

# Neutrophil Gelatinase–Associated Lipocalin Is a Predictor of the Course of Global and Renal Childhood-Onset Systemic Lupus Erythematosus Disease Activity

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**Objective.** To determine whether neutrophil gelatinase–associated lipocalin (NGAL) can predict worsening of global and renal disease activity in childhood-onset systemic lupus erythematosus (SLE).

**Methods.** One hundred eleven patients with childhood-onset SLE were enrolled in a longitudinal, prospective study with quarterly study visits and had at least 3 study visits. At each visit, global disease activity was measured using 3 external standards: the numerically converted British Isles Lupus Assessment Group (BILAG) index, the SLE Disease Activity Index 2000 update score, and the physician’s assessment of global disease activity. Renal and extrarenal disease activity were measured by the respective domain scores. The disease course over time was categorized at the most recent visit (persistently active, persistently inactive, improved, or worsening). Plasma and urinary NGAL levels were measured by enzyme-linked immunosorbent

assay, and urinary NGAL levels were standardized to the urinary creatinine concentration. The longitudinal changes in NGAL levels were compared with the changes in SLE disease activity using mixed-effect models.

**Results.** Significant increases in standardized urinary NGAL levels of up to 104% were detected up to 3 months before worsening of lupus nephritis (as measured by all 3 external standards). Plasma NGAL levels increased significantly by as much as 26% up to 3 months before worsening of global SLE disease activity as measured by all 3 external standards. Plasma NGAL levels increased significantly by 26% as early as 3 months prior to worsening of lupus nephritis as measured by the BILAG renal score.

**Conclusion.** Serial measurement of urinary and plasma NGAL levels may be valuable in predicting impending worsening of global and renal childhood-onset SLE disease activity.

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Lupus nephritis is very common in childhood-onset systemic lupus erythematosus (SLE) (1–3). The onset of lupus nephritis is usually early in the disease course within 2 years after the diagnosis of SLE is made (1,4). The outcome is poor, and ~10% of childhood-onset SLE patients with lupus nephritis develop end-stage renal disease within 10 years (5).

Neutrophil gelatinase-associated lipocalin (NGAL) is a candidate biomarker for the early detection of lupus nephritis (6). NGAL is one of the most highly up-regulated proteins in experimental acute kidney injury (7,8). Urinary NGAL and plasma NGAL levels are predictive of the development of acute kidney injury after cardiothoracic surgery, with levels increasing within 2 hours after the insult (9). Additionally, NGAL is a good biomarker for chronic kidney disease, since urinary and plasma NGAL levels correlate better (inversely) with the glomerular filtration rate than do serum creatinine levels (10).

Our group has previously shown that patients with childhood-onset SLE and biopsy-proven lupus nephritis have higher urinary NGAL levels than do healthy controls or patients with childhood-onset SLE without lupus nephritis, and that urinary NGAL levels correlate with renal disease activity (11). In cross-sectional comparisons, patients with childhood-onset SLE and worsening lupus nephritis have higher urinary NGAL levels than do patients with stable or improved lupus nephritis (12). The goal of the current study was to investigate the association of longitudinal changes in plasma and urinary NGAL levels with changes in renal, extrarenal, and global disease activity in childhood-onset SLE.

## PATIENTS AND METHODS

**Patients.** With the approval of the participating centers' institutional review boards, patients fulfilling at least 4 of 11 of the revised American College of Rheumatology classification criteria for SLE prior to age 18 years were enrolled in this prospective study (13). There were 3 categories of patients: 1) patients with newly diagnosed SLE, 2) patients with established SLE with biopsy-diagnosed lupus nephritis, and 3) patients with established SLE for at least 2 years without urinary changes suggestive of lupus nephritis. To be included in the analysis, patients ( $n = 111$ ) had to have had at least 3 study visits. The study was a prospective, longitudinal trial with study visits every 3 months. Some of the patients and samples were part of previous studies of renal biomarkers (12,14,15). A list of participating centers and medical professionals who contributed to this study, in addition to the authors, is shown in Appendix A.

**Laboratory assays.** Urine samples were centrifuged at 4,000g at 4°C to remove cellular debris before storing. Plasma and urine samples were frozen within 2 hours after collection

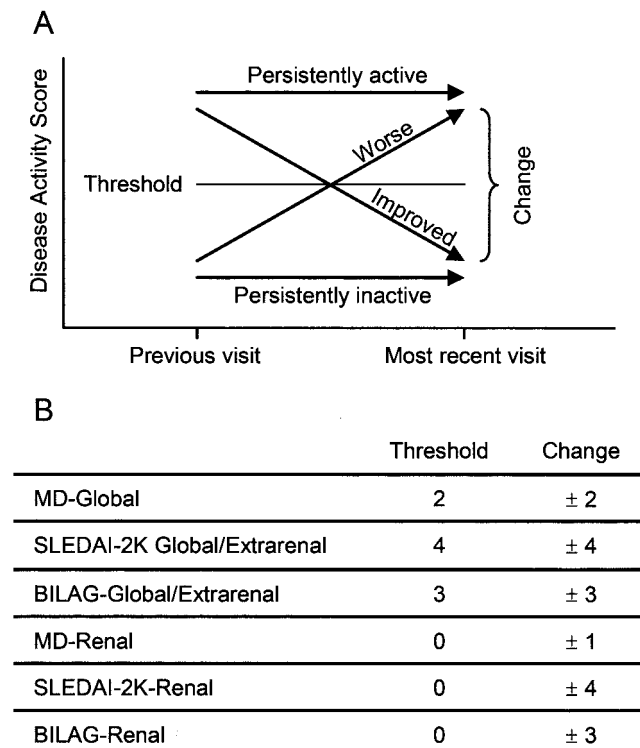
and stored at –80°C until the time of testing. Plasma and urinary NGAL levels were measured by enzyme-linked immunosorbent assay using a commercially available kit (Kit 036; AntibodyShop, Grusbakken, Denmark) as described in our previous report (12). Urine creatinine levels were measured using a quantitative colorimetric microplate assay kit (Oxford Biomedical Research, Oxford, MI). All measurements were made in duplicate. The laboratory personnel were blinded to the clinical data. Urinary NGAL excretion is presented as the amount of urinary NGAL in ng per mg of urine creatinine to correct for differences in NGAL due to urine dilution. The plasma NGAL concentration is presented in ng/ml plasma.

**Childhood-onset SLE disease activity measures.** At every study visit, global SLE disease activity was measured using 3 separate tools, as follows. The British Isles Lupus Assessment Group (BILAG) index (16) measures disease activity in 8 separate organ systems. While it was designed initially to reflect physicians' intention to treat, using 5 categories (A, B, C, D, E), for the present study we used the numerical conversion as proposed by Stoll et al (BILAG global, with a range of 0–72) (17). The second tool was the SLE Disease Activity Index 2000 update (SLEDAI-2K; global, with a range of 0–105) (18). The third tool was the physician's assessment of global disease activity (physician's global assessment), using a 10-cm visual analog scale (VAS; 0 = no disease activity and 10 = maximal disease activity). Similarly, for estimation of renal SLE disease activity, we used the following 3 separate measures: the BILAG renal domain score (range 0–9), the SLEDAI-2K renal domain score (range 0–16), and the physician's assessment of renal disease activity (physician's renal assessment; 10-cm VAS).

Extrarenal disease activity was measured using 2 tools: the BILAG global score minus the BILAG renal score (BILAG extrarenal score; range 0–63) and the SLEDAI-2K global score minus the SLEDAI-2K renal score (SLEDAI-2K extrarenal score; range 0–89). Both the BILAG and the SLEDAI-2K disease activity measures are sensitive to change in childhood-onset SLE (19).

**Course of disease activity.** The childhood-onset SLE disease course was categorized based on the change in disease activity at a reference time point (time 0). The respective disease activity scores were compared between 2 time points: the time of the most recent visit (time 0) and the time of the preceding visit (time –1). For example, a patient with 3 study visits could have 2 reference time points at which the disease course was determined (at the second visit and at the third visit). There were 4 categories of disease course: persistently active, persistently inactive, improved, or worsening. Details of how these categories were established are presented in Figure 1. The parameters to define the disease courses were chosen by 2 authors (HIB, PD) and were considered to represent a conservative estimate of minimal clinically important change in disease activity (20,21). The minimal clinically important change in disease activity in childhood-onset SLE is likely smaller than that in adult SLE; studies to prospectively validate these parameters in childhood-onset SLE are currently under way (22).

**Statistical analysis.** Levels of both plasma NGAL and urinary NGAL (standardized to the concentration of urine creatinine) were considered primary measures in this study. They were log-transformed in order to fit major assumptions of parametric statistical models in analyses. For each NGAL



**Figure 1.** Categorization of the disease course with childhood-onset systemic lupus erythematosus (SLE). **A**, The disease course was categorized as persistently active, persistently inactive, improved, or worsening. For patients to be categorized as having persistently active (inactive) disease, the disease activity score had to remain above (below) a predefined threshold and the change could not exceed a predefined magnitude. If the change exceeded a predefined magnitude, patients were categorized as having improved (if decreased score) or worsening (if increased score) disease activity. **B**, The predefined thresholds and required changes are shown. MD Global = physician's assessment of global disease activity measured on a 10-cm visual analog scale (VAS) (a value of 0 indicates inactive SLE); SLEDAI-2K Global/Extrarenal = SLE Disease Activity Index 2000 update global score (range 0–105)/extrarenal score (range 0–89) (a value of 0 indicates inactive SLE); BILAG Global/Extrarenal = British Isles Lupus Assessment Group global score (range 0–72)/extrarenal score (range 0–63) (a value of 0 indicates inactive SLE); MD Renal = physician's assessment of renal disease activity measured on a 10-cm VAS (a value of 0 indicates inactive SLE renal disease); SLEDAI-2K Renal = SLEDAI-2K renal score (range 0–16) (a value of 0 indicates inactive SLE renal disease); BILAG Renal = BILAG renal score (range 0–9) (a value of 0 indicates inactive SLE renal disease).

measure, its change corresponding to a disease course category was assessed using a mixed-effect model, adjusting for controlling covariates, mainly the demographics (23). Because each patient had multiple (at least 3) visits, a random effect (i.e., patients) was used in the mixed-effect model to account for within-patient correlation during repeated observations. Post hoc estimates of changes in mean values were performed simultaneously among all 4 categories of disease course and adjusted for individual Type I errors using Tukey's method.

Two types of changes in NGAL levels (the change between time –1 and time 0 and the change between time –2 and time –1) were analyzed in the mixed-effect models. Other numerical variables were summarized with mean  $\pm$  SD values, and binary or categorical variables were summarized with frequency values (in %). Relationships between NGAL measures and between disease activity scales were assessed using Pearson's and Spearman's correlation coefficients, respectively.

In order to determine whether NGAL levels at different time points could be predictive of a worsening disease course, we applied multiple logistic regression models using the dichotomized disease course (worsening versus not worsening) as the dependent variable, and we used measurements of NGAL levels at different time points as predictors, adjusting them for the patients' demographics. The predicted logit of worsening was then transformed into the "predicted probability of worsening" for each case. A receiver operating characteristic (ROC) curve was plotted by connecting sensitivity/specificity points under all possible probabilities of worsening. The area under the curve (AUC) was used to assess the overall accuracy. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also used to assess the discriminating and predicting performance of the NGAL measure using a specific threshold "predicted probability of worsening." Excel XP (Microsoft, Redmond, WA) and SAS 9.1 (SAS Institute, Cary, NC) programs were used for

**Table 1.** Characteristics of the 111 patients with childhood-onset SLE at the baseline study visit\*

Age, mean $\pm$ SD years	15.9 $\pm$ 3.4
Female	89 (80.2)
Number of visits, mean $\pm$ SD	5.2 $\pm$ 1.3
Time between visits, mean $\pm$ SD months	3.4 $\pm$ 1.5
Race/ethnicity	
White	55 (49.5)
African American	36 (32.4)
Asian	14 (12.6)
Hispanic	13 (11.7)
Biopsy-proven lupus nephritis†	63 (56.8)
WHO class II	2 (1.8)
WHO class III	14 (12.6)
WHO class IV	27 (24.3)
WHO class V	16 (14.4)
WHO class III + V	1 (0.9)
WHO class IV + V	3 (2.7)
Anti-dsDNA antibody positive	56 (50.5)
Medications at baseline	
Prednisone	84 (75.7)
Cyclophosphamide	12 (10.8)
Mycophenolate mofetil	43 (38.7)
Azathioprine	12 (10.8)
Methotrexate	3 (2.7)
Hydroxychloroquine	88 (79.3)
Angiotensin-converting enzyme inhibitor	33 (29.7)

\* Except where indicated otherwise, values are the number (%) of patients. WHO = World Health Organization; anti-dsDNA = anti-double-stranded DNA.

† Lupus nephritis was classified according to the revised 1995 criteria (24). Forty-nine of the 111 patients did not have systemic lupus erythematosus (SLE) renal involvement. All patients with physician-diagnosed SLE renal disease underwent a kidney biopsy.

**Table 2.** Disease activity and disease course during the study, as determined using the 3 external standards\*

	BILAG score			SLEDAI-2K score			Physician's assessment	
	Global, 0–72	Renal, 0–9	Extrarenal, 0–63	Global, 0–105	Renal, 0–16	Extrarenal, 0–89	Global, 10-cm VAS	Renal, 10-cm VAS
Disease activity during the study, mean ± SD	4.3 ± 4.0	1.4 ± 2.5	3.2 ± 3.3	4.9 ± 4.6	1.7 ± 3.2	3.2 ± 3.0	2.1 ± 2.6	1.2 ± 1.9
Observations per disease course, no. (%)								
Persistently active	83 (22.7)	106 (29.0)	60 (16.4)	65 (17.8)	35 (9.6)	46 (12.6)	47 (12.9)	59 (16.6)
Persistently inactive	141 (38.6)	205 (56.2)	187 (51.2)	181 (49.6)	239 (65.5)	249 (68.2)	218 (60.1)	166 (46.8)
Improved	86 (23.6)	35 (9.6)	71 (19.5)	71 (19.5)	53 (14.5)	40 (11.0)	63 (17.4)	88 (24.8)
Worsening	55 (15.1)	19 (5.2)	47 (12.9)	48 (13.2)	38 (10.4)	30 (8.2)	35 (9.6)	42 (11.8)

\* The 3 external standards for measuring disease activity were the British Isles Lupus Assessment Group (BILAG) index, the Systemic Lupus Erythematosus Disease Activity Index 2000 update (SLEDAI-2K), and the physician's assessment of disease activity (physician's assessment) on a visual analog scale (VAS). The disease course was categorized as persistently active, persistently inactive, improved, or worsening. For patients to be categorized as having persistently active (inactive) disease, the disease activity score had to remain above (below) a predefined threshold and the change could not exceed a predefined magnitude. If the change exceeded a predefined magnitude, patients were categorized as having improved (if decreased score) or worsening (if increased score) disease activity. Predefined thresholds and required changes are shown in Figure 1B. A total of 365 observations were made for each of the BILAG and SLEDAI-2K scores, while a total of 363 observations were made for the physician's global assessment and a total of 355 observations were made for the physician's renal assessment.

analysis. *P* values less than 0.05 were considered significant, and *P* values less than 0.1 were reported to show trends.

## RESULTS

### Baseline patient characteristics and treatments.

Table 1 summarizes the characteristics of the 111 patients included in the study. Their mean ± SD age was 15.9 ± 3.4 years, and the majority were female (80.2%). Lupus nephritis was classified according to the original system (24), since some biopsy samples were obtained prior to the introduction of the new system in 2004 (25). Biopsy-proven lupus nephritis (often class IV and class V) was present in 56.8% of the patients. Frequently used antiinflammatory medications included prednisone (75.7%), hydroxychloroquine (79.3%), mycophenolate mofetil (38.7%), cyclophosphamide (10.8%), and azathioprine (10.8%); 29.7% of patients were treated with angiotensin-converting enzyme inhibitors at baseline.

### Change in disease activity and in disease course.

Table 2 summarizes the mean disease activity during the study period and the proportions of the different disease courses at the reference time point. A total of 365 observations of reference time points were available for the longitudinal analyses. The most common disease course was a “persistently inactive” course, while a “worsening” course occurred less frequently (worsening of global disease activity 9.6–15.1%, worsening of renal disease activity 5.2–11.8%, worsening of extrarenal disease activity 8.2–12.9%).

**Correlation between different measurements of disease activity.** Using Spearman's rank correlation coefficients corrected for tied ranks, there were strong

correlations between global and extrarenal disease activity (BILAG global versus extrarenal scores:  $r = 0.79, P < 0.0001$ ; SLEDAI-2K global versus extrarenal scores:  $r = 0.74, P < 0.0001$ ), between global and renal disease activity (BILAG global versus renal scores:  $r = 0.59, P < 0.0001$ ; SLEDAI-2K global versus renal scores:  $r = 0.63, P < 0.0001$ ); physician's global assessment versus physician's renal assessment:  $r = 0.51, P < 0.0001$ ), and between the different tools (BILAG global score versus SLEDAI-2K global score:  $r = 0.60, P < 0.0001$ ; BILAG global score versus physician's global assessment:  $r = 0.57, P < 0.0001$ ; SLEDAI-2K global score versus physician's global assessment:  $r = 0.51, P < 0.0001$ ; BILAG renal score versus SLEDAI-2K renal score:  $r = 0.68, P < 0.0001$ ; BILAG renal score versus physician's renal assessment:  $r = 0.69, P < 0.0001$ ; SLEDAI-2K renal score versus physician's renal assessment:  $r = 0.69, P < 0.0001$ ; BILAG extrarenal score versus SLEDAI-2K extrarenal score:  $r = 0.47, P < 0.0001$ ). Renal and extrarenal disease activity were not correlated (BILAG renal score versus BILAG extrarenal score:  $r = 0.07, P = 0.16$ ; SLEDAI-2K renal score versus SLEDAI-2K extrarenal score:  $r = 0.01, P = 0.95$ ).

**Distribution of plasma and urinary NGAL levels and correlation between plasma NGAL levels and urinary NGAL levels.** Plasma NGAL and urinary NGAL levels were log-normally distributed in the study population (data not shown). Pearson's correlation using log-transformed plasma NGAL and urinary NGAL levels indicated that there was no correlation between plasma NGAL and urinary NGAL levels at any given

**Table 3.** Longitudinal levels of plasma NGAL and global, renal, and extrarenal disease course\*

Tool, disease course (no. of observations)	Mean (95% CI)			<i>P</i>	
	Time -2	Time -1	Time 0	Time -2 vs. time -1	Time -1 vs. time 0
<b>BILAG global score</b>					
Active (83)	57.0 (49.2–66.0)	57.8 (50.7–65.8)	60.9 (54.5–68.0)	NS	NS
Inactive (141)	52.7 (46.5–59.7)	52.7 (47.3–58.6)	53.9 (49.3–59.0)	NS	NS
Improved (86)	55.4 (47.6–64.6)	55.1 (47.9–63.3)	55.8 (49.7–62.7)	NS	NS
Worsening (55)	54.7 (46.3–64.8)	65.0 (56.1–75.4)	67.8 (59.7–77.0)	0.007	NS
<b>SLEDAI-2K global score</b>					
Active (65)	52.4 (45.0–61.1)	55.2 (48.2–63.3)	57.5 (51.2–64.7)	NS	NS
Inactive (181)	54.0 (47.7–61.2)	51.8 (46.5–57.7)	54.0 (49.4–58.9)	NS	NS
Improved (71)	59.7 (51.1–69.7)	59.1 (51.1–68.2)	58.2 (51.4–65.8)	NS	NS
Worsening (48)	52.6 (45.2–61.2)	63.2 (55.5–72.1)	65.2 (58.0–73.4)	0.001	NS
<b>BILAG renal score</b>					
Active (106)	54.0 (43.6–66.9)	55.7 (45.8–67.7)	67.7 (57.8–79.2)	NS	NS
Inactive (205)	53.4 (47.3–60.3)	52.1 (47.1–57.7)	54.0 (49.7–58.6)	NS	NS
Improved (35)	61.4 (51.7–72.9)	62.7 (53.4–73.7)	57.4 (49.8–66.2)	NS	NS
Worsening (19)	51.1 (42.8–61.1)	64.5 (55.5–75.0)	59.7 (51.7–68.8)	0.0001	NS
<b>SLEDAI-2K renal score</b>					
Active (35)	57.3 (48.7–67.3)	57.9 (50.3–66.6)	57.9 (51.3–65.3)	NS	NS
Inactive (239)	49.4 (43.0–56.7)	50.1 (44.4–56.4)	53.5 (48.6–59.0)	NS	0.05
Improved (53)	57.8 (50.0–66.8)	58.4 (51.1–66.6)	58.8 (52.7–65.8)	NS	NS
Worsening (38)	62.6 (52.0–75.3)	68.0 (57.3–80.7)	67.7 (68.0–79.2)	NS	NS
<b>BILAG extrarenal score</b>					
Active (60)	61.4 (52.0–72.5)	65.3 (56.3–75.6)	67.5 (59.5–76.6)	NS	NS
Inactive (187)	52.8 (46.8–59.6)	52.3 (47.2–58.0)	53.5 (49.2–58.1)	NS	NS
Improved (71)	53.6 (46.0–62.4)	55.7 (48.5–64.0)	59.1 (52.4–66.6)	NS	NS
Worsening (47)	55.3 (46.8–65.5)	62.9 (54.0–73.2)	64.5 (56.5–73.6)	0.07	NS
<b>SLEDAI-2K extrarenal score</b>					
Active (46)	52.3 (43.6–62.7)	56.6 (48.2–66.5)	59.4 (51.8–68.1)	NS	NS
Inactive (249)	55.0 (48.8–61.9)	54.8 (49.6–60.5)	55.2 (50.9–59.8)	NS	NS
Improved (40)	53.4 (44.0–64.9)	55.0 (45.8–66.1)	57.7 (49.4–67.3)	NS	NS
Worsening (30)	53.1 (44.4–63.5)	60.9 (51.7–71.6)	70.1 (60.9–80.8)	0.07	NS

\* Levels of plasma neutrophil gelatinase-associated lipocalin (NSAL) are shown as ng plasma NGAL/ml. Time -2 = time point 2 visits prior to the reference time point; time -1 = time point 1 visit prior to the reference time point; time 0 = reference time point at which the disease course was defined; 95% CI = 95% confidence interval; NS = not significant (see Table 2 for other definitions). See Table 2 for explanation of disease course (predefined thresholds and required changes are shown in Figure 1B).

study visit ( $r < 0.01$ ). Pearson's correlation using log-transformed standardized urinary NGAL levels (ng/mg creatinine) and log-transformed absolute urinary NGAL levels (ng/ml urine) demonstrated a high degree of correlation ( $r = 0.81$ ). In all remaining Results sections, urinary NGAL levels are presented standardized to the urine creatinine concentration.

**Longitudinal changes in plasma NGAL levels and change in global disease activity.** The relationship between the course of global disease activity (BILAG global score or SLEDAI-2K global score) and longitudinal plasma NGAL levels is shown in Table 3. Among patients who experienced worsening of global disease activity, there was a significant increase in plasma NGAL levels occurring between time -2 and time -1 (i.e., approximately 6 months to 3 months before the clinical diagnosis of a global flare was made). An identical pattern was observed when global disease

activity was measured with the physician's global assessment; between time -2 and time -1, patients with worsening disease activity experienced an increase in plasma NGAL level from 57.4 ng/ml (95% confidence interval [95% CI] 47.9–68.8) to 72.7 ng/ml (95% CI 62.0–85.2) ( $P < 0.001$ ). None of the other disease courses (persistently active, persistently inactive, or improved) was associated with a longitudinal change in plasma NGAL levels. Only 4 patients with a "worsening" disease course (as measured by the SLEDAI-2K global score) at time 0 already had a "worsening" disease course at time -1. When we excluded these patients from the analysis, similar results were obtained.

**Longitudinal changes in plasma NGAL levels and change in renal disease activity.** Patients with worsening renal disease activity as measured by the BILAG renal score experienced a significant increase in plasma NGAL level between time -2 and time -1. A

**Table 4.** Urinary NGAL levels over time and the future course of lupus nephritis\*

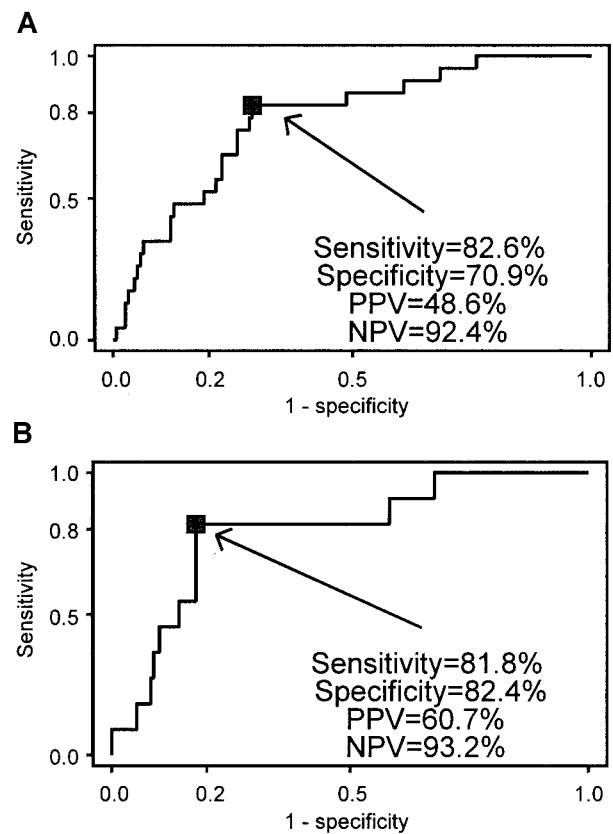
Tool, disease course (no. of observations)	Mean (95% CI)			P	
	Time -2	Time -1	Time 0	Time -2 vs. time -1	Time -1 vs. time 0
<b>BILAG renal score</b>					
Active (106)	12.7 (9.6–16.9)	14.7 (10.1–21.4)	16.9 (11.5–24.8)	NS	NS
Inactive (205)	8.3 (6.5–10.6)	10.8 (7.9–14.7)	16.0 (12.1–21.2)	NS	0.002
Improved (35)	8.6 (5.1–14.6)	9.0 (4.4–18.6)	8.4 (4.1–17.3)	NS	NS
Worsening (19)	11.1 (6.1–20.1)	22.6 (12.7–40.4)	43.8 (25.1–76.3)	0.01	0.02
<b>SLEDAI-2K renal score</b>					
Active (35)	24.8 (15.3–40.0)	23.4 (11.7–46.5)	25.9 (13.4–50.3)	NS	NS
Inactive (239)	7.6 (6.0–9.6)	9.6 (7.1–13.1)	13.5 (10.2–17.9)	NS	0.007
Improved (53)	13.3 (9.0–19.7)	11.4 (5.9–21.9)	14.8 (8.3–26.3)	NS	NS
Worsening (38)	10.3 (6.9–15.5)	17.5 (11.2–27.2)	27.3 (17.3–42.8)	0.03	0.06

\* Levels of urinary neutrophil gelatinase-associated lipocalin (NGAL) are shown as ng urinary NGAL/mg urinary creatinine. Time -2 = time point 2 visits prior to the reference time point; time -1 = time point 1 visit prior to the reference time point; time 0 = reference time point at which the disease course was defined; 95% CI = 95% confidence interval; NS = not significant (see Table 2 for other definitions). See Table 2 for explanation of disease course (predefined thresholds and required changes are shown in Figure 1B).

similar pattern was observed with the physician’s renal assessment; between time -2 and time -1, the group of patients with worsening disease activity experienced an increase in plasma NGAL level from 53.6 ng/ml (95% CI 42.2–68.1) to 73.4 ng/ml (95% CI 59.7–90.3) ( $P < 0.001$ ). Such increases did not reach significance when using the SLEDAI-2K renal score (see Table 3). Plasma NGAL level was not predictive of any other lupus nephritis disease course (active, inactive, or improved).

**Longitudinal changes in urinary NGAL levels and lupus nephritis disease course.** Between time -2 and time -1, patients with worsening SLEDAI-2K or BILAG renal scores experienced, on average, significant increases in urinary NGAL levels of 70% and 104%, respectively (Table 4). A similar increase was seen when renal disease activity was measured by the physician’s renal assessment; between time -2 and time -1, the group of patients with worsening lupus nephritis experienced an increase in urinary NGAL level from 10.1 ng/mg creatinine (95% CI 6.5–15.7) to 17.2 ng/mg creatinine (95% CI 10.7–27.8) ( $P = 0.04$ ), while no significant change of urinary NGAL level occurred during that interval in patients with any of the other disease courses. Only 4 patients with a “worsening” disease course at time 0 already had a “worsening” disease course at time -1. The exclusion of these patients from the analysis yielded similar results.

There was a significant concurrent increase in urinary NGAL level in patients who had persistently inactive disease at the reference time point. This significant increase was due to a subgroup of patients who experienced worsening at the subsequent time point according to the SLEDAI-2K renal score ( $P = 0.05$ ), while patients who continued to have inactive disease at



**Figure 2.** Receiver operating characteristic curves plotted by connecting sensitivity/specificity points under all possible probabilities of worsening of renal disease activity. Shown are the sensitivity and specificity of the predicted probability of worsening, estimated from multivariate logistic regression, using the SLEDAI-2K renal score as the external standard (area under the curve [AUC] = 0.78) (A) and the BILAG renal score as the external standard (AUC = 0.80) (B). PPV = positive predictive value; NPV = negative predictive value (see Figure 1 for other definitions).

the subsequent time point did not experience a significant increase in urinary NGAL level (data not shown). These properties of urinary NGAL level were summarized using ROC analysis, using a dichotomized outcome (worsening versus not worsening of lupus nephritis). The sensitivity, specificity, PPVs, and NPVs were calculated for the resulting “predicted probability of worsening” (Figure 2). When using the SLEDAI-2K renal score as the external standard, the resulting AUC was 0.78, and when using the BILAG renal score as the external standard, the resulting AUC was 0.80. There was no statistically important relationship between the course of global or extrarenal disease and urinary NGAL levels over time.

## DISCUSSION

The longitudinal data presented in this study demonstrate that an increase in urinary NGAL levels is predictive of worsening of childhood-onset SLE renal disease activity. Additionally, an increase in plasma NGAL levels is predictive of worsening of global and renal childhood-onset SLE disease activity. Therefore, urinary NGAL is an excellent candidate for a predictive biomarker for worsening of childhood-onset SLE renal disease activity, and plasma NGAL is an excellent candidate for a predictive biomarker for worsening of childhood-onset SLE renal disease activity and global disease activity.

SLE often follows a relapsing–remitting disease course (26). Due to the difficulty of predicting worsening of SLE disease activity, treatment is often only initiated once disease activity becomes severe and damage has occurred. Given the high morbidity and mortality in childhood-onset SLE with frequent and severe lupus nephritis (3,27), the identification of biomarkers that can predict worsening of lupus nephritis is highly desirable. The early recognition of worsening lupus nephritis, however, is difficult using routinely available laboratory tests. For example, levels of anti–double-stranded DNA (anti-dsDNA) antibodies may increase prior to the worsening of lupus nephritis (28–30), but only 50% of patients with childhood-onset SLE renal disease test positive for anti-dsDNA antibodies (2). In addition, levels of anti-dsDNA antibodies sometimes decrease concurrently with acute SLE flares, possibly due to increased tissue deposition (31), demonstrating a complex relationship between anti-dsDNA levels and SLE disease activity. Serum levels of the complement components C3 and C4 often decrease concurrently with renal flares, and thus have little predictive value (32).

Results of other routine tests used to evaluate renal function, such as serum creatinine, urine protein, and examination of the urine sediment, vary not only with lupus nephritis activity but also with the presence of renal damage (33).

Additionally, there was no statistically important relationship between the future course of global, renal, or extrarenal disease and either serum C3 and C4 levels or the urine protein:creatinine ratio in our patient population. This information has already been reported for the presented patient cohort (15).

One of the difficulties of studying biomarkers for lupus nephritis has been the absence of a noninvasive criterion standard. While kidney biopsy is the gold standard for diagnosing lupus nephritis, it is impractical to perform repeated biopsies to screen for worsening of lupus nephritis. Alternative external standards must be used for the assessment of lupus nephritis and global SLE disease activity, including the BILAG and SLEDAI-2K global and renal scores. For the present study, we quantified global and renal disease activity using 3 external standards (2 for extrarenal domains) to ensure that relationships found between NGAL levels and childhood-onset SLE disease courses were not spurious. The BILAG index has been developed from the perspective of physicians’ intention to treat to provide a snapshot of SLE activity by organ involvement rather than to supply a global disease activity score (34). Conversely, the SLEDAI-2K has been designed as a tool for assessing global SLE disease activity (18). There were strong correlations among the different tools for the assessment of global, renal, and extrarenal disease activity, supporting the concurrent validity of these measures in our study.

NGAL is a small, glycosylated (25-kd) protein produced in multiple normal tissues and organs, including epithelial tissues, endothelium, and bone marrow, and its production is increased in neoplastic and inflammatory conditions (35,36). Urinary NGAL levels increase markedly and immediately following acute kidney injury (37). Similarly, urinary NGAL levels are elevated with chronic kidney disease, correlating with disease severity (10,38).

Previously, we and others have shown that urinary NGAL is an excellent biomarker of concurrent lupus nephritis activity. Patients with active lupus nephritis have significantly higher urinary NGAL levels when compared cross-sectionally with patients with inactive lupus nephritis and healthy controls (11); patients with worsening lupus nephritis have higher urinary

NGAL levels when compared with patients with stable or improving lupus nephritis (12).

Our longitudinal prospective study has allowed us to examine whether NGAL could be a predictor of the future course of childhood-onset SLE. One impressive finding of our study is the marked increase in urinary NGAL levels up to 3 months prior to worsening lupus nephritis activity, irrespective of the external standard used. Our data also demonstrate a significant increase in plasma NGAL levels up to 3 months prior to worsening of global SLE disease activity and a significant increase in plasma NGAL levels prior to worsening of renal disease activity. Plasma NGAL levels also increased prior to worsening of extrarenal SLE disease activity, but changes did not reach statistical significance.

It is currently a subject of speculation why levels of urinary and plasma NGAL may increase before worsening of lupus nephritis becomes clinically detectable. One possibility is that the kinetics and specificity of the molecule may be different from those of other biomarkers. Urinary NGAL may be an immediate-early marker of general kidney injury, a notion supported by the findings reported in acute kidney injury. Another possibility is that NGAL may be produced after SLE-specific glomerular or tubular injury. While the most likely source of urinary NGAL in acute kidney injury is the distal tubules, the source of urinary NGAL in lupus nephritis is less clear. The observed increase in urinary NGAL levels may result from increased glomerular protein loss and disturbed reabsorption in the proximal nephron segment in addition to increased intrarenal production. Furthermore, based on results from experimental studies, the glomerulus may represent a source of NGAL. Mesangial cells treated *in vitro* with nephritogenic murine anti-dsDNA antibodies overexpress NGAL, indicating mesangial cells as a possible source (39). Additionally, a murine model of crescentic glomerulonephritis suggests that glomerular epithelial cells are a possible source of NGAL (40).

The increase in plasma NGAL levels prior to worsening of lupus nephritis, but not prior to worsening of extrarenal childhood-onset SLE, suggests a prominent role of lupus nephritis in increasing plasma NGAL levels. Similar findings are seen in other types of chronic kidney disease, with an inverse correlation between plasma NGAL levels and glomerular filtration rate (41). Several mechanisms may be postulated. Kidney injury results in dramatically increased NGAL messenger RNA expression in distant organs, especially the liver and lungs, and the overexpressed NGAL protein may constitute a distinct systemic pool (42). Additional con-

tributions to the systemic pool may derive from NGAL released from neutrophils and macrophages. Furthermore, any decrease in glomerular filtration rate resulting from kidney injury would be expected to decrease the renal clearance of NGAL, with subsequent accumulation in the systemic circulation. The relative contribution of these mechanisms to the rise in plasma NGAL levels after acute kidney injury and in lupus nephritis remains to be determined.

Some of the problems in the clinical use of NGAL may include its nonspecific nature (i.e., the fact that urinary NGAL levels also increase after various other types of kidney injury, including ischemic and toxic injury). We anticipate that urinary NGAL may eventually be used in concert with other biomarkers to help us to better understand the nature of the underlying renal insult. Studies to identify and validate additional biomarkers for lupus nephritis are currently under way (14,15,43).

In summary, we demonstrated that urinary NGAL levels may be predictive of the development or worsening of lupus nephritis in childhood-onset SLE. In addition, an increase in plasma NGAL levels may be predictive of worsening of global and renal disease activity. As with all initial biomarker validation studies, confirmation of our findings in other cohorts is warranted. Future studies in an independent patient cohort, preferably one with childhood-onset SLE and adult SLE, are needed to verify that NGAL is a predictive biomarker. The early identification of patients at risk would be extremely helpful in order to initiate treatment early with the eventual goal of avoiding long-term morbidity and mortality due to lupus nephritis and SLE.

#### AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Brunner had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Hinze, Suzuki, Devarajan, Brunner.

**Acquisition of data.** Klein-Gitelman, Passo, Olson, Singer, Haines, Onel, O'Neil, Silverman, Tucker, Brunner.

**Analysis and interpretation of data.** Hinze, Suzuki, Ying, Devarajan, Brunner.

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#### APPENDIX A: PARTICIPATING CENTERS AND MEDICAL PROFESSIONALS

Participating centers and medical professionals who contributed to this study, in addition to the authors of this article, are as

follows: British Columbia Children's Hospital, Vancouver, British Columbia, Canada: Drs. David Cabral, Jaime Guzman, Kristin Houghton, Peter Malleson, Ross Petty, and Stuart Turvey (data collection); Tony Hong and Dr. America Uribe (study coordinators). Cincinnati Children's Hospital Medical Center, Cincinnati, OH: Dr. Michael Bennett (discussion); Drs. Thelma Kathman and Qing Ma (technical assistance); Dr. Susan Thompson (sample storage); Drs. Bob Colbert, Thomas Griffin, Alexei Grom, and Daniel Lovell (data collection); Shannen Nelson (study coordinating center study nurse); Jamie Meyers-Eaton (study coordinator); Shweta Srivastava (sample processing); Dr. Amber Khan, Clinical Fellow (data entry); Aimee Baker (manuscript preparation). Hackensack University Medical Center, Hackensack, NJ: Drs. Yukiko Kimura, Suzanne Li, and Jennifer Weiss (data collection); Mary Ellen Riordan (study coordination). Hospital for Sick Children, Toronto, Ontario, Canada: Lawrence Ng (study coordinator). Medical College of Wisconsin, and Children's Research Institute, Milwaukee, WI: Dr. James Nocton, Dr. Calvin Williams, and Elizabeth Roth-Wojicki, PNP (data collection); Marsha Malloy (data collection and site coordination); Joshua Kapfhamer and Noshaba Khan (study coordinators). Northwestern University Feinberg School of Medicine, Chicago, IL: Blair Dina (study coordinator). Rainbow Babies & Children's Hospital, Cleveland, OH: Dr. Elizabeth Brooks (data collection); Michelle Walette (study coordinator). La Rabida Children's Hospital, Chicago, IL: Dr. Linda Wagner-Weiner (data collection); Becky Pupilava (study coordinator). University of Oklahoma Health Sciences Center, Oklahoma City: Drs. Michael Hendrickson and James N. Jarvis (data collection); Tracy Fuelling, Lisa Kempke, Linda Menifee, and Kathy Redmond (study coordinators).