killer cells. The urinary levels of TWEAK have been found to correlate with activity index of LN making this cytokine a potential biomarker in the SLE patient population.(7)

In this study we assessed the association of uMCP-1 and uTWEAK with renal histological findings (biopsy activity index, biopsy chronicity index, fibrosis and class of LN) and response to treatment in newly biopsied South African patients.

Methods

Study population and study design

This was a prospective observational study conducted at the renal clinic at Groote Schuur Hospital in Cape Town. We enrolled consenting adult patients (≥ 18 years) diagnosed with biopsy proven active LN. Patients received induction treatment using the National Health Institute (NIH) protocol of monthly pulsed cyclophosphamide or mycophenolate mofetil protocols for 6 months.(12) 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(13) to assess disease activity and the systemic lupus collaborating clinics / American college of

rheumatology damage index (SLICC/ACR Damage Index)(14) to assess irreversible impairment. Standard of care blood tests were performed at the hospital's accredited laboratory using standard methods and included a full blood count (FBC), creatinine, anti-nuclear antibodies (ANA), anti-Smith antibodies (anti-Sm), anti-double stranded deoxyribonucleic acid antibodies (anti-dsDNA) and complement (C3 and C4) at each visit. Estimation of glomerular filtration rate was calculated using the CKD-EPI equation.(15) 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 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.(16) Criteria proposed by Hill et al in 2000 was used to assess biopsy activity index (BAI), biopsy chronicity index (BCI) and interstitial fibrosis.(17) Proliferative LN was defined as histological classes III and IV and interstitial fibrosis was reported as present, irrespective of degree, if reported by the pathologist.

Measurement of Urinary MCP-1

MCP-1 was measured using the Human MCP-1 Quantikine ELISA Kit manufactured by R&D Systems, Minneapolis, United States of America. 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

TWEAK was measured using the TWEAK Human Instant ELISA Kit by eBioscience (Thermo Fisher Scientific), Vienna, Austria. 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 uL 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 optical density of 0.9 – 0.95. The absorbance of each microwell was measured at 450 nm as the primary wave length and wavelength correction at 630 nm.

Determining the Concentration of MCP-1 and TWEAK

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. These results were denoted “corrected” and expressed as picograms per milligram of creatinine (pg/mg Cr). In this study we report both absolute and corrected concentrations of the respective biomarkers and their utility.

Definition of remission status

Complete response in LN was defined as return of serum creatinine to previous baseline, plus a decline in the UPCR to <0.05 g/mmol. Partial response was defined as stabilization (±25%), or improvement of serum creatinine, but not to normal, plus a ≥50% decrease in UPCR. Alternatively, if there was nephrotic-range proteinuria then remission required a ≥50% reduction in UPCR, and a UPCR <0.300 g/mmol. Patients not meeting the above criteria were considered to have not attained remission.(18)

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 ± 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

Of the 20 participants enrolled with active lupus nephritis, 11 were new cases of LN with first time biopsy while 9 had pre-existing LN and biopsied for a disease flare, 14 (70%) were females and the overall mean age 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 (IQR 3 - 9) and 2 (IQR 1 - 4) respectively. The median and interquartile ranges for systolic and diastolic blood pressure were 132.5 mmHg (IQR 120.5 - 140.0) mmHg and 75.0 mmHg (70.0 - 87.0) mmHg, respectively. The median serum creatinine was 104 (IQR 56.5 – 153.5)umol/L with an estimated glomerular filtration of 64.7 (IQR 41.6 - 118.8) ml/min/1.73m^2 and UPCR was 0.37 (IQR 0.18 – 0.59) g/mmol. Corrected uMCP-1 and uTWEAK median values were 1093.0 (IQR 576.5 - 2013.5) pg/mgCr and 159.0 (IQR 88.5 - 295.5) pg/mgCr, respectively. A combination of steroids and cyclophosphamide were the main induction agents utilised (55%) (Table 1).

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

Urinary MCP-1 and TWEAK did not correlate with biopsy activity index (rho = 0.05, p = 0.85 and rho = 0.13, p = 0.61, respectively) (Table 2). 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 (Table 2). Biochemical results including C3 and C4, anti-dsDNA as well as SLEDAI and SLICC scores did not correlate with levels of both biomarkers (Figure 1 and 2).

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 significantly improved (Table 3). Overall, there was no significant reduction in uMCP-1 from baseline compared to the end of induction therapy (p = 0.06), however, the reduction in the urinary level of TWEAK was significant (p = 0.03) (Table 3).

Remission status at the end of induction phase of treatment

Following induction therapy, 85% of patients attained remission (partial remission (n = 7) or complete remission (n = 5). The median values for uMCP-1 for those attaining remission (n = 12) and no remission (n = 2) were 1012.5 (529 - 2013.5) pg/mgCr and 594 (453 - 735) pg/mgCr at baseline and 298 (226 - 351) pg/mgCr (p = 0.0186) and 1846 (729 - 2964) pg/mgCr (p = 0.654) at 6 months, respectively. Urinary TWEAK values for the remission and no remission groups were 142 (88.5 - 297) pg/mgCr and 126 (18 - 234) pg/mgCr at baseline and 36 (12.5 – 76.5) pg/mgCr (p = 0.015) and 166.5 (162 - 171) pg/mgCr (p = 0.65) at 6 months, respectively. Patients who achieved any form of remission had significantly lower urinary biomarker values (Table 3, Figure 3 and 4).

Discussion

Diagnosis and assessment of flares as well as treatment response in LN remains challenging. Kidney biopsy is the gold standard test for diagnosis and assessment of LN flares to guide treatment despite the invasive nature of this test and associated complications.(2,19,20) Furthermore, kidney biopsy as a diagnostic test is not readily available in all centres and some tests used to aid the decision on kidney biopsy lack specificity and sensitivity.(21) The need

therefore arises to look for better markers other than kidney biopsy, serum creatinine, complements, urine protein and sediment in assessing disease activity and treatment response in LN. Prior studies have suggested utility of urine biomarkers in patients with lupus nephritis.(7,11,22,23)

In this prospective study of South African patients of predominantly mixed ancestry, we found that both uMCP-1 and uTWEAK did not correlate with renal histological features. However, the urine biomarkers correlated with the remission status at the end of induction phase of treatment. The levels of both biomarkers significantly declined amongst patients who attained remission (complete or partial remission) while they increased in those that did not attain remission suggesting utility of these biomarkers in predicting treatment response and disease flares in patients with active lupus nephritis.

TWEAK is a cytokine belonging to the TNF superfamily of structurally related cytokines. TWEAK gene is expressed in multiple cells including mesangial, glomerular and tubular cells of the kidney.(24) During inflammation, the cytokine binds to its receptor Fn14 (fibroblast growth factor-inducible 14) on cell surfaces signalling through the NF-κB pathway to stimulate a wide array of other cytokines, chemokines and cell adhesion molecules.(25) Amongst the produced chemokines is MCP-1. This therefore explains the rise in both uTWEAK and uMCP-1 during intra-renal inflammation.

An earlier study by Xuejing et al of 46 patients with LN (34 active and 12 non-active) reported correlation between uTWEAK and biopsy activity index ($r = 0.825$, $P < 0.01$).(7) Another study of 44 patients with LN (22 active and 22 inactive) by Taha et al which assessed the histological correlation for urinary MCP-1 reported a correlation with activity index, however, no correlation with chronicity index was established as we also reported.(8) The divergent

histological findings in our study may be related to the smaller sample size. Also, the lack of correlation with histological features, particularly, chronicity index could be due to earlier diagnosis of LN as renal biopsy for in patients with lupus is readily performed at our centre.

Our study also showed that there was correlation between both uMCP-1 and uTWEAK, and remission status (partial, complete or no remission). Patients who had attained remission (complete or partial) had lower levels of both biomarkers at the end of induction phase of treatment while those who did not attain remission had higher values (Table 3). Singh et al assessed uMCP-1 in patients with LN flares and found a significant correlation between the biomarker and flares(26). Schwartz et al also reported high levels of uTWEAK in patients with active LN and reported that a rise was also seen during disease flares, thus highlighting the role of these biomarkers in the diagnosis and monitoring for flares.(9,27)

Finally, we did not find a correlation between uMCP-1 or uTWEAK with traditional serological markers (anti-dsDNA, serum complement levels and UPCR) commonly used in LN and disease activity score (SLEDAI) at baseline. Lack of correlation with traditional serological markers in our study differ from report from Alharazy et al who showed a positive correlation between uMCP-1 and proteinuria and SLEDAI score.(28) Another study by Salem et al did not find correlation between uTWEAK and serum complements, anti-dsDNA and proteinuria as seen in our study. However, Reyes-Martínez et al reported a significant correlation of uTWEAK and the serological markers.(29) The discrepancies in observations across studies require further evaluation. Major limitations of our study include the size of our sample as well as our study design which did not allow us to assess the role of different treatment regimen on levels of the biomarkers we assessed. Understanding the effects of treatment on urinary biomarker level could allow us to determine the “best” regimen for reducing intrarenal inflammation

which could in turn have an impact on patients' outcomes. Despite these limitations, our study strength is in showing correlation with remission status which therefore shows that they may be used for monitoring response to treatment.

Conclusion

Our study did not show correlation between uMCP-1 and uTWEAK with histological features of LN. However, both biomarkers were elevated in patients with active disease and reduced with the remission at the end of induction phase of treatment. These findings support that these biomarkers may be used for monitoring early treatment response. However, further prospective studies are needed to confirm the clinical utility of these biomarkers in monitoring disease activity and response to treatment with a larger sample size.

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Tables

Table 1: Demographic, clinical and biochemical features at baseline

Variable	n	Median
Age, *years (mean)*	20	29.8 ± 10.7
Gender, female (%)	14	70
Ethnicity (%)		
o Black	7	35
o Coloured	12	60
o White	1	5
Body mass index, *kg/m^2*	20	22.5 (20.7 - 25.6)
Systolic blood pressure, *mmHg*	20	132.5 (120.5 - 140.0)
Diastolic blood pressure, *mmHg*	20	75.0 (70.0 - 87.0)
SLEDAI	20	17 (13-26)
SLICC	20	1.5 (1-2)
Biopsy features (%)		
o Proliferative	14	70
o Non-proliferative	6	30
o Activity index	20	4 (3-9)
o Chronicity index	20	2 (1-4)
o Interstitial Fibrosis	10	50
Treatment (%)		
o CS	5	25
o CS + CYC	11	55
o CS + MMF	4	20
Haemoglobin, *g/dL*	20	9.25 (8.15 - 10.0)
Platelets, *x 10^9/L*	20	228.0 (148.5 - 278)
Serum creatinine, *umol/L*	20	104 (56.5 – 153.5)
Estimate glomerular filtration rate, *mL/min/1.73m^2*	20	64.7 (41.6 - 118.8)
UPCR, *g/mmol creatinine*	20	0.37 (0.18 – 0.59)
C3, *g/L*	20	0.47 (0.34 – 0.68)
C4, *g/L*	20	0.09 (0.05 – 0.14)
Anti-dsDNA Antibodies, *IU/ml*	20	247.5 (66.5 – 379)
Anti-smith Antibodies, *U/ml*	20	1.7 (0.6 - 42.7)
Urine biomarkers		
o Urine MCP-1, *pg/mL*	20	1440.1 (683.5 - 2729.5)
o Corrected Urine MCP-1, *pg/mg creatinine*	20	1093.0 (576.5 - 2013.5)
o Urine TWEAK, *pg/mL*	20	209.0 (117.0 - 312.0)
o Corrected Urine TWEAK, *pg/mg creatinine*	20	159.0 (88.5 - 295.5)

Key: SLEDAI: Systemic lupus erythematosus disease activity index, SLICC: Systemic Lupus International Collaborating Clinics, 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

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

	Corrected uMCP-1, pg/mg creatinine		Corrected uTWEAK, pg/mg creatinine	
Histologic feature	***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	0.27	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)	

Key: Proliferative LN, (Lupus nephritis classes III, IV, III+V and IV+V), Non-proliferative, (Lupus nephritis classes I, II and V), uMCP-1, urinary monocyte chemoattractant-1, uTWEAK, urinary tumour necrosis factor-like weak inducer of apoptosis

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 109/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/1.73m2*	64.7 (41.6 - 118.8)	92.3 (53.6 - 114.5	0.04
Corrected Urinary MCP-1, *pg/mg creatinine*			
• All	1092.7 (578.6 – 1848.0)	314.5 (197.0 - 622.0)	0.06
• Remission group	1012.5 (529 – 2013.5)	298 (226 – 351)	0.018
• No remission group	594 (453 – 735)	1845.5 (727 – 2964)	0.654
Corrected Urinary TWEAK, *pg/mg creatinine*			
• All	159.0 (88.5 - 295.5)	36.0 (17.0 - 88.0)	0.03
• Remission group	142 (88.5 – 297)	36 (12.5 – 76.5)	0.015
• No remission group	126 (18 – 234)	166.5 (162 – 171)	0.65

Key: eGFR, estimated glomerular filtration rate, SLEDAI, Systemic Lupus Erythematosus Disease Activity Index, rSLEDAI, renal Systemic Lupus Erythematosus Disease Activity Index, UPCR, urine protein creatinine ratio, MCP-1, monocyte chemoattractant- 1, TWEAK, tumor necrosis factor-like weak inducer of apoptosis

Legends to figures

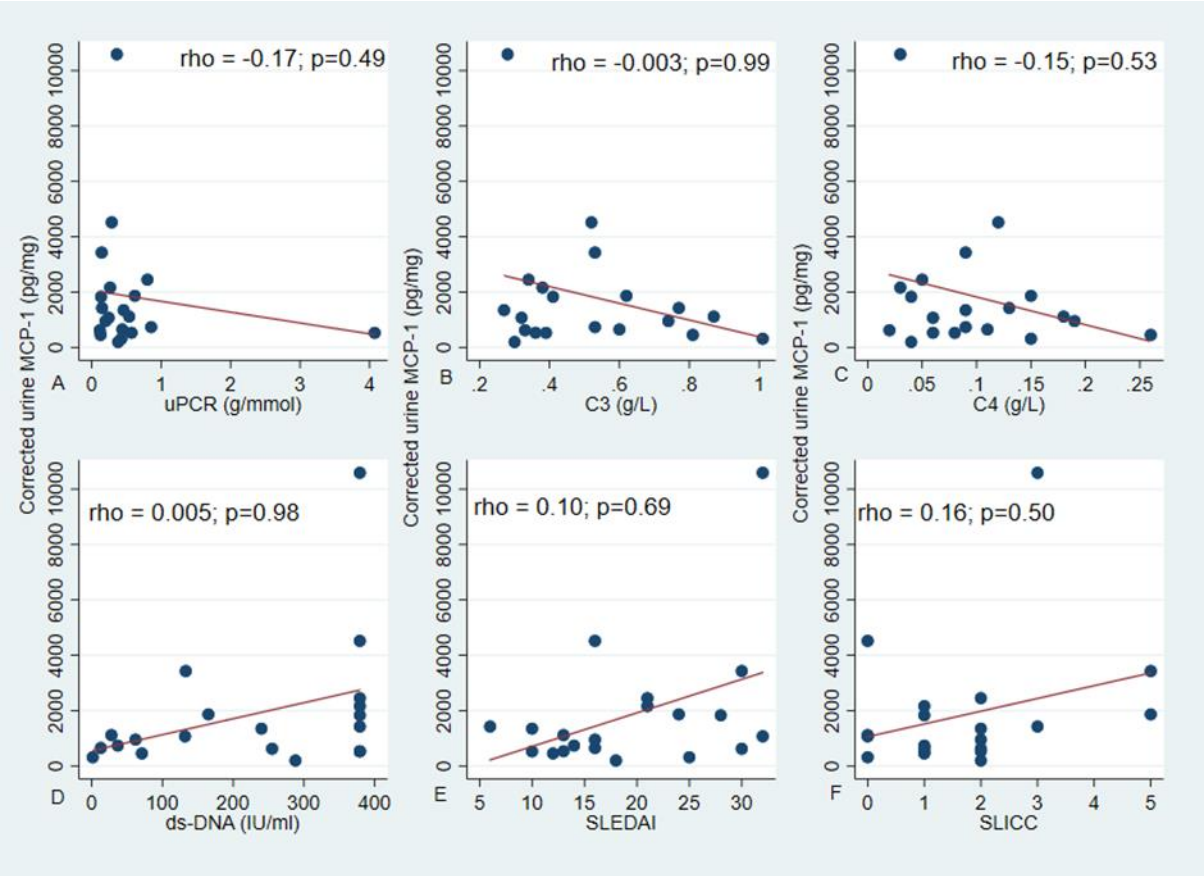

Figure 1: Correlation of urinary MCP-1 with biochemical parameters and disease activity and chronicity scores.

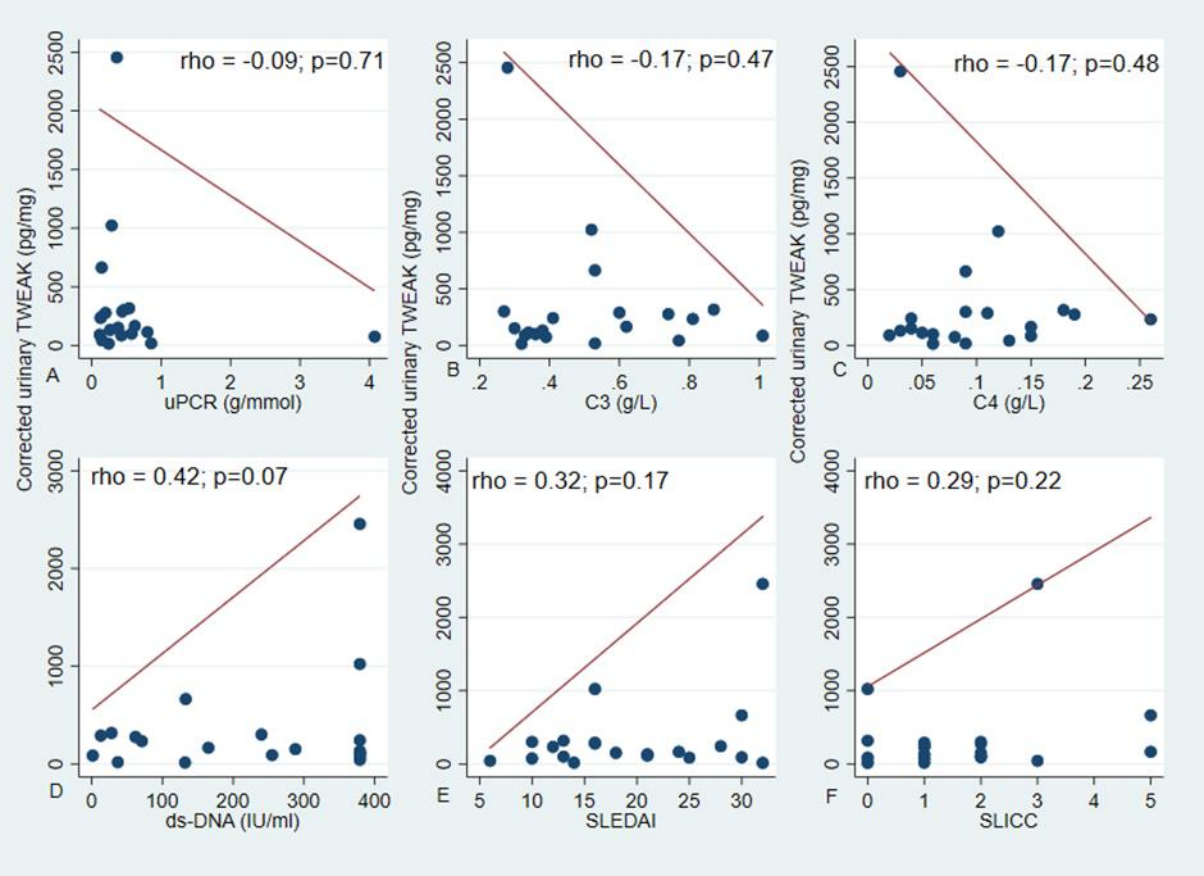

Figure 2: Correlation of urinary TWEAK with biochemical parameters and disease activity and chronicity scores.

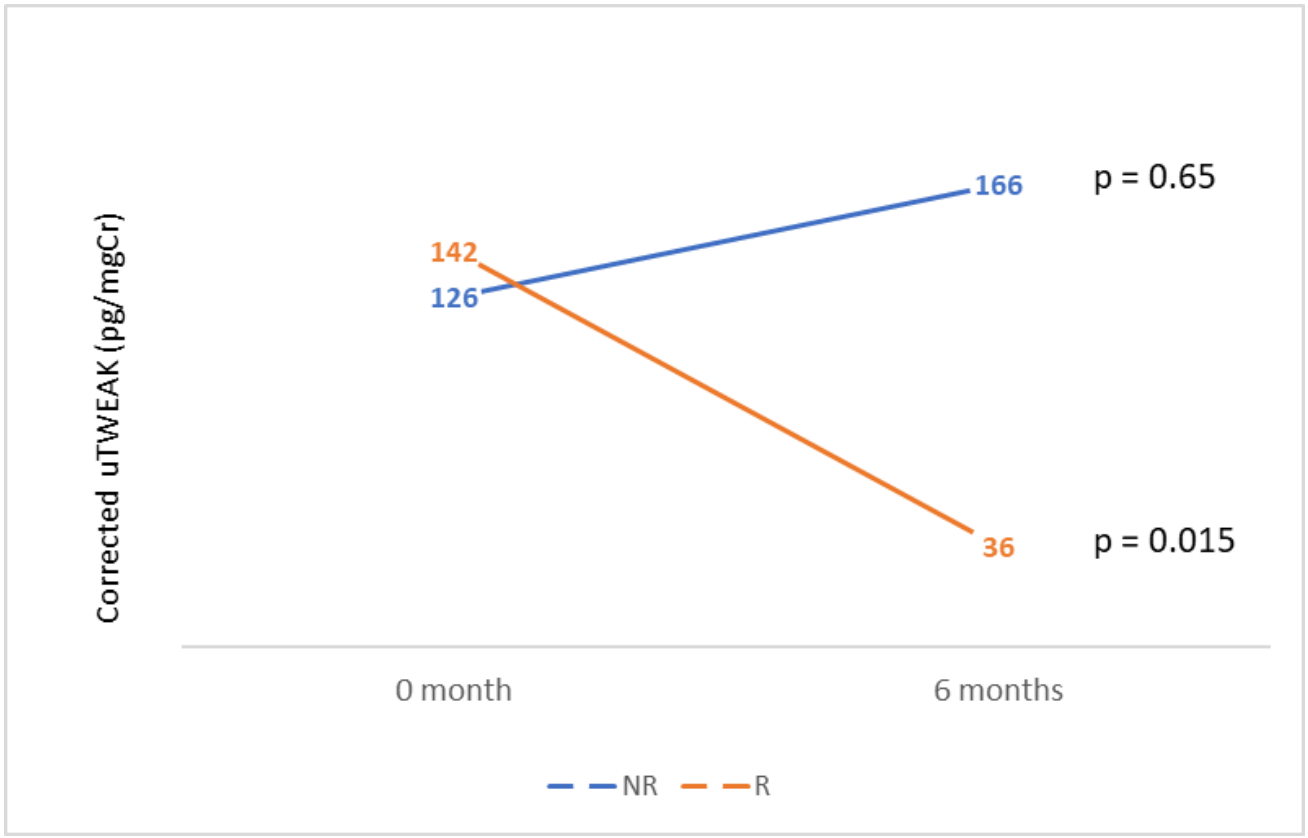

Figure 3: Change in corrected urinary TWEAK in relation to remission status
Key: NR, no remission, R, remission

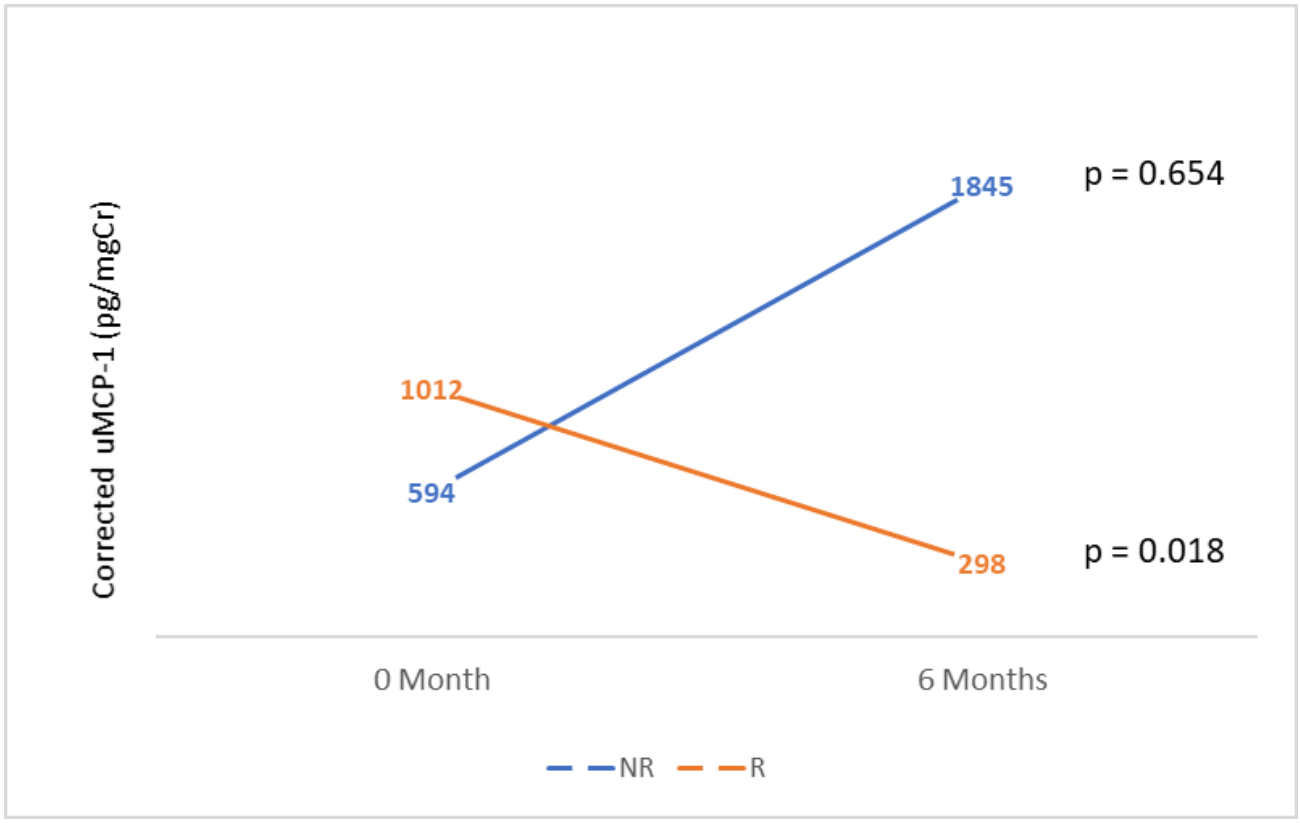

Figure 4: Change in corrected urinary MCP-1 in relation to remission status
Key: NR, no remission, R, remission

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- My mother – for continually praying for me
- My siblings for their constant encouragement.

Transparency declarations

None to declare.

Appendices

Appendix 1: BMC Nephrology- Journal Instruction to Authors of Research Articles

Criteria

Research articles should report on original primary research but may report on systematic reviews of published research provided they adhere to the appropriate reporting guidelines which are detailed in our editorial policies. Please note that non-commissioned pooled analyses of selected published research will not be considered.

BMC Nephrology strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories. Where a widely established research community expectation for data archiving in public repositories exists, submission to a community-endorsed, public repository is mandatory. A list of data where deposition is required, with the appropriate repositories, can be found on the Editorial Policies Page.

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Preparing your manuscript

The information below details the section headings that you should include in your manuscript and what information should be within each section.

Please note that your manuscript must include a 'Declarations' section including all of the subheadings (please see below for more information).

Title page

The title page should:

- present a title that includes, if appropriate, the study design e.g.:
    - "A versus B in the treatment of C: a randomized controlled trial", "X is a risk factor for Y: a case control study", "What is the impact of factor X on subject Y: A systematic review"
    - or for non-clinical or non-research studies a description of what the article reports
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    - if a collaboration group should be listed as an author, please list the Group name as an author. If you would like the names of the individual members of the Group to be searchable through their individual PubMed records, please

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- indicate the corresponding author

Abstract

The Abstract should not exceed 350 words. Please minimize the use of abbreviations and do not cite references in the abstract. Reports of randomized controlled trials should follow the CONSORT extension for abstracts. The abstract must include the following separate sections:

- Background: the context and purpose of the study
- Methods: how the study was performed, and statistical tests used
- Results: the main findings
- Conclusions: brief summary and potential implications
- Trial registration: If your article reports the results of a health care intervention on human participants, it must be registered in an appropriate registry and the registration number and date of registration should be in stated in this section. If it was not registered prospectively (before enrolment of the first participant), you should include the words 'retrospectively registered'. See our editorial policies for more information on trial registration

Keywords

Three to ten keywords representing the main content of the article.

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The Background section should explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

Methods

The methods section should include:

- the aim, design and setting of the study
- the characteristics of participants or description of materials
- a clear description of all processes, interventions and comparisons. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses
- the type of statistical analysis used, including a power calculation if appropriate

Results

This should include the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as tables and figures.

Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study.

Conclusions

This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study reported.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations should be provided.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations':

- Ethics approval and consent to participate
- Consent for publication
- Availability of data and material
- Competing interests
- Funding
- Authors' contributions
- Acknowledgements
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The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

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

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

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Hao Z, AghaKouchak A, Nakhjiri N, Farahmand A. Global integrated drought monitoring and prediction system (GIDMaPS) data sets. figshare. 2014. http://dx.doi.org/10.6084/m9.figshare.853801

With the corresponding text in the Availability of data and materials statement:

The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]. [Reference number]

Competing interests

All financial and non-financial competing interests must be declared in this section.

See our editorial policies for a full explanation of competing interests. If you are unsure whether you or any of your co-authors have a competing interest, please contact the editorial office.

Please use the authors initials to refer to each authors' competing interests in this section.

If you do not have any competing interests, please state "The authors declare that they have no competing interests" in this section.

Funding

All sources of funding for the research reported should be declared. The role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

Authors' contributions

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Please use initials to refer to each author's contribution in this section, for example: "FC analysed and interpreted the patient data regarding the haematological disease and the transplant. RH performed the histological examination of the kidney and was a major contributor in writing the manuscript. All authors read and approved the final manuscript."

Acknowledgements

Please acknowledge anyone who contributed towards the article who does not meet the criteria for authorship including anyone who provided professional writing services or materials.

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Endnotes

Endnotes should be designated within the text using a superscript lowercase letter and all notes (along with their corresponding letter) should be included in the Endnotes section. Please format this section in a paragraph rather than a list.

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Example reference style:

Article within a journal

Smith JJ. The world of science. Am J Sci. 1999;36:234-5.

Article within a journal (no page numbers)

Rohrmann S, Overvad K, Bueno-de-Mesquita HB, Jakobsen MU, Egeberg R, Tjønneland A, et al. Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. BMC Medicine. 2013;11:63.

Article within a journal by DOI

Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000; doi:10.1007/s801090000086.

Article within a journal supplement

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When preparing figures, please follow the formatting instructions below.

- Figures should be numbered in the order they are first mentioned in the text, and uploaded in this order. Multi-panel figures (those with parts a, b, c, d etc.) should be submitted as a single composite file that contains all parts of the figure.
- Figures should be uploaded in the correct orientation.
- Figure titles (max 15 words) and legends (max 300 words) should be provided in the main manuscript, not in the graphic file.
- Figure keys should be incorporated into the graphic, not into the legend of the figure.
- Each figure should be closely cropped to minimize the amount of white space surrounding the illustration. Cropping figures improves accuracy when placing the figure in combination with other elements when the accepted manuscript is prepared for publication on our site. For more information on individual figure file formats, see our detailed instructions.
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- maximum height of 225 mm for figure and legend
- image resolution of approximately 300 dpi (dots per inch) at the final size

Figures should be designed such that all information, including text, is legible at these dimensions. All lines should be wider than 0.25 pt when constrained to standard figure widths. All fonts must be embedded.

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- Tables larger than one A4 or Letter page in length can be placed at the end of the document text file. Please cite and indicate where the table should appear at the relevant location in the text file so that the table can be added in the correct place during production.
- Larger datasets, or tables too wide for A4 or Letter landscape page can be uploaded as additional files. Please see [below] for more information.
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- Table titles (max 15 words) should be included above the table, and legends (max 300 words) should be included underneath the table.
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If you have any questions or are experiencing a problem with tables, please contact the customer service team at info@biomedcentral.com.

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As the length and quantity of data is not restricted for many article types, authors can provide datasets, tables, movies, or other information as additional files.

All Additional files will be published along with the accepted article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files, if requested, should be sent by email to the journal's editorial email address, quoting the manuscript reference number. Please do not send completed patient consent forms unless requested.

Results that would otherwise be indicated as "data not shown" should be included as additional files. Since many web links and URLs rapidly become broken, BioMed Central requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. Do not include any individual participant details. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission. Each additional file should be cited in sequence within the main body of text.

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- File name (e.g. Additional file 1)
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- Title of data
- Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

Appendix 2: Ethics Approval Letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 404 7682 • **Facsimile** [021] 406 6411
Email: noel.isaacs@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

27 June 2017

HREC REF: 332/2017

Prof I Okpechi
Division of Nephrology & Hypertension
E13 Renal Unit
NGSH

Dear Prof Okpechi

PROJECT TITLE: CORRELATION BETWEEN URINARY BIOMARKERS (uMCP-1 AND uTWEAK), RENAL HISTOLOGY AND EARLY RESPONSE TO THERAPY IN NEWLY BIOPSIED PATIENTS WITH LUPUS NEPHRITIS (Masters-candidate Dr M Molol) sub-study linked to 402/2014

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th June 2018.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student Dr M Molol will be involved in this study.

Please note that for all studies approved by the HREC, the principal investigator must obtain appropriate institutional approval before the research may occur.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB0001938

Appendix 3: Data Abstraction Sheet (Visit 1)

TWEAK STUDY (LUPUS BIOMARKER STUDY)

ALUGEN

INSERT PATIENT STICKER HERE

STUDY NUMBER					
VISIT 1	**VISIT DATE:**				
DOB	DD/MM/YYYY	Study arm	ACTIVE / QUIESCENT		
GENDER	M / F	SLEDAI SCORE			
ETHNICITY	B / W / C / I / A	SLICC SCORE			
SMOKING	Ex / Y / N	Urinalysis	Protein	RBC	
ALCOHOL USE	Ex / Y / N		WBC	Nitrite	
OCCUPATION	Student / Employed/ Unemployed/	Urine microscopy	RBC casts	WBC casts	
YEARS OF EDUCATION	0 – 25 (----------)		Granular casts	Hyaline casts	
HIGHEST QUALIFICATION	Pry / Sec / Tertiary / Post-grad	FBC			
DATE OF SLE DIAGNOSIS		ANA			
NUMBER OF KIDNEY BIOPSIES		Anti-Ds-DNA			
DATE LAST KIDNEY BIOPSY		Anti-Sm			
LN – CLASS (MOST RECENT)		C3			
WEIGHT (KG)		C4			
HEIGHT (M)		UPCR			
SBP (MMHG)		Creatinine			
DBP (MMHG)		TWEAK			
CURRENT REMISSION STATUS	CR / PR / NR	MCP-1			
CURRENT MEDICATIONS	CQ	STEROIDS	CYC	MMF	AZA

Appendix 4: Data Abstraction Sheet (Visit 2)

TWEAK STUDY (LUPUS BIOMARKER STUDY)

ALUGEN

INSERT PATIENT STICKER HERE

Study number					
VISIT 2	**VISIT DATE:**				
WEIGHT (kg)					
SBP (mmHg)					
DBP (mmHg)					
STUDY ARM	ACTIVE / QUIESCENT				
SLEDAI SCORE					
URINALYSIS	PROTEIN	RBC			
	WBC	NITRITE			
FBC					
UPCR					
SERUM CREATININE					
U-TWEAK					
U-MCP-1					
CURRENT REMISSION STATUS	CR / PR / NR				
CURRENT MEDICATIONS	CQ	STEROIDS	CYC	MMF	AZA

Appendix 5: Consent Form

INFORMED CONSENT FOR PARTICIPATION IN A STUDY ENTITLED: "Correlation between urinary biomarkers (uMCP-1 and uTWEAK), renal histology and early response to therapy in newly biopsied patients with lupus nephritis".

Study Number:	e.g.	M	M	W	1	A	0	0	1

Study Aim:

The purpose of this study is to assess if there is any relationship between levels of particles called Tumor Necrotic Factor-like weak inducer of apoptosis (TWEAK) and monocyte chemoattractant protein-1 (MCP-1) in urine and the kidney biopsy findings in patients with systemic lupus erythematosus (SLE) the involves the kidneys (Lupus Nephritis) and whether the levels of these particles can be used to determine whether patients will respond to treatment after 6 months from the time of kidney biopsy. The results of this study will be used to guide doctors treating patients with lupus nephritis on whether they can treat patients with this disease without having to a kidney biopsy while basing the decision only on the urine levels of the particles named above. There are studies that have been done that show that there could be a relationship between the severity of this disease and the levels in urine of these particles.

Involvement:

You will be asked, by consenting, allow the study person to collect your blood and urine to test them for these particles. You will also be asked to come back after 6 months of treatment and have the levels these particles to be re-measured. The samples collected will be stored in a fridge at -80 degrees celcius until a point when all the collected samples will be measures.

Confidentiality:

Any information that is obtained in connection with this study and that can be identified with you will remain strictly confidential. However, the data may be seen by the ethical review committee and may be published without disclosing your identity.

Withdrawal from the study:

Your participation in this study is voluntary; you therefore have a right to withdraw from the study anytime. In case of withdrawal your information will be destroyed and will not be used as part of the data

Risks and Benefits:

There are no risks involved in participating in the study. At each visit during the study you will be compensated with R50 which you are expected to use for transportation to the study site. However, the results from this study will help us to determine whether urinary TWEAK and MCP-1 correlate with kidney biopsy results and response to treatment.

Contacts:

If you have questions regarding your rights as a research participant, you may wish to contact the principal investigators: A/Prof. Ikechi Okpechi and Dr Mothusi Walter Moloi, University of Cape Town, E13, Division of Nephrology and Hypertension, Groote Schuur Hospital (Tel: +27 214043310)

The study has been approved by the Human Research and Ethics Committee, Faculty of Health Sciences, University of Cape Town. If you have further questions on your rights as a study participant you may consult: Human Research Ethics Committee, E53 Room 46, Old Main Building, Groote Schuur Hospital, (Tel: +27 21 6501236)

Written Consent / Agreement

I..... (name of the patient) being ..... years old (with a sound mind to consent) have been informed about the study entitled: **"Correlation between urinary biomarkers (uMCP-1 and uTWEAK), renal histology and early response to therapy in newly biopsied patients with lupus nephritis"**. I have been given the opportunity to ask questions concerning the study and these have been answered to my satisfaction. I understand that I may at any time during the study revoke my consent and withdrawal from the study without any penalty. I also understand that giving consent to enrol into the study does not take away my legal rights in case of negligence or other legal fault by anyone who is involved in the study. I therefore volunteer to participate in this study

Signature of participant or thumb print.....Date ----------

Signature of witness .....Date ----------

Name of the patient obtaining consent.....

Signature of the person obtaining consent: .....Date ----------

Appendix 6: SLEDAI-2K disease activity questionnaire

SLEDAI-2K disease activity questionnaire:

For an item to be scored the indicated weight, the manifestation must have been **Present in the past 10 days.**

Weight	Tick if present	Descriptor	Definition
8		Seizure	Recent onset, exclude metabolic, infectious or drug causes.
8		Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes
8		Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features, inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.
8		Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, or optic neuritis. Exclude hypertension, infection, or drug causes.
8		Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8		Lupus headache	Severe, persistent headache; may be migrainous, but must be nonresponsive to narcotic analgesia.
8		CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8		Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4		Arthritis	≥ 2 joints with pain and signs of inflammation (i.e., tenderness, swelling or effusion).
4		Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4		Urinary casts	Heme-granular or red blood cell casts.
4		Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4		Proteinuria	>0.5 gram/24 hours
4		Pyuria	>5 white blood cells/high power field. Exclude infection.
2		Rash	Inflammatory type rash.
2		Alopecia	Abnormal, patchy or diffuse loss of hair.
2		Mucosal ulcers	Oral or nasal ulcerations.
2		Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2		Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2		Low complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory
2		Increased DNA binding	Increased DNA binding by Farr assay above normal range for testing laboratory.
1		Fever	>38◦C. Exclude infectious cause.
1		Thrombocytopenia	<100,000 platelets / x10^9/L, exclude drug causes.
1		Leukopenia	< 3,000 white blood cells / x10^9/L, exclude drug causes.

TOTAL SCORE: _____

Physician Global Activity Assessment:

No Activity	Mild Activity	Moderate Activity	Severe Activity

TWEAK-MCP-1 STUDY

Appendix 7: Systemic Lupus International Collaborating Clinics Damage Index (SLICC Scoring sheet)

Systemic Lupus International Collaborating Clinics Damage Index

(Circle the appropriate score)

ITEM	0	1	2	3
Ocular (either eye, by clinical assessment)				
- Any cataract ever	0	1		
- Retinal change or optic atrophy	0	1		
Neuropsychiatric				
- Cognitive impairment (e.g. memory deficit, poor calculation etc)	0	1		
- Cerebrovascular accident (score 2 if >1)	0	1	2	
- Cranial or peripheral neuropathy (excluding optic)	0	1		
- Transverse myelitis	0	1		
Renal				
- Estimated or measured glomerular filtration rate< 50%	0	1		
- Proteinuria >3.5 gm/24hours or UPCR >0.35g/mmol	0	1		
- End-stage renal disease (regardless of dialysis or transplantation	0			3
Pulmonary				
- Pulmonary hypertension (right ventricular prominence, or loud P2	0	1		
- Pulmonary fibrosis (physical and radiograph)	0	1		
- Shrinking lung (radiograph)	0	1		
- Pleural fibrosis (radiograph)	0	1		
- Pulmonary infarction (radiograph)	0	1		
Cardiovascular				
- Angina or coronary artery bypass	0	1		
- Myocardial infarction ever (score 2 if > 1)	0	1	2	
- Cardiomyopathy (ventricular dysfunction)	0	1		
- Valvular disease (diastolic murmur, or systolic murmur >3/6)	0	1		
- Pericarditis for 6 months, or pericardiectomy	0	1		
Peripheral vascular				
- Claudication for 6 months	0	1		
- Minor tissue loss (pulp space)	0	1		
- Significant tissue loss ever (e.g. loss of digit or limb)(score 2 if > 1 site)	0	1	2	
- Venous thrombosis with swelling, ulceration, or venous stasis	0	1		
Gastrointestinal				
- Mesenteric insufficiency	0	1		
- Infarction or resection of bowel below duodenum spleen, liver, or gall bladder ever (score 2 if > 1 site)	0	1	2	
- Chronic peritonitis	0	1		
- Stricture or upper gastrointestinal tract surgery ever	0	1		
Musculoskeletal				
- Muscle atrophy or weakness	0	1		
- Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	0	1		
- Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	0	1		
- Avascular necrosis (score 2 if > 1)	0	1	2	
- Osteomyelitis	0	1		
Skin				
- Scarring chronic alopecia	0	1		
- Extensive scarring or panniculum other than scalp and pulp space	0	1		
- Skin ulceration (excluding thrombosis) for > 6 months	0	1		
Premature gonadal failure	0	1		
Diabetes regardless of treatment	0	1		
Malignancy (exclude dysplasia) (score 2 if > 1site)	0	1	2	

TWEAK/MCP-1 STUDY