Urinary Vascular Cell Adhesion Molecule, But Not Neutrophil Gelatinase-associated Lipocalin, Is Associated with Lupus Nephritis

ADNAN N. KIANI, TIANFU WU, HONG FANG, XIN J. ZHOU, CHUL W. AHN, LAURENCE S. MAGDER, CHANDRA MOHAN, and MICHELLE PETRI

ABSTRACT. ***Objective.*** Vascular cell adhesion molecule-1 (VCAM-1), an adhesion molecule, is involved in the progression of glomerular and tubulointerstitial injury. Neutrophil gelatinase-associated lipocalin (NGAL), a member of the lipocalin superfamily, has been shown to rise in both acute and chronic kidney damage. Both VCAM-1 and NGAL have been found at high levels in the urine of patients with active lupus nephritis. We investigated both as potential biomarkers for lupus nephritis.

Methods. VCAM-1 and NGAL were measured by ELISA during 1 to 8 clinic visits in 107 patients with systemic lupus erythematosus (SLE; 91% women, 51% black, 36% white, 4% Asian, 4% Hispanic, and 5% others) for a total of 190 visits. Patients' mean age was 41 years. We analyzed the relationship between these potential urine biomarkers and the urine protein/creatinine ratio (urine Pr/Cr), the Systemic Lupus International Collaborating Clinics (SLICC) renal activity score, SLE Disease Activity Index renal descriptors, and other clinical variables.

Results. VCAM-1 levels were strongly associated with the physician's global estimate of disease activity ($p = 0.0002$), the renal visual analog scale ($p < 0.0001$), the urine Pr/Cr ($p < 0.0001$), and SLICC renal activity score ($p < 0.0001$). VCAM-1 levels were also associated with a urine Pr/Cr ≥ 0.5 ($p < 0.0001$). NGAL was not associated with any measure of disease activity or with lupus serologies.

Conclusion. Urine VCAM-1 had a strong association with measures of disease activity, including multiple renal activity descriptors. In contrast to previous SLE studies, NGAL failed to show any association with lupus nephritis. (First Release April 15 2012; J Rheumatol 2012;39:1231–7; doi:10.3899/jrheum.111470)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS VASCULAR CELL ADHESION MOLECULE
NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN LIPOCALIN LUPUS NEPHRITIS

From the Division of Rheumatology, Johns Hopkins University School of Medicine, Baltimore, Maryland; University of Texas Southwestern Medical Center, Dallas, Texas; and University of Maryland School of Medicine, Baltimore, Maryland, USA.

The Hopkins Lupus Cohort is supported by a grant from the National Institutes of Health (NIH AR43727). This research was also supported by grant UL1 RR025005 from the National Center for Research Resources (NCRR).

A.N. Kiani, MD, MPH, Instructor in Medicine, Division of Rheumatology, Johns Hopkins University School of Medicine; T. Wu, PhD, Assistant Professor of Medicine, University of Texas Southwestern Medical Center; H. Fang, MD, MS, Research Program Coordinator, Division of Rheumatology, Johns Hopkins University School of Medicine; X.J. Zhou, MD, Professor of Pathology; C.W. Ahn, PhD, Professor of Clinical Sciences, University of Texas Southwestern Medical Center; L.S. Magder, MPH, PhD, Professor of Epidemiology and Public Health, University of Maryland School of Medicine; C. Mohan, MD, PhD, Professor of Medicine, University of Texas Southwestern Medical Center; M. Petri, Professor of Medicine, Division of Rheumatology, Johns Hopkins University School of Medicine.

Dr. Kiani and Dr. Wu contributed equally to this report, as co-first authors. Dr. Petri and Dr. Mohan are co-senior authors.

Address correspondence to Dr. M. Petri, Division of Rheumatology, Johns Hopkins University School of Medicine, 1830 East Monument St., Suite 7500, Baltimore, MD 21205, USA. E-mail: mpetri@jhmi.edu

Accepted for publication February 2, 2012.

Lupus nephritis (LN) is a common manifestation among patients with systemic lupus erythematosus (SLE), occurring in over 50%. One major determinant of poor prognosis among patients with SLE is renal involvement, LN being both more common and more severe in non-white patients[1,2,3]. Despite new immunosuppressive agents available for treatment, no significant improvement has been seen in survival in patients with SLE or in the incidence of endstage renal disease from LN[4,5,6].

Renal biopsy remains the "gold standard" to determine SLE renal activity[7], but carries its own risks. Neither clinical nor serological data can accurately predict the histopathological lesions of LN. There remains a need for noninvasive biomarkers that associate with renal activity and are able to predict renal flares.

Studies of murine lupus have shown the importance of adhesion molecules, cytokines, and chemokines in the pathogenesis of glomerular inflammation[8,9]. However, to be clinically practical, biomarkers of LN would need to be measured in the urine, since urine collection is much easier and less invasive compared to repetitive renal biopsies.

Neutrophil gelatinase-associated lipocalin (NGAL;

lipocalin-2) is a member of the lipocalin family and is extensively studied in both animals and humans[10,11,12,13,14]. In a murine model for human SLE (chronic graft-vs-host disease), increased levels of NGAL were found after induction of disease[11]. Increased levels of NGAL have also been found in other inflammatory conditions, including plasma of patients with vasculitis and Kawasaki syndrome[15,16]. Cancers, including breast, colorectal, pancreatic, and ovarian tumors, have shown increased NGAL expression as well[17,18,19,20].

Brunner, *et al* have shown that urinary NGAL levels correlated with renal disease activity in pediatric lupus[12]. Pitashny, *et al* found that urinary NGAL levels were higher in patients with LN, correlating with both the renal SLE Disease Activity Index (SLEDAI) descriptors and the urine protein to creatinine ratio (urine Pr/Cr)[13]. However, urinary NGAL is not specific for LN, being found in multiple types of acute renal injury[21,22,23,24].

Vascular cell adhesion molecule-1 (VCAM-1) is a member of the immunoglobulin superfamily that is highly expressed in kidneys, both in murine SLE models and in human LN[25,26,27,28,29]. VCAM-1 is also expressed in normal kidneys, but levels increase many-fold in diseased states[29,30,31]. High VCAM-1 expression was found in the endothelium of renal transplant rejection biopsy specimens. Antibodies directed against VCAM-1 prolonged allograft survival[32]. In a study of 30 SLE patients with LN, 20 SLE patients without LN, and 20 controls, high urinary VCAM-1 levels were seen in patients with Class III, IV, and V disease compared to those without LN and controls[33]. In another study, elevated urinary levels of VCAM-1 were seen in 38 SLE patients with varying SLEDAI scores (0 to 18) versus patients with rheumatoid arthritis and healthy controls. These levels correlated with SLEDAI scores, especially renal SLEDAI descriptors, and the urine Pr/Cr ratio, and were higher in patients with active LN versus those with no renal disease[25]. Serum levels of VCAM-1 have also been shown to be associated with SLE disease activity[26,34].

Our primary goal was to relate the levels of VCAM-1 and NGAL with levels of proteinuria. We also evaluated these 2 promising urinary biomarkers for LN prospectively in a longitudinal study of SLE patients with and without LN and correlated them with renal pathology activity and chronicity indices.

MATERIALS AND METHODS

Our study was approved by the Johns Hopkins University School of Medicine Institutional Review Board. All patients gave informed consent. The patients were part of the Hopkins Lupus Cohort, a prospective longitudinal study of lupus activity and outcomes. Consecutive patients with biopsy-proven LN or proteinuria were invited to participate, as were a subset of consecutive patients without these conditions. The cohort protocol required at least quarterly visits, but patients were seen more often depending on disease activity. At each visit, disease activity indices — the physician's global assessment, Safety of Estrogens in Lupus Erythematosus: National Assessment (SELENA)-SLEDAI[35], and renal activity on a 0 to 3 visual analog scale (VAS)[36] — were calculated by one rheumatologist. Vital signs and laboratory tests were recorded: weight, blood pressure, complete blood count, platelets, erythrocyte sedimentation rate, creatinine, cholesterol, C3, C4, anti-dsDNA, anticardiolipin, lupus anticoagulant by Russell's viper venom time test confirmation, urinalysis, urine Pr/Cr ratio; as well as treatment [prednisone, immunosuppressives, hydroxychloroquine, angiotensin-converting enzyme (ACE) inhibitor and dose, angiotensin receptor blocker and dose]. Urine samples were collected at each visit.

Quantifying renal disease activity. We used the Systemic Lupus International Collaborating Clinics (SLICC) Renal Activity Score to quantify renal disease activity[37]. This score was derived from a regression analysis using the physician rating of renal activity as the "gold standard." It is calculated as follows: proteinuria 0.5 to 1 g/day (3 points), proteinuria 1 to 3 g/day (5 points), proteinuria > 3 g/day (11 points), urine red blood cells ≥ 5/high power field (hpf; 3 points), and urine white blood cells (WBC) ≥ 5 /hpf (1 point).

Urine collection, preparation, and biomarker measurement. Urine "clean-catch midstream" samples of 25–50 ml were collected into appropriate sterile containers and a protease inhibitor was added. Samples were placed on ice or refrigerated at 4°C within 1 h of collection. Aliquots and the remaining urine sample in the centrifuge tube were frozen at –80°C and batched for shipment to the University of Texas Southwestern Medical Center laboratory (Dallas, TX, USA). VCAM-1 and NGAL were measured by ELISA (R&D Systems, Minneapolis, MN, USA). The results were reported as pg/ml (VCAM-1) and ng/ml (NGAL).

Statistical methods. We normalized our measures of urinary VCAM-1 and NGAL by dividing by the urine creatinine concentration to adjust for the variable urine concentration. Then we transformed each variable by taking the $\log_e$(variable + 1) prior to calculating means and performing inference. This was done because distributions of NGAL and VCAM-1 were skewed. We used a mixed-effects model to measure the associations between biomarkers and clinical variables, including a random effect for the patient, to account for the correlation between repeated observations from the same patient. For difference between subgroups (low, medium, high) with respect to the proportion of visits with urine Pr/Cr ratio ≥ 0.5, a General Estimating Equations method was used to calculate p values accounting for repeated observations from the same patient. To assess the association between biomarkers and biopsy results, we chose the closest visit date to the biopsy date when the US National Institutes of Health (NIH) renal activity index (0–24) and chronicity indices (0–12) were calculated. We then used linear regression models to examine associations between these indices and clinical predictors.

RESULTS

Data were obtained from 107 patients with SLE (91% women). VCAM-1 and NGAL were measured at 1 to 8 visits per patient over the course of a 23-month period for a total of 190 visits. Twenty-four percent had 2 visits, 21% had ≥ 3, and 1% had 5, 6, and 8 visits each. Of 45% of patients with followup visits, 11% had disease flares.

Patients were white (36%), black (51%), Hispanic (4%), Asian (4%), and other ethnicity (5%). The mean age was 41 years. Cumulative clinical manifestations included malar rash (51%), discoid rash (26%), photosensitivity (47%), oral ulcers (38%), arthritis (73%), serositis (54%), renal disorder [83%; 78% had LN based on International Society of Nephrology (ISN) biopsy class, 77% based on proteinuria, and 75% based on both biopsy and proteinuria], neurological disorder (11%), immunologic disorder (93%), and positivity for antinuclear antibodies (99%).

Table 1 shows the association between the biomarkers and various clinical conditions observed at each visit. VCAM-1 levels were strongly associated with the physician's global estimate of disease activity and the renal VAS. VCAM-1 was

Table 1. Mean log-transformed and normalized (by urine creatinine) VCAM-1 and NGAL, by clinical variables at each visit.

Clinical Variables at Each Visit	VCAM-1		NGAL	
	Mean (SD)	p*	Mean (SD)	p*
Age, yrs				
21–44 (n = 115)	0.21 (0.40)	0.07	0.17 (0.40)	0.43
45–70 (n = 75)	0.11 (0.17)		0.12 (0.17)	
Sex				
Female (n = 175)	0.17 (0.34)	0.79	0.16 (0.31)	0.31
Male (n = 15)	0.14 (0.15)		0.07 (0.15)	
Ethnicity				
White (n = 62)	0.15 (0.20)	0.66	0.11 (0.16)	0.46
African American (n = 108)	0.17 (0.39)		0.18 (0.37)	
Other (n = 20)	0.23 (0.34)		0.11 (0.13)	
Hematuria				
Present (n = 20)	0.20 (0.14)	0.73	0.13 (0.12)	0.80
Absent (n = 170)	0.17 (0.35)		0.15 (0.31)	
Proteinuria				
Present (n = 28)	0.27 (0.29)	0.15	0.13 (0.14)	0.48
Absent (n = 162)	0.15 (0.33)		0.15 (0.32)	
Pyuria				
Present (n = 8)	0.20 (0.13)	0.76	0.18 (0.14)	0.74
Absent (n = 182)	0.17 (0.34)		0.15 (0.30)	
Anti-dsDNA				
Present (n = 64)	0.18 (0.20)	0.75	0.13 (0.12)	0.44
Absent (n = 126)	0.17 (0.38)		0.16 (0.36)	
Low C3 or C4				
Present (n = 84)	0.20 (0.25)	0.46	0.12 (0.13)	0.21
Absent (n = 105)	0.15 (0.38)		0.17 (0.38)	
Leukopenia				
Present (n = 5)	0.22 (0.22)	0.64	0.18 (0.10)	0.82
Absent (n = 185)	0.17 (0.33)		0.15 (0.30)	
Urine protein/creatinine ratio				
≥ 0.5 (n = 76)	0.26 (0.24)	< 0.0001	0.14 (0.15)	0.34
< 0.5 (n = 106)	0.07 (0.10)		0.13 (0.17)	
Renal failure				
Ever (n = 15)	0.11 (0.15)	0.57	0.15 (0.17)	0.95
Never (n = 175)	0.18 (0.34)		0.15 (0.31)	
Hydroxychloroquine				
Yes (n = 146)	0.17 (0.36)	0.97	0.16 (0.33)	0.48
No (n = 44)	0.18 (0.23)		0.11 (0.12)	
Prednisone				
Yes (n = 155)	0.19 (0.36)	0.068	0.15 (0.32)	0.78
No (n = 35)	0.07 (0.07)		0.13 (0.17)	
Mycophenolate mofetil				
Yes (n = 99)	0.16 (0.17)	0.72	0.12 (0.14)	0.24
No (n = 91)	0.18 (0.45)		0.18 (0.41)	
Azathioprine				
Yes (n = 16)	0.45 (0.93)	0.0017	0.36 (0.89)	0.014
No (n = 174)	0.15 (0.20)		0.13 (0.16)	
Diabetes mellitus				
Present (n = 20)	0.08 (0.08)	0.30	0.17 (0.22)	0.69
Absent (n = 170)	0.18 (0.34)		0.15 (0.31)	
Use of ACE/ARB inhibitor				
Yes (n = 132)	0.14 (0.15)	0.0488	0.13 (0.16)	0.16
No (n = 58)	0.24 (0.55)		0.20 (0.48)	
SLICC Renal Activity Score				
≥ 4 (n = 64)	0.26 (0.25)	< 0.0001	0.14 (0.14)	0.17
< 4 (n = 107)	0.09 (0.13)		0.12 (0.15)	
SLEDAI				
≥ 4 (n = 53)	0.21 (0.24)	0.30	0.13 (0.13)	0.60
< 4 (n = 137)	0.16 (0.36)		0.16 (0.34)	

Table 1. Continued.

Clinical Variables at Each Visit	VCAM-1 Mean (SD)	p*	NGAL Mean (SD)	p*
Physician's Global Assessment				
≥ 1.5 (n = 89)	0.27 (0.45)	0.0002	0.18 (0.39)	0.23
< 1.5 (n = 101)	0.08 (0.11)		0.12 (0.17)	
Renal activity (VAS)				
≥ 1.5 (n = 75)	0.31 (0.47)	< 0.0001	0.19 (0.42)	0.19
< 1.5 (n = 115)	0.08 (0.10)		0.12 (0.16)	

* p values are based on a mixed-effects model because some patients contributed multiple observations. VCAM-1: vascular cell adhesion molecule-1; NGAL: neutrophil gelatinase-associated lipocalin; ACE: angiotensin-converting enzyme; ARB: angiotensin receptor blocker; SLICC: Systemic Lupus International Collaborating Clinics; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; VAS: visual analog scale.

also associated with the urine Pr/Cr ratio ($p < 0.0001$) and with the SLICC Renal Activity Score ($p < 0.0001$). SELENA-SLEDAI results (Table 1) showed no association of NGAL or VCAM-1 with SLEDAI when analyzed categorically. However, when we analyzed SLEDAI as a continuous variable, only VCAM-1 had a borderline association ($p = 0.0544$). NGAL was not associated with any measure of disease activity or with lupus serologies (Table 1). There was no strong relationship between the biomarkers and diabetes mellitus, current use of ACE inhibitor, or use of an angiotensin receptor blocker (Table 1). When we redid the analyses after excluding patients with diabetes mellitus and those receiving ACE inhibitor or angiotensin receptor blocker, all the results were qualitatively similar to those reported above.

Table 2 shows relationships between the biomarkers and urine Pr/Cr > 0.5. Among those with medium or high urinary VCAM-1, 63% and 87%, respectively, had urine Pr/Cr ≥ 0.5, compared to only 15% of those with low VCAM-1 ($p < 0.0001$).

Table 3 shows the sensitivity, specificity, and positive and negative predictive values of various rules, based on the biomarkers, for identifying patients with high levels of renal activity (i.e., Renal Activity Score ≥ 4).

Table 2. Proportion (%) of patient visits with urine protein/creatinine ratio ≥ 0.5 in subgroups defined by level of VCAM-1 and NGAL (normalized to urine creatinine).

Subgroups Defined by Biomarker Levels at Each Visit	Proportion (%) of Visits with Urine Protein/Creatinine Ratio ≥ 0.5	p*
VCAM-1**		
Low	15/97 (15)	< 0.0001
Medium	34/54 (63)	
High	27/31 (87)	
NGAL**		
Low	43/104 (41)	0.2810
Medium	18/49 (37)	
High	15/26 (57)	

* For difference between subgroups (low, medium, high) with respect to the proportion of visits with urine protein/creatinine ratio ≥ 0.5, calculated using a GEE approach that accounts for repeated measures from the same patients. ** Low: ≤ 0.1; medium: 0.1–0.3; high: ≥ 0.3 for VCAM-1 and NGAL. VCAM-1: vascular cell adhesion molecule-1; NGAL: neutrophil gelatinase-associated lipocalin.

Twenty-six patients had a renal biopsy within 3 months of the biomarker measurement. Tables 4 and 5 show the relationship between biomarker values and biopsy results within the last 3 and 6 months, respectively. Our results showed there was a higher percentage of patients with high VCAM-1 levels in Class V disease versus other ISN classes, although the results were not statistically significant.

Table 6 shows the association between the renal biopsy NIH activity and chronicity indices and clinical renal predictors. The NIH activity index was associated with the SLICC renal activity score ($p = 0.005$), 24-hour urine protein ($p < 0.0001$), and urine WBC count ($p < 0.0006$), whereas the NIH chronicity index score was associated with serum creatinine ($p < 0.0001$). There was no correlation between NGAL and VCAM-1 and the renal biopsy activity index ($p = 0.50$ and $p = 0.25$) or chronicity index ($p = 0.14$ and $p = 0.23$).

DISCUSSION

We analyzed urinary levels of VCAM-1 and NGAL and their associations with lupus serologies and markers of disease activity. VCAM-1 is an adhesion molecule that, although weakly expressed in normal kidneys, is increased many-fold in immune-mediated renal disease[29,38]. Our study found that an increased urinary VCAM-1 level correlated with the urine Pr/Cr ratio and the SLICC Renal Activity Score, as well as with other measures of disease activity. Similar findings have been reported in other studies[26,34,39]. However, our study included a larger patient population with serial sample collection over a longer period, in contrast to previous studies that had smaller patient populations, short followup, and serum sampling as opposed to urine sampling[26,34]. In contrast with 2 studies[26,34], we found no association of urinary VCAM-1 with low complement. We did find urinary VCAM-1 to be higher in Class V LN.

Activity and chronicity indices have provided useful information on renal survival in patients with SLE[40,41]. In our

Table 3. Sensitivity, specificity, and positive and negative predictive values of various rules for identifying patients with a SLICC renal activity score of 4 points or more based on VCAM-1 and NGAL.

Definition of a "Positive" Test	Sensitivity, %	Specificity, %	Positive Predictive Value, %	Negative Predictive Value, %
VCAM-1/creatinine ratio				
> 0.1	79	73	66	84
> 0.2	54	88	75	74
> 0.3	34	93	75	68
NGAL/creatinine ratio				
> 0.1	45	57	43	61
> 0.2	28	75	45	61
> 0.3	20	86	54	62

VCAM-1: vascular cell adhesion molecule-1; NGAL: neutrophil gelatinase-associated lipocalin; SLICC: Systemic Lupus International Collaborating Clinics.

Table 4. Analysis of renal biopsies by International Society of Nephrology class within 3 months.

Urine Biomarker	Class II, n (%)	Class III, n (%)	Class IV, n (%)	Class V, n (%)
VCAM-1*				
Low (n = 8)	0 (0)	3 (38)	2 (25)	3 (38)
Medium (n = 7)	2 (29)	3 (43)	2 (29)	4 (57)
High (n = 11)	0 (0)	3 (27)	3 (27)	9 (82)
NGAL*				
Low (n = 15)	2 (13)	5 (33)	4 (27)	9 (60)
Medium (n = 8)	0 (0)	3 (38)	2 (25)	5 (63)
High (n = 3)	0 (0)	1 (33)	1 (33)	2 (67)

* Low: ≤ 0.1; medium: 0.1–0.3; high: ≥ 0.3 for VCAM-1 and NGAL. VCAM-1: vascular cell adhesion molecule-1; NGAL: neutrophil gelatinase-associated lipocalin.

Table 5. Analysis of renal biopsies by International Society of Nephrology (ISN) class within 6 months.

Urine Biomarker	Class II, III, IV n (%)	p	Class V, n (%)	p
VCAM-1*				
Low (n = 12)	8 (67)	> 0.99	6 (50)	0.12
High (n = 21)	14 (67)		16 (76)	
NGAL*				
Low (n = 16)	12 (75)	0.45	10 (63)	0.71
High (n = 16)	10 (63)		11 (69)	

* Low: ≤ 0.1; high: ≥ 0.1 for VCAM-1 and NGAL. VCAM-1: vascular cell adhesion molecule-1; NGAL: neutrophil gelatinase-associated lipocalin.

Table 6. Association between renal biopsy National Institutes of Health (NIH) activity and chronicity indices and clinical renal predictors.

Variable	Estimated Effect on Mean Renal Biopsy NIH Activity Index per 1 Unit of Variable	p
Urinalysis protein	1.366 ± 0.768	0.08
Urinalysis red blood cells/hpf	0.144 ± 0.075	0.06
Urinalysis white blood cells/hpf	0.191 ± 0.056	0.0006
Urine creatinine level	0.016 ± 0.012	0.18
Urine protein/creatinine ratio	0.238 ± 0.355	0.50
24-h protein	4.020 ± 0.345	< 0.0001
Serum creatinine	–0.231 ± 1.697	0.47
SLICC renal activity score	0.437 ± 0.156	0.005
Log-normalized VCAM	–1.329 ± 1.154	0.25
Log-normalized NGAL	–0.878 ± 1.316	0.50
	Estimated Effect on Mean Renal Biopsy NIH Chronicity Index per 1 Unit of Variable	p
Urinalysis protein	–0.361 ± 0.357	0.31
Urinalysis red blood cells/hpf	0.017 ± 0.038	0.66
Urinalysis white blood cells/hpf	0.009 ± 0.026	0.71
Urine creatinine level	–0.003 ± 0.005	0.60
Urineprotein/creatinine ratio	–0.172 ± 0.157	0.28
24-h protein	–1.043 ± 0.603	0.084
Serum creatinine	2.511 ± 0.549	< 0.0001
SLICC renal activity score	–0.036 ± 0.083	0.66
Log-normalized VCAM	–0.627 ± 0.518	0.23
Log-normalized NGAL	–0.845 ± 0.577	0.14

SLICC: Systemic Lupus International Collaborating Clinics; VCAM-1: vascular cell adhesion molecule-1; NGAL: neutrophil gelatinase-associated lipocalin; hpf: high power field.

study the renal biopsy NIH activity index correlated with renal predictors, including the SLICC renal activity score, 24-hour urine protein, and urine WBC counts. As expected, the renal biopsy NIH chronicity index was associated with the serum creatinine findings. Serum creatinine and a high NIH renal biopsy chronicity index have been shown to have independently increased the risk for the development of chronic renal disease or death[42]. In our study there was a strong association between the 2. In contrast, urine levels of VCAM-1 and NGAL did not correlate with renal pathology activity or chronicity indices. However, in a separate unpublished study by our group, we noted that urine VCAM-1 levels correlate significantly with renal pathology activity index when the kidneys are biopsied on the same day the urine is collected.

In our study, in contrast to previous studies, urinary NGAL was not associated with either lupus serologies or any markers of renal disease activity. Two studies that did find an asso-

ciation of urinary NGAL with LN were cross-sectional[12,13]. Our study included a larger number of patients with multiple visits. It is possible that the different measures used to assess renal function/injury and the difficulty in reproducibly differentiating cumulative renal damage from acute active disease in the absence of a renal biopsy could explain different results reported from previous studies.

Urinary VCAM-1, but not urinary NGAL, appears to be a promising biomarker for lupus nephritis. Validation of urinary VCAM-1 in combination with other biomarkers, including concurrent renal biopsies, might allow this marker to be used in routine SLE clinical care.

ACKNOWLEDGMENT

We thank Elhaum Khobahy for excellent technical assistance.

REFERENCES

1. Austin HA, Boumpas DT, Vaughan EM, Balow JE. High-risk features of lupus nephritis: Importance of race and clinical and histological factors in 166 patients. Nephrol Dial Transplant 1995;10:1620-8.
2. Dooley MA, Hogan S, Jennette C, Falk R. Cyclophosphamide therapy for lupus nephritis: Poor renal survival in black Americans. Glomerular Disease Collaborative Network. Kidney Int 1997;51:1188-95.
3. Bastian HM, Alarcon GS, Roseman JM, McGwin G Jr, Vila LM, Fessler BJ, et al. Systemic lupus erythematosus in a multi-ethnic US cohort (LUMINA) XLII: Factors predictive of new or worsening proteinuria. Rheumatology 2007;46:683-9.
4. Cameron JS. Lupus nephritis. J Am Soc Nephrol 1999;10:413-24.
5. Ward MM. Changes in the incidence of end-stage renal disease due to lupus nephritis, 1982-1995. Arch Intern Med 2000;160:3136-40.
6. Ward MM. Changes in the incidence of endstage renal disease due to lupus nephritis in the United States, 1996-2004. J Rheumatol 2009;36:63-67.
7. Moroni G, Pasquali S, Casanova S, Zucchelli P, Ponticelli C. Clinical and prognostic value of serial renal biopsies in lupus nephritis. Am J Kidney Dis 1999;34:530-9.
8. Wuthrich RP. Vascular cell adhesion molecule-1 (VCAM-1) expression in murine lupus nephritis. Kidney Int 1992;42:903-14.
9. Wu T, Xie C, Bhaskarabhatla M, Yan M, Leone A, Chen SS, et al. Excreted urinary mediators in an animal model of experimental immune nephritis with potential pathogenic significance. Arthritis Rheum 2007;56:949-59.
10. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14:2534-43.
11. Ka SM, Rifai A, Chen JH, Cheng CW, Shui HA, Lee HS, et al. Glomerular crescent-related biomarkers in a murine model of chronic graft versus host disease. Nephrol Dial Transplant 2006;21:288-98.
12. Brunner HI, Mueller M, Rutherford C, Passo MH, Witte D, Grom A, et al. Urinary neutrophil gelatinase-associated lipocalin as a biomarker of nephritis in childhood-onset systemic lupus erythematosus. Arthritis Rheum 2006;54:2577-84.
13. Pitashny M, Schwartz N, Qing X, Hojaili B, Aranow C, Mackay M, et al. Urinary lipocalin-2 is associated with renal disease activity in human lupus nephritis. Arthritis Rheum 2007;56:1894-903.
14. Devarajan P. Review: Neutrophil gelatinase-associated lipocalin: A troponin-like biomarker for human acute kidney injury. Nephrology 2010;15:419-28.
15. Ohlsson S, Wieslander J, Segelmark M. Increased circulating levels of proteinase 3 in patients with anti-neutrophilic cytoplasmic autoantibodies-associated systemic vasculitis in remission. Clin Exp Immunol 2003;131:528-35.
16. Biezeveld MH, van Mierlo G, Lutter R, Kuipers IM, Dekker T, Hack CE, et al. Sustained activation of neutrophils in the course of Kawasaki disease: An association with matrix metalloproteinases. Clin Exp Immunol 2005;141:183-8.
17. Stoesz SP, Friedl A, Haag JD, Lindstrom MJ, Clark GM, Gould MN. Heterogeneous expression of the lipocalin NGAL in primary breast cancers. Int J Cancer 1998;79:565-72.
18. Nielsen BS, Borregaard N, Bundgaard JR, Timshel S, Sehested M, Kjeldsen L. Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel diseases. Gut 1996;38:414-20.
19. Furutani M, Arii S, Mizumoto M, Kato M, Imamura M. Identification of a neutrophil gelatinase-associated lipocalin mRNA in human pancreatic cancers using a modified signal sequence trap method. Cancer Lett 1998;122:209-14.
20. Lim R, Ahmed N, Borregaard N, Riley C, Wafai R, Thompson EW, et al. Neutrophil gelatinase-associated lipocalin (NGAL) an early-screening biomarker for ovarian cancer: NGAL is associated with epidermal growth factor-induced epithelio-mesenchymal transition. Int J Cancer 2007;120:2426-34.
21. Mishra J, Ma Q, Kelly C, Mitsnefes M, Mori K, Barasch J, et al. Kidney NGAL is a novel early marker of acute injury following transplantation. Pediatr Nephrol 2006;21:856-63.
22. Trachtman H, Christen E, Cnaan A, Patrick J, Mai V, Mishra J, et al. Urinary neutrophil gelatinase-associated lipocalcin in D+HUS: A novel marker of renal injury. Pediatr Nephrol 2006;21:989-94.
23. Mishra J, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin: A novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol 2004;24:307-15.
24. Bachorzewska-Gajewska H, Malyszko J, Sitniewska E, Malyszko JS, Dobrzycki S. Neutrophil-gelatinase-associated lipocalin and renal function after percutaneous coronary interventions. Am J Nephrol 2006;26:287-92.
25. Wu T, Xie C, Wang HW, Zhou XJ, Schwartz N, Calixto S, et al. Elevated urinary VCAM-1, P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. J Immunol 2007;179:7166-75.
26. Ikeda Y, Fujimoto T, Ameno M, Shiiki H, Dohi K. Relationship between lupus nephritis activity and the serum level of soluble VCAM-1. Lupus 1998;7:347-54.
27. Wuthrich RP. Vascular cell adhesion molecule-1 (VCAM-1) expression in murine lupus nephritis. Kidney Int 1992;42:903-14.
28. Nakatani K, Fujii H, Hasegawa H, Terada M, Arita N, Ito MR, et al. Endothelial adhesion molecules in glomerular lesions: Association with their severity and diversity in lupus models. Kidney Int 2004;65:1290-300.
29. Seron D, Cameron JS, Haskard DO. Expression of VCAM-1 in the normal and diseased kidney. Nephrol Dial Transplant 1991;6:917-22.
30. Brady HR. Leukocyte adhesion molecules and kidney diseases. Kidney Int 1994;45:1285-300.
31. Hillis GS, Duthie LA, Brown PA, Simpson JG, MacLeod AM, Haites NE. Upregulation and colocalization of connexin 43 and cellular adhesion molecules in inflammatory renal disease. J Pathol 1997;182:373-9.
32. Bechtel U, Scheuer R, Landgraf R, Konig A, Feucht HE. Assessment of soluble adhesion molecules (sICAM-1, sVCAM-1, sELAM-1) and complement cleavage products (sC4d, sC5b-9) in urine. Clinical monitoring of renal allograft recipients.

Transplantation 1994;58:905-11.
33. Abd-Elkareem MI, Al Tamimy HM, Khamis OA. Increased urinary levels of the leukocyte adhesion molecules ICAM-1 and VCAM-1 in human lupus nephritis with advanced renal histological changes: Preliminary findings. Clin Exp Nephrol 2010;14:548-57.
34. Spronk PE, Bootsma H, Huitema MG, Limburg PC, Kallenberg CG. Levels of soluble VCAM-1, soluble ICAM-1, and soluble E-selectin during disease exacerbations in patients with systemic lupus erythematosus (SLE); a long term prospective study. Clin Exp Immunol 1994;97:439-44.
35. Petri M, Kim MY, Kalunian KC, Grossman J, Hahn BH, Sammaritano LR, et al: OC-SELENA Trial. Combined oral contraceptives in women with systemic lupus erythematosus. N Engl J Med 2005;353:2550-8.
36. Petri M, Hellmann D, Hochberg M. Validity and reliability of lupus activity measures in the routine clinic setting. J Rheumatol 1992;19:53-9.
37. Petri M, Kasitanon N, Lee SS, Link K, Magder L, Bae SC, et al; Systemic Lupus International Collaborating Clinics. Systemic lupus international collaborating clinics renal activity/response exercise: Development of a renal activity score and renal response index. Arthritis Rheum 2008;58:1784-8. (Erratum in: Arthritis Rheum 2008;58:2823).
38. Alpers CE, Hudkins KL, Davis CL, Marsh CL, Riches W, McCarty JM, et al. Expression of vascular cell adhesion molecule-l in kidney allograft rejection. Kidney Int 1993;44:805-16.
39. Molad Y, Miroshink E, Sulkes J, Pitlik S, Weinberger A, Monselise Y. Urinary soluble VCAM-1 in systemic lupus erythematosus: A clinical marker for monitoring disease activity and damage. Clinic Exp Rheumatol 2002;20:403-6.
40. Austin HA, Muenz LR, Joyce KM, Antonovych TT, Balow JE. Diffuse proliferative lupus nephritis: Identification of specific pathologic features affecting renal outcome. Kidney Int 1984;25:689-95.
41. Austin HA, Muenz LR, Joyce KM, Antonovych TA, Kullick ME, Klippel JH, et al. Prognostic factors in lupus nephritis. Contribution of renal histologic data. Am J Med 1983;75:382-91.
42. Contreras G, Pardo V, Cely C, Borja E, Hurtado A, De La Cuesta C, et al. Factors associated with poor outcomes in patients with lupus nephritis. Lupus 2005;14:890-5.