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The role of urinary neutrophil gelatinase-associated lipocalin in lupus nephritis

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ARTICLE INFO

ABSTRACT

Background: Urine neutrophil gelatinase-associated lipocalin (uNGAL) has been proposed as a potential biomarker for lupus nephritis (LN) activity. We determined the association between uNGAL with LN activity in systemic lupus erythematosus (SLE) patients compared to the current standard markers of SLE.
Methods: A total of 100 SLE patients with biopsy-proven LN were recruited—47 with active and 53 inactive LN. uNGAL levels were measured. Renal function test, urinary parameters, lupus serology and calculated renal SLE Disease Activity Index-2K (renal SLEDAI-2K) were analyzed to determine their associations with uNGAL.
Results: Normalized uNGAL levels (ng/mg creatinine) were significantly higher in patients with active LN compared to those with inactive disease (p = 0.01). uNGAL and renal SLEDAI-2K were associated (r = 0.32, p = 0.001). Multiple logistic regression showed that only serum creatinine and renal SLEDAI-2K were independent predictors of uNGAL levels (p = 0.03 and 0.02 respectively). Analysis of the receiver operating characteristic (ROC) curve showed that uNGAL was a potential biomarker for LN.
Conclusions: uNGAL was increased in active LN especially in LN flares. Serial measurements of uNGAL levels may be of value in monitoring response of LN to treatment and for predicting LN flares.

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1. Introduction

Lupus nephritis (LN) is one of the most common and severe complications of systemic lupus erythematosus (SLE) affecting approximately 50%–70% of patients [1–3]. LN is associated with significant morbidity and mortality [1,4] particularly among patients of Hispanic, African–American [5] and Asian ethnicities [6]. Recent data report that patients develop end stage renal disease (ESRD) some 10 years after the first episode of LN [7,8]. Death due to ESRD occurs in 5% to 25% within 5 years in the more severe forms of LN [9]. However ESRD in LN has been delayed or prevented following the use of cyclophosphamide [10]. The survival of lupus patients has improved markedly due to advances in SLE/LN therapy. This also has been reported in the Asian population [11,12].

Renal biopsy is the 'gold' standard for the diagnosis of LN and its severity. However, it is a relatively 'invasive' procedure and repeated serial renal biopsies are not always practical in real life practice especially in frequent relapsers or in patients with associated severe hematologic or cerebral manifestations. Furthermore, it may not always reliably represent the global status of the kidney [13].

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa protein that is produced in many tissues and organs including epithelial tissues, endothelium and bone marrow and its production is increased in neoplastic and inflammatory conditions [14]. Several reports have shown that NGAL levels are an early biomarker of acute kidney injury (AKI) in a variety of acute clinical settings. These include post cardiac surgery [15], in critically ill patients [16] and post contrast for coronary angiography in adult and pediatric patients [17–19]. Experimental models of LN have shown that antibody-mediated nephritis stimulates local expression of NGAL which plays a role in the pathogenesis of nephritis by promotion of inflammation and apoptosis. Thus pharmacologic blockade of NGAL formation may be a novel therapeutic intervention in antibody-mediated nephritis and deserves further exploration [20]. In humans, studies have also demonstrated that NGAL is a potential biomarker of LN activity [21–24]. We therefore aimed to determine the association between uNGAL with renal disease activity in SLE patients with biopsy-proven LN compared to the current standard markers viz proteinuria and renal SLEDAI-2K.

2. Patients and methods

This was a cross-sectional, observational study which recruited SLE patients with biopsy proven LN [25]. These patients were divided into two groups based on the presence or absence of LN activity. Patients

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with relapse/flare of LN were included in the active group. We excluded LN patients with ESRD or who required chronic dialysis or had undergone renal transplantation and those with clinical LN in whom a renal biopsy could not be performed as well as pregnant patients. Informed consent was obtained from all recruited subjects. The study protocol was approved by the Medical Research and Ethics Committee of the Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

2.1. SLE Disease Activity Indices (SLEDAI-2K) and laboratory testing

Bombardier et al. [26] developed a scoring system of the SLE Disease Activity Index (SLEDAI). The score ranges from 0 to 105, with higher scores representing increased disease activity. This was revised by Gladman et al. [27] – SLEDAI-2K – so as to capture ongoing, new or recurrent disease activity. SLEDAI-2K is divided into: a) SLEDAI-2K — global score (range 0–150), b) SLEDAI-2K — extrarenal score (range 0–63) and c)SLEDAI-2K — renal score (range 0–16).

Laboratory tests included full blood count, blood urea nitrogen (BUN), serum creatinine, estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) formula, urinalysis by dipstick, urine microscopy, urine protein creatinine index (uPCI), serum complement 3 and 4 levels (C3, C4) and anti-dsDNA antibody titers (anti-dsDNA Ab).

2.2. Definition of LN activity

2.2.1. Active LN was defined by the presence of one or more of the following criteria:

2.2.1.1. Proteinuria with or without any of the following features [28] including presence of hematuria or red cell casts, and increase in serum creatinine or decline in eGFR.

Proteinuria was measured as spot morning urine protein creatinine index (uPCI) and was positive if the value was >1000 mg/mmol (NR ≤ 200).

2.2.1.2. Renal SLEDAI score ≥4 [27].

The score included the presence of any one/more of the following feature/s in the urinalysis: proteinuria, hematuria, leucocyturia or urinary red cell casts after exclusion of stones, concurrent urinary tract infection or other causes [26].

2.2.2. Relapse/flare of LN

Relapse/flare of LN was defined as recurrence of renal disease activity after a period of remission ≥ 3 months for the purpose of this study [28].

2.2.3. Remission

Remission was defined as absence of disease activity and no change in immunosuppressive therapy for at least 3 months [28].

2.2.4. Inactive LN

Inactive LN was defined by the presence of one or more of the following criteria:

2.2.4.1. Proteinuria (uPCI) <500 mg/mmol with/without serum albumin ≥35 g/l, inactive urine sediments (<5 red cells/HPF) and no red cell casts and no leucocyturia (<5 white cells/HPF) and stable serum creatinine (unless due to other causes e.g. renin-angiotension system (RAS) blockade) [28].

2.2.4.2. Renal SLEDAI score 0 or <4.

2.3. Laboratory testing for uNGAL

All fresh urine samples collected were immediately centrifuged to remove sediments and frozen in aliquots at −80 °C for later NGAL batch testing. The commercially available enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems was used. It is a sandwich monoclonal ELISA designed to measure human Lipocalin-2/NGAL in urine, cell culture supernates, serum, plasma and saliva. Briefly, standard or sample was added to each well and left to incubate for 2 h at 2–8 °C. The plates were washed 4 times to remove any unbound substances. Conjugate was then added to each well and incubated for 2 h at 2–8 °C, then the plates were washed four times and Substrate Solution was added to each well. After incubation for 30 min at room temperature, the Stop solution was added to each well. Finally, the absorbance was read at 450 nm with the correction wave length set at 540 nm. uNGAL levels are reported as ng/mg of urinary creatinine (normalized value) so as to standardize uNGAL to renal creatinine excretion.

3. Statistical analysis

Kolmogorov–Smirnov test with p > 0.05 was used to determine the normality of the data. Normally distributed continuous variables are expressed as mean [±SD]. Non-normally distributed variables are presented as median [interquartile range (IQR)]. Categorical variables are presented as counts (percentages). Pearson's χ^2 test was used to compare categorical variables and a 2-sided independent-sample *t* test was used to compare normally distributed variables. The Mann–Whitney *U* test and Kruskal–Wallis tests were used for non-normally distributed variables. Correlations between uNGAL levels with relevant laboratory parameters were examined using Spearman's correlation coefficients. Furthermore, multiple logistic regression was used to analyze the association of uNGAL with relevant laboratory parameters as well as LN duration and medications.

Receiver operator characteristic curves were constructed for uNGAL and the usual standard markers used for predicting LN activity. The area under the curve (AUC) with associated 95% confidence interval (CI) of each parameter served as a measure of the discriminatory capacity of uNGAL and anti-dsDNA Ab titers for early detection of LN activity. The best cut-off value for each test was determined using the maximization of the Youden index (sensitivity + specificity − 1) [29]. The SPSS software ver 18.0 was used for statistical analysis. All tests were 2-sided and p < 0.05 was considered statistically significant.

4. Results

4.1. Clinical characteristics of the study patients

After screening, 100 patients were eligible for inclusion in the study. Their demographic and clinical characteristics are presented in Table 1. All had established SLE by ACR criteria with biopsy-proven LN. There were 47 patients with active LN and 53 with inactive LN. As expected, there were significant differences between the two groups in the following parameters: serum albumin, urinary proteinuria, uPCI, SLEDAI-2K (global) and SLEDAI-2K (renal). Detailed findings are shown in Table 2.

4.2. Association of various measures of SLE and LN activity

There were significant correlations between SLEDAI-2K global with both renal (r = 0.78, p < 0.001) and extrarenal scores (r = 0.48, p < 0.001). However, the SLEDAI-2K (renal) and SLEDAI-2K (extrarenal) were not correlated (r = -0.05, p = 0.58).

4.3. Distribution of uNGAL levels

uNGAL levels were significantly higher in patients with active LN compared to those with inactive LN. The uNGAL was 195.80 ng/mg creatinine (IQR 21.07–1413) in active LN compared to 83.66 ng/mg creatinine (IQR 0–746.5) in inactive LN (p = 0.01) as illustrated in Fig. 1.

Table 1
Demographic and clinical characteristics of study patients with lupus nephritis.

Parameters	All subjects	Active LN	Inactive LN	p value
	n = 100	n = 47	n = 53	
Demographic				
Age, mean ± SD y	36.90 ± 10.62	36.40 ± 9.97	37.33 ± 11.24	NS
Female: no. (%)	92 (92)	43 (46.7)	49 (92.5)	NS
Male: no. (%)	8 (8)	4 (8.5)	4 (7.5)	
Race: no. (%)				
Malay	41(41)	24 (51.1)	17 (32.1)	NS
Chinese	55 (55)	21 (44.7)	34 (64.2)	
Indian	4 (4)	2 (4.3)	2 (3.8)	
LN duration (years) median (IQR)	7 (1–24)	7 (1–124)	7 (1–17)	NS
Biopsy- proven lupus nephritis no (%)				
WHO class I	1 (1)	1 (2.1)	0 (0)	0.71
WHO class II ± V	6 (6)	3 (6.4)	3 (5.7)	
WHO class III ± V	34 (34)	15 (31.9)	19 (35.8)	
WHO class IV ± V	52 (52)	26 (55.3)	26 (49.1)	
WHO class V	5 (5)	1 (2.1)	4 (7.5)	
WHO class VI	2 (2)	1 (2.1)	1 (1.9)	
Activity index median (IQR)	8 (0–19)	9 (0–16)	8 (0–19)	NS
Chronicity index median (IQR)	3 (0–15)	3.58 (0–9)	3 (1–15)	NS
CKD stage				
Stage 1 (eGFR >90)	61 (61)	25 (53.2)	36 (67.9)	NS
Stage 2 (eGFR 60–89)	22 (22)	10 (21.3)	12 (22.6)	
Stage 3 (eGFR 30–59)	14 (14)	9 (19.1)	5 (9.4)	
Stage 4 (eGFR 15–29)	3 (3)	3 (6.4)	0 (0%)	
Medications, no (%)				
Prednisolone	95 (95)	43 (91.5)	52 (98.1)	NS
Cyclophosphamide	8 (8)	8 (17)	0 (0)	0.002
Mychophenolic acid	22 (22)	12 (25.5)	10 (18.9)	NS
Cyclosporine/Tacrolimus	30 (30)	19 (40.4)	11 (20.8)	0.03
Azathioprine	36 (36)	12 (25.5)	24 (45.3)	0.04
Hydroxychloroquine	42 (42)	20 (42.6)	22 (41.5)	NS
Renin angiotension system blockers (ACEI/ARB/Spironolactone)	68 (68)	29 (61.7)	39 (73.6)	NS
Aspirin	13 (13)	44 (83)	9 (17)	NS

Abbreviations: IQR: interquartile range; LN: lupus nephritis; ACEI: angiotension converting enzyme inhibitors; ARBs: angiotension receptor blockers.

4.4. Association of uNGAL with various laboratory parameters and LN disease activity

uNGAL was associated with uPCI (r = 0.34, p = 0.001), leucocyturia (r = 0.21, p = 0.03), SLEDAI-2K (global) (r = 0.19, p = 0.05) and
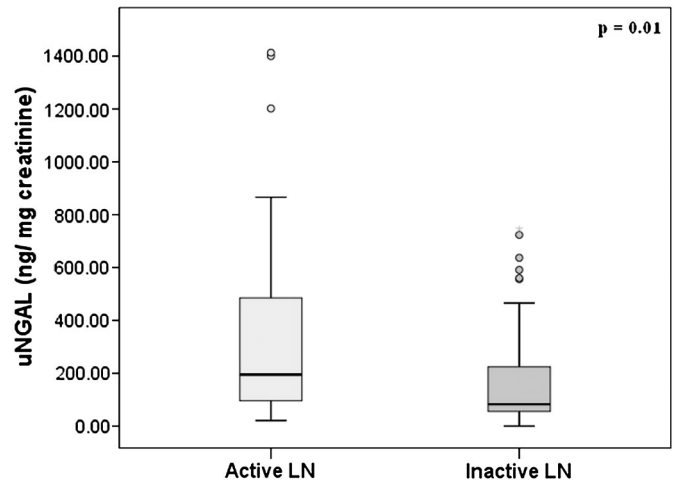

Fig. 1. Median uNGAL levels in the two patients groups. uNGAL levels are significantly increased in patients with active LN (n = 47) compared to inactive LN (p = 0.01).

SLEDAI-2K (renal) (r = 0.32, p = 0.001; Fig. 2). These are summarized in Table 3.

4.5. Correlation between uNGAL with LN disease duration and treatment

uNGAL levels were associated with LN duration (r = 0.27, p = 0.03) and did not differ with the use of corticosteroids or immunosuppressive medications. Proteinuria (uPCI) and uNGAL levels were not different between those patients who received renin angiotension system (RAS) blockers (ACE-inhibitors and/or ARBs and/or spironolactone) compared with those who did not (p = 0.18 and 0.27 respectively).

4.6. Independent predictors of uNGAL levels

Multiple logistic regression was performed to assess the independent predictors of uNGAL levels. uNGAL levels were converted from numerical to categorical variable (high or low levels) based on its cutoff value from the ROC curve. All parameters with a p value ≤ 0.1 in Table 3 as well as LN duration and medications were entered into the regression model.

Only serum creatinine (odds ratio (OR) = 1.04; 95% CI, 1.003–1.09, p = 0.03) and SLEDAI-2K renal score (OR = 1.58; 95% CI, 1.05–2.36, p = 0.02) were independent predictors of uNGAL levels. Notably,

Table 2
Laboratory parameters and SLEDAI-2K scores between the patient groups.

Parameters[a]	Active LN	Inactive LN	p value
	n = 47	n = 53	
Hemoglobin (NR 14.0–17.0 g/dl)[b]	12.2 (8.6–16.6)	12.3 (8.5–15.6)	NS
Total WBC (NR 4.0–10.0 × 10^9/l)[c]	7.7 ± 3.6	7.1 ± 2.65	NS
Platelets (NR 150–400 × 10^9/l)[c]	234 ± 112	256 ± 83.5	NS
Anti-dsDNA titres (NR < 30 IU)[b]	35.18 (1.73–195.97)	24.24 (0.81–279.21)	NS
Serum C3 (NR 79–152 mg/dl)[c]	100.5 ± 36.39	109.62 ± 39.94	NS
Serum C4 (NR 16–38 mg/dl)[c]	21.46 ± 12.82	22.94 ± 11	NS
Serum albumin (NR 35–50 g/l)[c]	37.78 ± 5.54	41.88 ± 3.59	<0.001
Serum creatinine (NR 44–80 μmol/l)[b]	69 (33–252)	63 (41–158)	NS
eGFR (NR > 60 ml/min/1.73 m^2)[c]	93.61 ± 46.01	99.75 ± 31.54	NS
Urinary protein (NR < 300 mg/l)[b]	167.00 (8.30–1811)	45.30 (1.33–494.5)	<0.001
uPCI (NR < 200 mg/mmol creatinine)[b]	1100 (100–5100)	200 (100–600)	<0.001
uNGAL (ng/mg creatinine)[b]	195.80 (21.07–1413)	83.66 (0–746.5)	0.01
SLEDAI-2K (global: 0–105)[b]	8 (0–18)	2 (0–10)	<0.001
SLEDAI-2K (renal: 0–16)[b]	4 (0–16)	0 (0–3)	<0.001
SLEDAI-2K (extra-renal: 0–89)[b]	2 (0–10)	2 (0–10)	NS

[a] Abbreviations: NR: normal range; LN: lupus nephritis; WBC: white blood cell count; anti-dsDNA: anti-double-stranded DNA; C3: complement 3; C4: complement 4: eGFR: estimated glomerular filtration rate; uPCI: urine protein creatinine index; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index-2K.
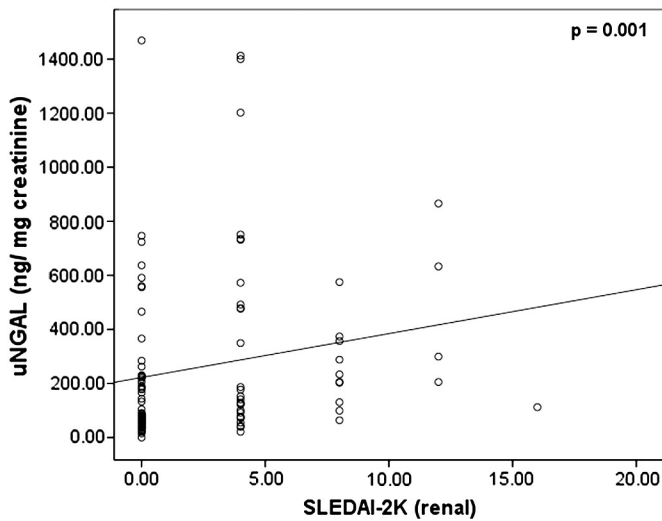[b] Median (interquartile range).
[c] Mean (SD).

Fig. 2. Correlation between uNGAL and SLEDAI-2K (renal) (r = 0.23, p = 0.001).

corticosteroids, immunosuppressive agents and RAS blockers were not independently associated with uNGAL levels (Table 4).

4.7. ROC curve analysis of uNGAL levels to identify SLE patients with active LN

ROC curves were constructed to assess the potential diagnostic and predictive values of uNGAL for identifying SLE patients with active LN as illustrated in Fig. 3A. The area under the curve (AUC) for uNGAL was 0.83 (95% CI: 0.74–0.92: p = 0.001). The Youden index was highest (0.57) at a cut-off value at 91.25 ng/mg creatinine. At this point uNGAL had a sensitivity of 0.89 and a specificity of 0.67. ROC curves for uNGAL compared with standard biochemical markers for LN activity were also constructed. The AUC for proteinuria (uPCI) was 0.94 (95% CI: 0.90–0.99: p < 0.001) and that for SLEDAI-2K renal score was 0.96 (95% CI: 0.90–1: p < 0.001). These were higher than those for uNGAL (Fig. 3B). Whereas the AUCs for hematuria and leucocytuuria were lower than those for uNGAL. In comparison, the AUCs for the serological markers (anti-dsDNA Ab titers and serum complement), serum albumin and

serum creatinine were lower than that for uNGAL for detection of LN activity. The AUCs for anti-dsDNA Ab titers, C3, C4 were 0.48 (p = 0.78), 0.39 (p = 0.09) and 0.43 (p = 0.35) respectively. Whereas the AUC for serum albumin was 0.23 (p = 0.004) and for serum creatinine was 0.59 (p = 0.14).

5. Discussion

In SLE patients, lupus nephritis is a common and severe affliction which carries significant morbidity and mortality. In this modern era, active severe LN often responds to judiciously administered immuno-suppressive treatment. However these medications themselves are also associated with significant morbidity and even mortality while uncontrolled LN leads to chronic or end stage kidney disease and even death. Current routine laboratory markers lack both sensitivity and specificity for LN disease activity in particular for flares and for response to treatment. This study was designed to evaluate the role of uNGAL as an early biomarker for this purpose and compared the uNGAL levels between those patients with active LN to those with inactive renal disease.
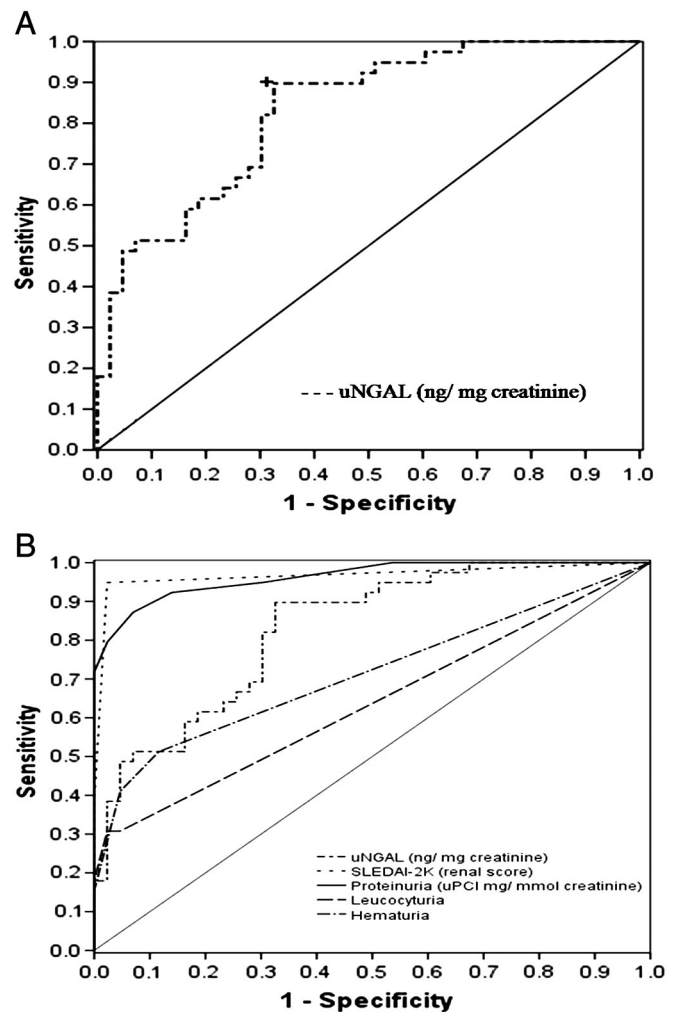
Table 3
Association between uNGAL and various parameters in the study patients.

Spearman's rho variable	uNGAL (ng/mg creatinine)	
	r	p
Hemoglobin	−0.19	NS
White blood cell	0.07	NS
Platelets	0.10	NS
Serum albumin	−0.11	NS
Serum creatinine	0.17	NS
eGFR	−0.18	NS
C3 (mg/dl)	−0.09	NS
C4 (mg/dl)	−0.02	NS
Anti-dsDNA Ab (IU)	0.17	NS
Urinary protein (mg/l)	0.07	NS
Urine protein creatinine index (uPCI)	0.34	0.001
Leucocyturia	0.21	0.03
Hematuria	0.10	NS
SLEDAI-2 K global score	0.19	0.05
SLEDAI -2 K renal score	0.32	0.001
SLEDAI-2 K-extra renal score	−0.12	NS

Abbreviations: r: Spearman's rho correlation coefficient; eGFR: estimated glomerular filtration rate; C3: complement 3; C4: complement 4; anti-dsDNA: anti–double-stranded DNA; uPCI: urine protein creatinine index; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index-2K.

Fig. 3. Receiver operating characteristic curves of uNGAL, proteinuria, SLEDAI-2K renal score, leucocyturia and hematuria for the diagnosis of LN activity in SLE patients. (A) The interrupted curve represents the uNGAL; the area under the curve (AUC) was 0.83 (p = 0.001). The symbol represents the best cut-off value for uNGAL (91.25 ng/mg creatinine) with sensitivity of 0.89 and specificity of 0.67. (B) The AUC for proteinuria was 0.94 (95% CI: 0.90–0.99: p < 0.001) and that for SLEDAI-2K renal score was 0.96 (95% CI: 0.90–1: p < 0.001). These AUCs were greater than that for uNGAL. The AUC for hematuria was 0.68 (p = 0.004) and for leucocyturia was 0.63 (p = 0.03).

Table 4
Multivariable association between uNGAL with relevant laboratory parameters and LN treatment.

	B	SE	p	OR	95% CI	
					Lower	Upper
Hemoglobin	−0.25	0.18	NS	0.77	0.53	1.12
Serum creatinine	0.04	0.02	0.03	1.04	1.003	1.09
eGFR	0.02	0.01	NS	1.02	0.99	1.05
uPCI	1.62	5.96	NS	5.07	0.01	60.67
Leucocyturia	0.03	0.14	NS	1.03	0.78	1.36
Anti ds DNA Ab titers	0.005	0.005	NS	1.00	0.99	1.01
SLEDAI-2K renal score	0.45	0.20	0.02	1.58	1.05	2.36
SLEDAI-2K global score	−0.20	0.13	NS	0.81	0.63	1.05
LN duration	0.001	0.06	NS	1.001	0.87	1.13
Prednisolone	−1.61	2.09	NS	0.19	0.003	12.13
Cyclophosphamide	1.17	1.49	NS	3.22	0.17	60.42
Mychophenolic acid	0.45	0.82	NS	1.56	0.31	7.86
Azathioprine	−0.46	0.71	NS	0.62	0.15	2.54
Cyclosporine or Tacrolimus	−0.75	0.74	NS	0.46	0.11	2.005
Rennin angiotension system blockers	0.72	0.64	NS	2.06	0.58	7.38

R^2 0.22 (Hosmer & Lemeshow's), 0.28 (Cox & Snell), 0.38 (Nagelkerke). Model x^2 = 27.79, p = .023.
B: beta; eGFR: estimated glomerular filtration rate; uPCI: urine protein creatinine index; SLEDAI-2 K: Systemic Lupus Erythematosus Disease Activity Index-2K; Renin angiotension system blockers: (angiotension converting enzyme inhibitors, angiotension receptor blockers, spironolactone).

Most of the previous studies have investigated the role of uNGAL in LN patients compared to SLE patients without renal involvement or healthy controls and showed that uNGAL may be a potential biomarker for LN disease activity [21–24]. Brunner et al. [21] studied 35 pediatric patients with SLE and found that uNGAL levels were significantly higher in patients with SLE compared to those with juvenile idiopathic arthritis (JIA). uNGAL was also higher in patients with (biopsy proven) LN but not in those without. Pitashny et al. [22] studied a cohort of adults with SLE (32/70 had active LN) found that uNGAL was higher in patients with LN compared to those without. Rubinstein et al. [23] in a longitudinal study showed that uNGAL level was a significant predictor of LN activity in these patients. In particular, it was a predictor for flares in patients with biopsy-proven LN. Suzuki et al. [24] in a longitudinal study of childhood-onset SLE patients demonstrated that uNGAL was a useful biomarker for LN activity. Hinze et al. [31] in another longitudinal study of childhood-onset SLE patients also found uNGAL to be predictive of worsening LN. These authors also reported that plasma NGAL may be predictive of worsening global and renal disease activity [30]. Our finding of elevated uNGAL levels in active LN thus concurs with those reported in the literature. SLE patients with renal flares also had the highest uNGAL levels thus providing further evidence for uNGAL as a predictor for LN activity.

Since immunosuppressive agents are the standard treatment of severe LN, their use was significantly associated with LN disease activity. This was an expected but nonetheless important finding. However, there were no associations between uNGAL levels and the use of these medications at the prevailing doses. These findings are in contrast to those reported by other authors [21,22]. Pitashny et al. [22] reported an association between uNGAL levels and steroid dose. Whereas Brunner et al. [21] demonstrated associations between uNGAL with cyclophosphamide and azathioprine use. This discrepancy can be explained by the fact that these authors had compared uNGAL levels in SLE patients with and without renal involvement. Whereas our study cohort all had severe LN but with varying levels of activity.

Contrary to the findings reported by Brunner et al. [22] and Suzuki et al. [24] we found that uNGAL levels were not associated with the use of RAS blockers although there were trends toward lower uNGAL levels among our patients who received these medications. However, this is an observation only as this was not the objective of our study.

In general, the effect of RAS blockers agents on uNGAL has not been extensively studied.

In multiple logistic regression, serum creatinine and SLEDAI-2K renal score were independent predictors of uNGAL levels. None of the immunosuppressive medications or RAS blockers were associated with uNGAL levels.

Due to the delay between the urine collection for uNGAL assays and renal biopsies, we could not correlate this biomarker with LN classes as the histological features of LN may change with time and treatment.

It is well known that renal and extrarenal lupus manifestations often occur independently of each other. This is demonstrated by the highly significant correlation between the SLEDAI-2K (global) and SLEDI-2K (renal) scores but not between SLEDI-2K (renal) and SLEDI-2K (extrarenal) scores. This fact is also reflected in the uNGAL levels of our LN patients. Our findings are similar to those reported by Pitashny et al. [22] and Brunner et al. [21] whereas Pitashny et al. [22] found that uNGAL correlated with parameters of renal disease activity rather than with extrarenal or global disease activity. In our cohort, uNGAL also correlated with SLEDAI-2K (global) suggesting that high uNGAL in active LN reflected the renal components of the SLEDAI-2K score [22]. Brunner et al. [21] also reported similar findings to ours.

In our patients, uNGAL levels were associated with leucocyturia – an index of renal parenchymal inflammation – as urinary tract infection (UTI) had been excluded both clinically and by laboratory investigations. uNGAL levels also correlated with proteinuria and this finding concurs with those reported by Brunner et al. [21] and Pitashny et al. [22].

Increased uNGAL levels in LN may reflect the underlying tubular dysfunction and may be a useful predictor of LN flares. However the possibility of increasing leakage of NGAL by the glomerular capillary cannot be excluded. Bolignano et al. [32] hypothesized that the increase in uNGAL levels in chronic renal proteinuric disease is due to excessive protein loss which in turn leads to saturation of renal tubular cell transporters resulting in reduced tubular reabsorption of NGAL. The persistent proteinuria will activate the intratubular complement cascade which further damages tubular cells thus enhancing the impaired reabsorption of NGAL. In turn, the injured tubular cells may produce NGAL as a compensatory mechanism against complement-induced oxidative stress and apoptosis [32].

This was an observational study and all patients with active LN would already have been started on multi-targeted therapy as per standard practice at our center. This may explain their elevated levels of uNGAL albeit 'subdued' at the higher normal ranges. This implies that uNGAL may be useful for following the response of LN to treatment. Of greater interest was our finding that patients with 'flares' of their LN had the highest uNGAL levels, again lending support to uNGAL being a predictor of flare or relapse of LN.

The AUCs for proteinuria and SLEDAI-2K were greater than those for uNGAL. This was expected as both are standard parameters used for the definition of active LN. However, the other parameters (hematuria, leucocyturia, serum albumin and creatinine) showed poorer performances for detection of LN activity. Although anti-dsDNA Ab titers and serum complement levels are commonly used in clinical practice to evaluate LN activity, we did not find any correlation between these parameters with LN disease activity. Furthermore there were no associations between these markers with uNGAL. These findings are consistent with those reported by many authors [21,22]. Anti-dsDNA Ab titers have a low sensitivity (~50%) for predicting LN activity or renal flares [33]. Serum complement levels are also unreliable indicators when used in isolation and need to be interpreted in conjunction with other laboratory parameters for a more accurate evaluation of LN activity [34]. Data from our ROC analysis showed that anti-dsDNA Ab titers and serum complement levels have lower performances compared to uNGAL levels. These results concur with those reported by Rubinstein et al. [23] who demonstrated in a longitudinal study that uNGAL levels were superior to anti-dsDNA Ab titers in predicting renal flares in patients with biopsy-proven LN.

The main limitation of our study was the time lag between urine collection for uNGAL and renal biopsy. Thus it was not possible to correlate uNGAL with the histological classes of LN. We probably would have obtained more conclusive results if urine samples for the biomarker were taken simultaneously with renal biopsies for those patients who developed LN flares or who had persistently active or refractory LN despite the usual standard treatment.

In conclusion, our study showed that uNGAL was increased in active LN especially LN flares. Since only a few small longitudinal studies have been performed to date, the further evaluation of uNGAL in a large prospective longitudinal study to confirm its potential application as a superior or additional biomarker for non invasive monitoring of LN disease activity and for the detection of renal flares is warranted.

Acknowledgments

References

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