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Urinary NGAL-Positive Acute Kidney Injury and Poor Long-term Outcomes in Hospitalized Patients

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Introduction: Neutrophil gelatinase–associated lipocalin (NGAL) is a widely studied biomarker of renal tubular injury. Urinary NGAL (uNGAL) during acute kidney injury (AKI) predicts short-term adverse outcomes. However, the long-term predictive value is unknown.

Methods: We performed a prospective observational study of 145 patients with hospital-acquired AKI according to Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) criteria and analyzed the long-term predictive value of uNGAL at the time of AKI. We defined a composite outcome of all-cause mortality and the development of end-stage renal disease (ESRD).

Results: In all, 61 AKI patients died and 22 developed ESRD within 6 months. The uNGAL levels were significantly higher in patients with poor long-term outcomes. uNGAL levels ≥362 μg/l (highest quartile) and uNGAL levels between 95 and 362 μg/l (third quartile) were associated with hazard ratios of 3.7 (95% confidence interval, 2.1–6.5) and 1.9 (1.1–3.5), respectively, compared with uNGAL levels <95 μg/l (lower quartiles). After 6 months, 67% and 43% of patients within the highest and third uNGAL quartile, respectively, had either progressed to ESRD or died, compared to only 21% of patients with uNGAL in the lower 2 quartiles ($P < 0.001$). In multivariable Cox regression analyses accounting for conventional predictors, uNGAL was the strongest independent predictor of adverse long-term outcomes. The association of uNGAL levels and poor long-term outcomes remained significant in the subgroup of 107 AKI survivors discharged without requiring dialysis ($P = 0.002$).

Discussion: These data indicate that elevated uNGAL levels at AKI diagnosis predict poor long-term outcomes.

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Acute kidney injury (AKI) is common[1] and carries a significant risk of mortality[2,3] and end- stage renal disease (ESRD).[4–6] Currently, AKI is classified according to the dynamics of serum creatinine and the absolute urinary output, as reflected by the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE), Acute Kidney Injury Network (AKIN), or Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury criteria.[7–14] The presence and severity of AKI according to these criteria are associated with poor short-term outcomes. For instance, in a study of more than 2000 intensive care unit patients with AKI, mortality rates increased with higher RIFLE stages, ranging from 20% in RIFLE-R to 49.5% in RIFLE-F.[15] In hospitalized patients with and without AKI, mortality increased from 4.4% in patients without AKI to >40% in RIFLE-F AKI.[16]

Current AKI classifications have limited performance in differentiating the pathophysiology underlying renal functional decay. Novel biomarkers of renal tubular injury, including neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule–1 (KIM1), liver fatty-acid binding protein (L-FABP), and interleukin-18 (IL-18), have been shown to assist with diagnosing and risk stratifying AKI.[17–23] Based on

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a growing body of data to support the use of AKI biomarkers, the 10th Acute Dialysis Quality Initiative (ADQI) conference recommended that new biomarkers such as NGAL be included when diagnosing and staging AKI because of their sensitivity and prognostic importance.[24,25] NGAL is a 25-kDa protein belonging to the lipocalin family. NGAL is produced in renal epithelia in response to nephron injury. NGAL has utility in diagnosing AKI early in various clinical settings, such as critically ill patients in the intensive care unit,[26–30] septic patients,[31,32] and patients with AKI after contrast agent exposure[33,34] and after surgery.[35–41] NGAL effectively discriminates patients in the emergency room with intrinsic AKI from patients with prerenal azotemia, those with chronic kidney disease (CKD), and those with normal renal function.[21] High NGAL levels are associated with adverse outcomes, including in-hospital mortality and death.[21] Although most studies have addressed the utility of NGAL in predicting short-term poor clinical outcomes in AKI,[21,37,42–45] the role of NGAL in predicting long-term outcomes is unclear. Here, we provide evidence that high urinary NGAL (uNGAL) levels at the time of AKI diagnosis predict long-term adverse outcomes, including mortality and the development of ESRD.

MATERIALS AND METHODS

Study Design and Patient Cohort

During 2008 to 2009, a total of 145 patients with hospital-acquired AKI were enrolled in a prospective observational study to assess the utility of uNGAL, measured at the time of AKI, to predict in-hospital outcome and diagnosis, as previously reported.[46] Briefly, all hospitalized patients meeting the RIFLE criteria were eligible and were generally enrolled at the time of nephrology consultation. Based on diagnostic adjudications, 32 patients had prerenal AKI and 75 patients had intrinsic AKI (the remaining 38 patients could not be classified based on the available clinical data).[46] We assessed long-term outcomes of death or development of ESRD in this cohort.

Definitions

AKI was defined according to creatinine-based RIFLE criteria (either a >50% increase in serum creatinine concentration or a >25% decrease in glomerular filtration rate compared with baseline). ESRD was defined as a sustained (>3 months) requirement of renal replacement therapy. Follow-up of patients was achieved using the hospital electronic database; if information was insufficient, we contacted the general practitioner or nephrologist of the patient.

Study Duration

We performed the observation from November 2008 until August 2012. The median follow-up period was 6 months.

Outcome

The composite outcome included new-onset ESRD and all-cause mortality. Time to event was calculated between the date of study inclusion (day of urinary sampling) and the first occurrence of an aspect of the composite outcome (ESRD or death). We compared the predictive value of uNGAL levels to conventional predictors of adverse AKI outcomes (serum creatinine, RIFLE, and AKIN stages) as well as demographic factors and comorbidities.

uNGAL Measurements

Urinary samples were obtained, and uNGAL was determined on stored samples (−80°C) using a standardized clinical platform (Abbott Architect), as reported in our previous publication.[46] Briefly, the assay used a chemiluminescent microparticle immunoassay using a noncompetitive, 2-antianalyte antibody sandwich, which included a microparticle reagent prepared by covalently attaching an antianalyte antibody to paramagnetic particles and a conjugate reagent prepared by labeling a second antianalyte antibody with acridinium. The chemiluminescent signal was calibrated using known quantities of recombinant NGAL. As reported earlier, coefficients of variation were 3.0% for uNGAL at a concentration of 385 ng/ml.[47]

Informed Consent and Ethics

The Charité University Ethics Committee approved the study (EA3/011/08), and written informed consent was obtained from patients at the time point of enrollment.[46]

Statistical Analysis

We performed Kaplan–Meier estimation and log-rank tests to compare mortality and ESRD incidence as well as a composite of both endpoints in patient subsets stratified by NGAL quartile or creatinine quartile and by RIFLE stage visually and statistically. Cox regression analysis was used to identify predictors of adverse outcomes including the following covariates: demographic variables (age, gender), comorbidites (CKD, hypertension, diabetes), RIFLE and AKIN classes of AKI, creatinine at the time of AKI, and uNGAL at the time of AKI. Univariable Cox regression was used to identify individual significant predictors. Significant predictors were then combined in a multivariable Cox regression analysis. Backward elimination was used to construct a final Cox model. P values for between-group comparisons were calculated using χ^2 test,

Kruskal–Wallis test, or Mann–Whitney *U* test (as appropriate). A *P* value <0.05 was considered to be statistically significant. To identify the prediction ability of uNGAL and serum creatinine regarding occurrence of the composite outcome (ESRD and death) within 6 months, we performed receiver operating characteristic (ROC) analyses. Statistical analyses were performed using SPSS 22 and R 3.1.1 software.

RESULTS

We studied 145 patients with hospital-acquired AKI according to RIFLE criteria at the time of AKI diagnosis. The association of uNGAL in these patients with a diagnosis of intrinsic AKI and with short-term in-hospital outcomes has been previously reported.[46] We now report the results of clinical long-term follow-up of these patients (median follow-up, 6 months; range, <1 to 45 months). During the follow-up period, 22 patients (15.2%) developed ESRD (defined as a sustained requirement of renal replacement therapy), and 61 patients (42.1%) died. A total of 74 patients (51%) experienced the composite outcome of ESRD or death.

Patients were stratified according to NGAL quartiles at the time of AKI diagnosis. Baseline characteristics by NGAL quartile are shown in Table 1. Age, sex, and comorbidities were distributed similarly among NGAL quartiles. Patients within the highest uNGAL quartile had higher serum creatinine levels on inclusion and higher frequencies of stage 3 AKI. Based on diagnostic adjudications that we had reported earlier in this cohort and consistent with the observations reported therein,[46] patients in the higher uNGAL quartiles were more likely to have a diagnosis of intrinsic AKI, whereas patients with low uNGAL values were more likely to have prerenal AKI. We found a stepwise increase in the frequency of the composite outcome according to quartiles of uNGAL levels. In all, 78% of patients with NGAL levels ≥362 μg/l (corresponding to the highest quartile) experienced the composite outcome of death or ESRD during long-term follow-up, compared to 54.1% in the third NGAL quartile (95–362 μg/l), 38.9% in the second NGAL quartile (38–94 μg/l), and 33.3% in the lowest NGAL quartile (<37 μg/l). This finding indicated that NGAL levels at the time of AKI were strongly associated with poor long-term outcomes. These observations were in close agreement with cutoffs defined in our earlier studies.[21,46]

We next compared patients who developed the composite outcome with all other patients. The 2 groups had similar demographic features, comorbidities, and creatinine levels on AKI diagnosis and displayed similar distributions regarding AKI severity based on creatinine criteria, as shown in Table 2. In contrast, NGAL levels at the time of AKI diagnosis were significantly higher in patients who later experienced the composite outcome (629.0 vs. 221.5 μg/l; $P = 0.005$). To assess the diagnostic accuracy of uNGAL in diagnosing occurrence of the composite outcome (ESRD or death) at 6 months, we performed ROC analyses. The ROC–area under the curve (AUC) for uNGAL was 0.73 (95% confidence interval [CI] = 0.65–0.82), which was substantially higher than the ROC-AUC for serum creatinine (0.58; 95% CI = 0.48–0.68). At a cutoff of 362 μg/l (75th percentile), uNGAL showed a sensitivity of 44% and specificity of 87% in

Table 1. Patient characteristics according to NGAL quartile at the time of AKI

Patient characteristics by NGAL quartile	NGAL quartile 1 (<38 μg/l)	NGAL quartile 2 (38–95 μg/l)	NGAL quartile 3 (95–362 μg/l)	NGAL quartile 4 (>362 μg/l)	*P* value
Mean age, yr (SD)	65.8 (13.3)	70.8 (9.9)	67.3 (17.4)	66.9 (15.9)	0.664[a]
Female sex, n (%)	13 (36.1%)	17 (47.2%)	17 (45.9%)	12 (33.3%)	0.536[b]
History of chronic kidney disease, n (%)	8 (22.2%)	9 (25.0%)	12 (32.4%)	9 (25.0%)	0.779[b]
Hypertension, n (%)	30 (83.3%)	30 (83.3%)	33 (89.2%)	25 (69.4%)	0.168[b]
Diabetes, n (%)	12 (33.3%)	10 (27.8%)	13 (35.1%)	7 (19.4%)	0.452[b]
Median baseline creatinine (95% CI)	83.5 (71.1–95.0)	89.5 (74.0–96.0)	88.0 (80.0–100.0)	83.0 (72.0–100.0)	0.870[a]
Median serum creatinine (95% CI)	189.5 (155.0–224.0)	176.0 (164.6–229.5)	207.0 (188.5–238.0)	216.5 (192.0–315.0)	0.061[a]
RIFLE-F, n (%)	9 (25%)	11 (30.6%)	10 (27.0%)	13 (36.1%)	0.361[b]
AKIN-3, n (%)	9 (25%)	11 (30.6%)	10 (27.0%)	14 (38.9%)	0.272[b]
Prerenal AKI, n (%)	16 (44.4%)	10 (27.8%)	5 (13.5%)	1 (2.8%)	<0.001[b]
Intrinsic AKI, n (%)	7 (19.4%)	12 (33.3%)	25 (67.6%)	31 (86.1%)	<0.001[b]
Development of ESRD during follow-up, n (%)	2 (5.6%)	4 (11.1%)	5 (13.5%)	11 (30.6%)	0.021[b]
Death during follow-up, n (%)	11 (30.6%)	12 (33.3%)	17 (45.9%)	21 (58.3%)	0.065[b]
Death or ESRD during follow-up, n (%)	12 (33.3%)	14 (38.9%)	20 (54.1%)	28 (77.8%)	0.001[b]

AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; CI, confidence interval; ESRD, end-stage renal disease; NGAL, neutrophil gelatinase–associated lipocalin; RIFLE, Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease.
Median follow-up for outcomes: 6 months.
[a]Kruskal–Wallis test.
[b]χ^2 Test.

Table 2. Patient characteristics according to outcome

Patient characteristics by outcome	Total cohort	Outcome		*P* value
		Neither ESRD nor death	Composite (ESRD or death)	
Total number, n	145	71	74	
Mean age, yr (SD)	67.7 (14.4)	66,9 (15.0)	68.5 (13.9)	0.585[a]
Female sex, n (%)	59 (40.7%)	31 (43.7%)	28 (37.8%)	0.475[b]
History of chronic kidney disease, n (%)	38 (26.2%)	16 (22.5%)	22 (29.7%)	0.325[b]
Hypertension, n (%)	118 (81.4%)	58 (81.7%)	60 (81.1%)	0.925[b]
Diabetes, n (%)	42 (29.0%)	21 (29.6%)	21 (28.4%)	0.874[b]
Median baseline creatinine (μmol/l) (95% CI)	87 (79–91)	88 (78–95)	86.5 (77–99)	0.291[a]
Median serum creatinine on inclusion (μmol/l) (95% CI)	202 (189–220)	188 (174–224)	205.5 (192–229)	0.284[a]
RIFLE stage F, n (%)	43 (29.7%)	19 (26.8%)	24 (32.4%)	0.646[b]
AKIN stage 3, n (%)	44 (30.3%)	19 (26.8%)	25 (33.8%)	0.506[b]
Median NGAL (μg/l) (95% CI)	95.4 (71.0 −120,5)	56.0 (46.6–78.4)	208.1 (114.9–329.9)	<0.001[a]
Prerenal AKI, n (%)	32 (22.1%)	21 (29.6%)	11 (14.9%)	0.033[b]
Intrinsic AKI, n (%)	75 (51.7%)	27 (38.0%)	48 (64.9%)	0.001[b]

AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; CI, confidence interval; ESRD, end-stage renal disease; NGAL, neutrophil gelatinase−associated lipocalin; RIFLE, Risk, Injury, Failure, Loss of kidney function.
Median follow-up for outcomes: 6 months.
[a]Mann−Whitney *U* test.
[b]χ^2 Test.

predicting adverse long-term outcomes within the next 6 months. In comparison, serum creatinine at a cutoff of 264 umol/l (75th percentile) had a sensitivity of 31% and a specificity of 79%.

To compare the impact of uNGAL levels with conventional predictors of poor outcomes in patients with AKI (age, sex, comorbidities, serum creatinine, and RIFLE or AKIN stage), we used Cox regression analyses. Univariate analysis using candidate predictors suggested that pre-existing CKD and uNGAL >362 μg/l on AKI diagnosis were predictors of the composite outcome. Conversely, serum creatinine on inclusion and RIFLE or AKIN stage were not predictive of the composite outcome.

Next, we performed multivariable Cox regression and performed backward selection, as shown in Table 3. uNGAL > 362 μg/l and pre-existing CKD were independent significant predictors of the composite outcome. uNGAL >362 μg/l was associated with an adjusted hazard ratio of 3.03 (95% CI = 1.86–4.94).

Kaplan−Meier analyses showed that patients within the upper 2 uNGAL quartiles had significantly increased occurrences of adverse long-term outcomes compared to patients in the lower uNGAL quartiles ($P < 0.001$), as shown in Figure 1. After 6 months, 67% of patients in the highest uNGAL quartile had either progressed to ESRD or died, compared to 43% of patients in the third uNGAL quartile, and only 21% of patients in the lower quartiles ($P < 0.001$). Cox regression analyses showed that the highest uNGAL quartile and the third uNGAL quartile were associated with hazard ratios of 3.7 (95% CI = 2.1–6.5; $P < 0.001$) and 1.9 (95% CI = 1.1–3.5; $P < 0.05$), respectively, compared with the lower uNGAL quartiles. In contrast, strata of creatinine levels were not significantly associated with the combined endpoint ($P = 0.534$). Therefore, high NGAL at presentation with AKI is an independent predictor of long-term all-cause mortality and the development of ESRD.

Table 3. Univariable and multivariable Cox regression analysis on the entire cohort of AKI patients (N = 145)

Prediction of composite outcome (death or ESRD)	Univariable Cox regression			Multivariable model using significant covariates		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age (per 10 yr)	1.06	0.9–1.25	0.493			
Female sex	0.96	0.6–1.53	0.850			
History of chronic kidney disease	1.61	0.97–2.67	0.065	1.77	1.06–2.96	0.028
Hypertension	1	0.56–1.8	0.993			
Diabetes mellitus	0.94	0.57–1.56	0.806			
Serum creatinine on inclusion >264 μmol/l	1.15	0.67–1.95	0.614			
RIFLE class (1-3) on inclusion	1.13	0.82–1.55	0.448			
AKIN class (1-3) on inclusion	1.15	0.87–1.51	0.335			
uNGAL on inclusion > 362 μg/l	2.87	1.77–4.66	<0.001	3.03	1.86–4.94	<0.001

AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; CI = confidence interval; ESRD, end-stage renal disease; RIFLE, Risk, Injury, Failure, Loss of kidney function; uNGAL, urinary neutrophil gelatinase−associated lipocalin.

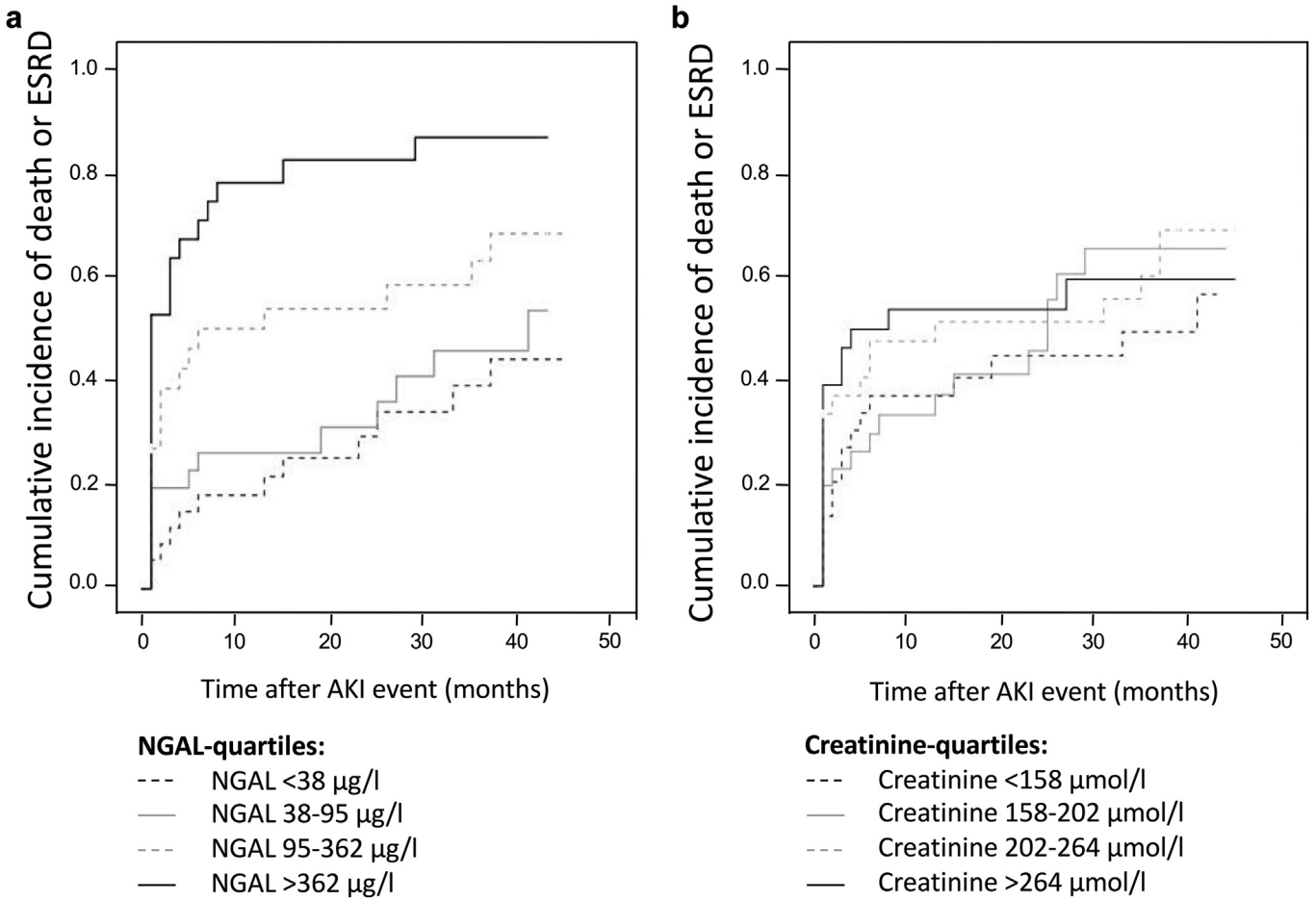

Figure 1. Cumulative incidence of the composite outcome of death or end-stage renal disease in acute kidney injury patients stratified by neutrophil gelatinase–associated lipocalin (NGAL) (a) and creatinine (b). The upper quartiles of NGAL, but not creatinine, are associated with an increasing incidence of the composite outcome.

Since part of the predictive ability of NGAL levels may be assigned to its powerful prediction of poor short-term outcomes,[46] we conducted a subgroup analysis to remove the influence of short-term outcomes, including only patients who had survived the AKI episode and who were discharged without a requirement for renal replacement therapy (N = 107). Of these patients, 5 developed ESRD and 32 died during long-term follow up. Cox regression showed that NGAL at the time of AKI diagnosis remained an independent predictor of the combined endpoint in these patients and that creatinine had no association with long-term outcomes in these patients, as shown in Table 4. Kaplan–Meier analyses for this subgroup

Table 4. Univariable and multivariable Cox regression analysis on the subpopulation of patients who had survived the AKI episode and who were discharged without a requirement for renal replacement therapy

	Univariate Cox regression			Multivariable model using significant covariates		
Prediction of composite outcome (death or ESRD)	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age (per 10 yr)	1.39	1.06–1.83	0.019	1.42	1.09–1.85	0.010
Female sex	0.84	0.42–1.69	0.628			
History of chronic kidney disease	1.3	0.57–2.98	0.534			
Hypertension	1.49	0.58–3.85	0.406			
Diabetes mellitus	0.88	0.42–1.82	0.728			
Serum creatinine on inclusion >264 µmol/l	0.66	0.26–1.70	0.392			
RIFLE class (1–3) on inclusion	0.77	0.49–1.22	0.259			
AKIN class (1–3) on inclusion	0.8	0.53–1.23	0.308			
uNGAL on inclusion >362 µg/l	3.48	1.70–7.12	0.001	3.93	1.90–8.13	<0.001

AKI, acute kidney injury; AKIN, Acute Kidney Injury Network; CI, confidence interval; ESRD, end-stage renal disease; RIFLE, Risk, Injury, Failure, Loss of kidney function; uNGAL, urinary neutrophil gelatinase–associated lipocalin.

showed that the third- and fourth-quartile uNGAL results were associated with significantly greater hazards of experiencing the composite outcome (P = 0.002), as shown in Figure 2. In contrast, increasing creatinine quartiles were not significantly associated with outcomes in this subgroup. These data indicate that NGAL-positive AKI predicts poor long-term outcomes even in patients who initially recover from their AKI episode.

The predictive ability of uNGAL regarding long-term outcomes may be driven by the ability of uNGAL to distinguish prerenal and intrinsic AKI. As reported in our previous investigation,[46] 32 patients within our cohort had prerenal AKI and 75 patients had intrinsic AKI at the time of enrollment in the study (the remaining 38 patients could not be classified based on the available clinical data). We used Kaplan–Meier analyses to compare long-term outcomes in patients with prerenal AKI and in those with intrinsic AKI. As shown in Figure 3, patients with intrinsic AKI progressed to ESRD or death more frequently than patients with prerenal AKI (Figure 3, P = 0.003 by log-rank test).

DISCUSSION

This study is the first to demonstrate that uNGAL levels measured at the time of AKI diagnosis in the hospital are predictive of death or the development of ESRD in the long term. Although the fact that AKI has an important impact on patients' long-term outcomes is well known,[17–22,48] our study shows that uNGAL is superior to creatinine levels or AKI stage in assessing the long-term prognosis. In addition, our study is the first to assess the relative predictive value of uNGAL when compared to other predictors in the setting of AKI. The difference in long-term outcomes may be driven by the distinct pathophysiology of prerenal and intrinsic AKI that is reflected by uNGAL levels.[46]

Previous studies have linked high NGAL levels with adverse long-term outcomes in other clinical settings. For instance, Ralib *et al.* showed an association of uNGAL at the time of intensive care unit admission and

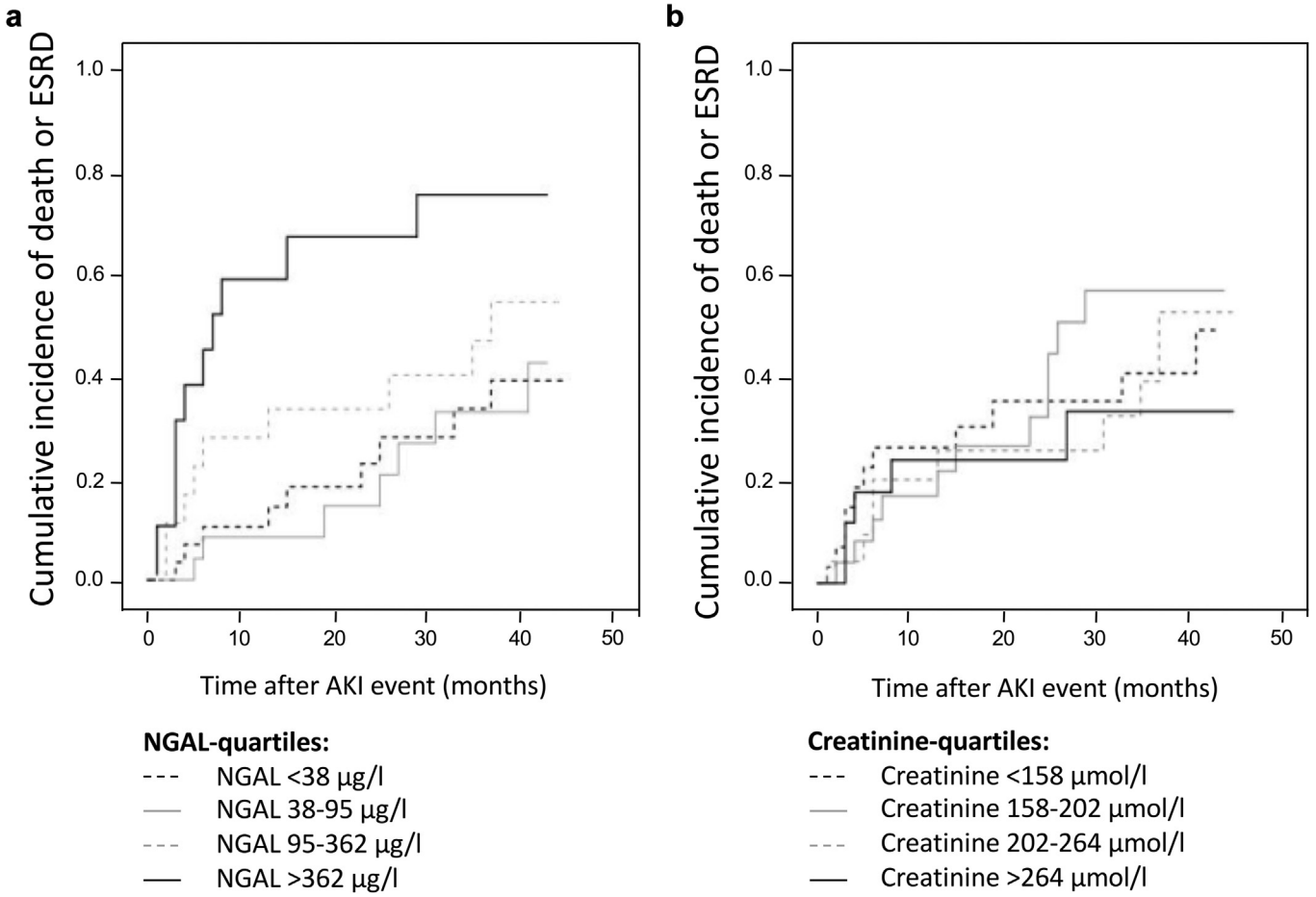

Figure 2. Subgroup analysis of cumulative incidence of the composite outcome of death or end-stage renal disease in acute kidney injury (AKI) patients stratified by neutrophil gelatinase–associated lipocalin (NGAL) (a) and creatinine (b). Only patients who had survived the AKI episode and who were discharged without a requirement for renal replacement therapy were included (N = 107). The upper quartiles of NGAL, but not creatinine, remain associated with an increasing incidence of the composite outcome.

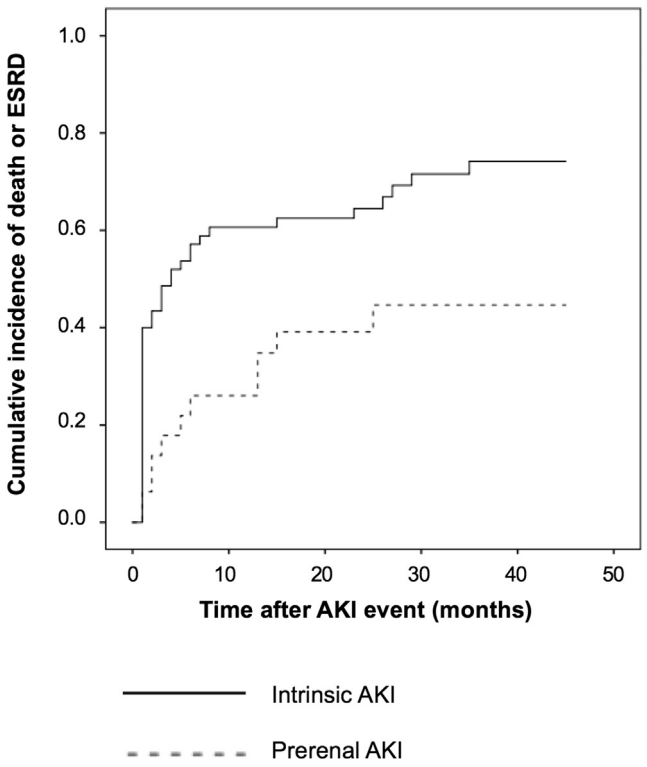

Figure 3. Cumulative incidence of the composite outcome of death or end-stage renal disease in patients with intrinsic and prerenal acute kidney injury (AKI; $P = 0.003$ by log-rank test).

1-year mortality, but did not consider the presence or absence of an AKI diagnosis.[49] Similarly, a recent long-term follow-up analysis of the Translational Research Investigating Biomarker Endpoints in AKI study in patients undergoing cardiac surgery found an association of perioperative peak uNGAL concentrations with long-term mortality rates. Indeed, these authors observed a stepwise increase in mortality rates by tertile of uNGAL, irrespective of the presence or absence of postoperative AKI.[50] Srisawat *et al.*[51] showed in their study that elevated plasma NGAL levels in patients with AKI due to pneumonia were associated with short-term renal nonrecovery. However, mortality as an outcome was not investigated, and uNGAL was not measured. Another study in critically ill patients with AKI requiring RRT[52] showed that relative changes in urinary NGAL values during the first 2 weeks after AKI predicted recovery of the kidney function with a ROC-AUC of 0.7. This moderate performance may be explained by the relative short follow-up of 60 days and by the fact that mortality was not an aspect of the study endpoint. Plasma NGAL predicted all-cause mortality in patients with hepatorenal syndrome.[53] In patients after hepatobiliary surgery, uNGAL predicted worsening of the kidney function but not mortality during 6 months of follow-up.[54] In a community-based study, uNGAL had a strong association with ESRD and all cause mortality in diabetic individuals at the 14-year follow-up.[55] Furthermore, several studies provide evidence that NGAL may be helpful in assessment of long-term (from 6 months up to 3 years) mortality in patients with chronic heart failure,[56,57] acute heart failure,[58,59] ST-elevation myocardial infarction,[60] and elective percutaneous intervention due to stable angina pectoris.[61] Together, these studies support a role for uNGAL measurements in assessing long-term prognosis and lend further plausibility to the findings reported herein.

In our study, neither RIFLE nor AKIN stage currently used to risk stratify patients with acute kidney injury contributed to the prediction of long-term mortality or ESRD. This may be due to the fact that our study population included only patients with established AKI and hence lacked an internal comparator population without AKI. In addition, the relatively small sample size of our study makes it difficult to find significant differences in long-term outcomes between different AKI stages.

In addition, serum creatinine at AKI diagnosis displayed weak associations with long-term outcomes when compared to uNGAL. Although there was a slight increase in serum creatinine in patients groups in increasing uNGAL quartiles (from a median of 189.5 μmol/L in the lowest quartile to a median of 216.5 μmol/L in the highest quartile; $P = 0.061$), there was still a large overlap of serum creatinine values between groups in different uNGAL quartiles (see the interquartile ranges of serum creatinine values in Table 1). In fact, the correlation of uNGAL and serum creatinine was weak (correlation coefficient = 0.272), as was the correlation between uNGAL quartile and serum creatinine quartile (correlation coefficient = 0.189). This explains why uNGAL quartiles and creatinine quartiles showed a markedly different association with long-term outcomes.

Other studies have clearly defined the impact of AKI on long-term outcomes, including death and ESRD. For instance, in adult cardiac surgery patients, the presence of AKI according to RIFLE criteria predicted 90-day mortality with an ROC-AUC of 0.82.[62] There was a stepwise increased mortality with advancing stages of AKI. Children undergoing cardiac surgery had a higher 4-year mortality with AKI RIFLE F than without AKI (odds ratio = 3.82, CI 1.89–7.75).[63] A prospective study on adult cardiac surgery patients demonstrated that modified RIFLE I and F stages were independent predictors of long-term mortality (hazard ratio = 2.35 and 3.09, respectively) compared to patients without AKI.[64] In patients with acute myocardial infarction, AKI severity by RIFLE classification during the first week after hospitalization was an independent predictor of

2-year mortality (hazard ratio = 8.1 and 19.3 for RIFLE I and F respectively).[65] However, these results were not replicated in all studies. In a population-based study, RIFLE predicted recovery of renal function, renal replacement therapy requirement, hospital length of stay, and in-hospital mortality, but not mortality at 90 days or 6 months.[66] Also, 2-year mortality of neonates undergoing cardiac surgery was not different whether or not they developed AKI, regardless of AKIN stage.[67]

Our observations also underline the importance of distinguishing the pathophysiology of AKI to differentiate intrinsic from prerenal AKI, potentially aided by biomarkers such as uNGAL. We found that patients with an adjudicated diagnosis of intrinsic AKI displayed significantly more adverse long-term outcomes with a cumulative incidence of $> 60\%$ during a 2-year follow-up. This incidence was significantly higher than that in patients with prerenal AKI, stressing the importance of distinguishing between different entities of AKI, not only for clinical management but also for prognostic assessment. Surprisingly, however, long-term outcomes were also remarkably frequent in patients who were assigned a diagnosis of prerenal AKI, with 2-year mortality approaching 40%. Although this may reflect adverse effects of prerenal azotemia, a more likely explanation lies in the fact that patients with prerenal AKI have severe comorbidities that predispose them to adverse long-term outcomes. These observations are consistent with previous analyses showing not only that the duration of AKI has a positive association with mortality but that even transient AKI is associated with an elevated risk of death.[68]

In addition, we observed that pre-existing CKD was a significant predictor of long-term outcomes in our cohort. We anticipated a role for pre-existing CKD as a predictor of long-term outcomes. Notably, a large multicenter study[69] demonstrated that patients with AKI on CKD during hospitalization had significantly worse long-term survival over a median follow-up of 4.8 years than patients with AKI but without CKD.

Our study has limitations. We conducted our analysis in a relatively small cohort at a single center. The definition of AKI in our study relied solely on creatinine dynamics. Urinary output was not assessed in our study, as most of our patients had AKI outside the intensive care unit setting. In addition, our study was not sufficiently powered to assess the individual components of the composite endpoint (mortality and ESRD) separately. This issue will need to be addressed in larger future cohorts. In addition, we do not have detailed information regarding the cause of death for most patients. However, our analysis including only patients who were alive and dialysis free on hospital discharge indicates that even when the initial episode of critical illness is survived, patients with NGAL-positive AKI remain at an increased risk for death or ESRD. Hence, further studies are needed to assess the long-term prognostic impact of uNGAL in patients with established AKI.

Long-term management of patients with AKI is currently suboptimal.[70,71] Evidence exists that patients who experience AKI episodes are not followed up systematically in the health care system. This state of affairs is concerning, especially given that multiple potential medical interventions exist. For instance, patients with post-AKI CKD might benefit from adequate blood pressure control,[72,73] an adaptation of glucose-lowering strategies,[74] and statin therapy.[75–79] The current uncertainty regarding post-AKI management may be due in part to the wide range of outcomes in post-AKI patients, ranging from complete restitution of kidney function to quick progression to CKD. A parameter that predicts adverse clinical courses in the setting of AKI would help to prioritize patients' follow-up, for example by nephrologists, during patients' hospital stay. Hence, determining uNGAL levels at the time of AKI diagnosis, in addition to improving diagnostic assessment,[21,46] may help in the selection of patients who require close medical attention after their hospital stay.

DISCLOSURE

KMSO has received license revenue related to the use of the NGAL assay. All the other authors declared no competing interests.

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