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EDITORIAL COMMENT

Early Detection of Acute Kidney Injury With Neutrophil Gelatinase-Associated Lipocalin*

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Acute kidney injury (AKI) after cardiac procedures and in critically ill patients has been consistently associated with increased cost of care, complications, permanent kidney dysfunction, and higher mortality (1,2). There is an immediate and present need to detect acute AKI before the rise in serum creatinine (Cr) and the development of oliguria, because by that time patients are often resistant to therapeutic interventions (3).

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For decades, the rise in serum Cr has been the only detectable sign of a reduction in glomerular filtration. Creatinine has had the disadvantages of being linked to creatine and the overall body muscle mass—hence, differing according to body size in addition to the rate of renal elimination (4). Furthermore, the kidney both filters and secretes Cr. Finally, the assays used to measure Cr have not been standardized across laboratories; therefore, studies reporting values from multiple centers have inherent variation in values attributed to differences in measurement technique (5). Cystatin C is a cysteine protease inhibitor that is synthesized and released into the blood at a relatively constant rate by all nucleated cells. It is freely filtered by the glomerulus, completely reabsorbed by the proximal tubule, and not secreted into urine. Its blood levels are not affected by age, sex, race, or muscle mass; thus, it seems to be a better indicator of glomerular function than serum Cr in patients with CKD. However, in AKI, it rises only slightly before serum Cr (6). The field of nephrology in the United States has been devoid of approved blood or urine markers of AKI, unlike cardiac biomarkers indicating myocardial injury and overload (troponin, creatine kinase myocardial band, and natriuretic peptides). The concept of measuring markers of the acute injury process is crucial to the early upstream identification of AKI before there is serious loss of organ function (7). Ideally, markers that give insight into the pathophysiologic mechanisms for AKI would be desirable, because there could be tandem development of therapeutic approaches.

Neutrophil gelatinase-associated lipocalin (NGAL), otherwise known as siderocalin or lipocalin-2, is normally secreted by renal tubular cells, lymphocytes, and cardiomyocytes and is a physiologic response to the presence of catalytic (labile, poorly liganded) iron in the cytosol or pericellular space that itself is liberated from organelles as a result of ischemic or toxic injury. It is a 25-kDa protein that acts as a natural siderophore that scavenges cellular and pericellular labile iron, thus reducing its availability for bacterial growth. Iron is the most common metal element in the human body, and there are elaborate transport (heme, transferrin, ferritin, ferroportin, electron transport chain) and management systems for its use in a variety of critical cellular systems, including oxygen transport and cellular respiration (8,9). It has been recently understood that the process of oxidative stress resulting in cell dysfunction, accelerated apoptosis, and death is reliant on the cytosolic and extracellular presence of labile or catalytic iron, liberated from its binding proteins. There are several steps in generation of reactive oxygen species. Oxygen might be reduced, forming superoxide anion, which can undergo reduction by superoxide dismutase to form hydrogen peroxide—which itself can then be reduced through several pathways. The net reaction is slow, and in the presence of reduced transition metals such as ferric iron (Fe^{+3}), a Haber–Weiss reaction results in the rapid formation of the highly damaging hydroxyl radical from the superoxide anion. Likewise, in the presence of ferrous iron (Fe^{+2}), a Fenton-type reaction converts hydrogen peroxide to the hydroxyl radical. It has been theorized that a common element to all forms of oxidative stress to the heart and kidneys involves the availability of unbound or poorly liganded iron (10). With the bleomycin detectable assay, Lele et al. (9) have recently demonstrated the release of catalytic iron into the blood in patients with acute coronary syndromes. In this study, the appearance of catalytic iron preceded the rise in serum troponin and had an area under the receiver operating characteristic curve for the detection of acute myocardial infarction over 0.90. Local cellular and tissue availability of catalytic iron might determine the degree and severity of organ injury in the setting of most hypoxic and other toxic insults (8). Therefore, a putative final common pathway for common sources of organ injury including ischemia, neurohormonal activation, chemotoxicity, and sepsis involves

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the loss of control over normal iron management and the transient tissue and organ system exposure to catalytic iron, which is attenuated by NGAL (11).

In this issue of the *Journal*, Haase et al. (12), as part of a previous larger meta-analysis, identified and invited authors of 18 published studies of clinical outcomes of AKI patients according to NGAL (measured either in the blood or urine) and Cr status. Of those, 7 authors returned complete datasets consisting of 1,217 patients (11). The major findings were that 41.1% of patients diagnosed with AKI would have been missed with Cr alone. Compared with patients declared negative with respect to both NGAL and Cr results, those positive for both had a 2-fold longer stay in the ICU and in-hospital, more dialysis initiation, and a tripled in-hospital mortality. In the discordant groups, NGAL tended to identify increased risk when the Cr was not significantly changed, with the greatest value being in the NGAL(+) but Cr(−) group. These data suggest that a marker of the renal response to oxidative stress (NGAL) and damage in addition to a marker of decreased renal filtration function (Cr) are complementary in the diagnosis and prognosis of AKI across a variety of hospitalized populations.

The reason this study is important is, first, because it consistently showed prognostic significance of NGAL value in a large number of AKI patients with wide range of clinical situations, including critically ill patients and post-cardiac surgery patients as well as pediatric and adult population. Neutrophil gelatinase-associated lipocalin typically detected AKI 36 to 48 h earlier than Cr, thus opening up a possibility of therapeutic interventions of AKI. Second, this analysis identified new important subgroups of AKI patients according to NGAL and Cr values, which seem to be complementary. Specifically, NGAL(−)/Cr(+) patients might have pre-renal azotemia or tubulo-glomerular feedback mechanisms at work without acute tubular necrosis, which might require totally a different therapeutic approach, as opposed to those who have started to secrete increased quantities of NGAL. Conversely, identification of the NGAL(+)/Cr(−) patient is a critical step forward, because the production of NGAL signals a response to catalytic iron-dependent oxidative stress before there is a measurable decrement in organ function. We recognize that a shortcoming of NGAL as a singular test is that baseline levels rise with chronic renal disease, and thus it becomes a marker of disease progression and might lose its ability to detect AKI from an elevated baseline in more seriously ill populations (13,14).

With the advent of NGAL and the detection of AKI within a few hours of initiation, it is now conceivable to test preventive or immediate therapeutic interventions. The change in NGAL might serve as an experimental marker in phase 2 trials of preventive approaches including catalytic iron chelators (e.g., deferiprone), other antioxidant agents such as N-acetylcysteine (for contrast exposure), or B-type natriuretic peptide in the perioperative period after cardiac surgery (15,16). Once NGAL has started to rise in critical care and post-surgical scenarios, treatment trials could be organized to test forms of continuous renal replacement therapy (CRRT) in the period of time surrounding the renal insult. Conceptually, use of CRRT would provide 3 important protective mechanisms that cannot be achieved pharmacologically: 1) ensures euvolemia and avoids hypo- or hyper-volumia; 2) provides sodium and solute (nitrogenous waste products) removal; and 3) by both aforementioned mechanisms, it might work to avoid both passive renal congestion and a toxic environment for the kidneys and allow their optimal function during a systemically vulnerable period (17). Despite these advantages, there remains a lack of clinical trial data supporting CRRT or other forms of extracorporeal solute removal, largely because the intervention is applied too late in the clinical course where the detection of AKI is dependent on the rise in serum Cr and development of volume overload.

The identification of NGAL as a protective siderophore and its role in limiting the ability of catalytic iron to propagate oxidative stress reactions in acute organ injury syndromes seems to be a major advance. Additional biomarkers, including kidney injury molecule-1, interleukin 18, liver-type fatty acid binding protein, renal tubular enzymes, and multiple proprietary markers under investigation, might add to the internal validity and assist in understanding the time course and recovery of AKI. These markers used together as a panel might be particularly valuable in the population where Cr has not yet changed. The discovery of NGAL and its clinical introduction in Europe last year has emerged as an ideal circumstance in the biomarker field, where both diagnostic and therapeutic advances are on the horizon.

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Key Words: acute kidney injury (AKI) ■ biomarker ■ creatinine ■ mortality ■ neutrophil gelatinase-associated lipocalin (NGAL) ■ renal replacement therapy (RRT).

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The Outcome of Neutrophil Gelatinase-Associated Lipocalin-Positive Subclinical Acute Kidney Injury

A Multicenter Pooled Analysis of Prospective Studies

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Objectives The aim of this study was to test the hypothesis that, without diagnostic changes in serum creatinine, increased neutrophil gelatinase-associated lipocalin (NGAL) levels identify patients with subclinical acute kidney injury (AKI) and therefore worse prognosis.

Background Neutrophil gelatinase-associated lipocalin detects subclinical AKI hours to days before increases in serum creatinine indicate manifest loss of renal function.

Methods We analyzed pooled data from 2,322 critically ill patients with predominantly cardiorenal syndrome from 10 prospective observational studies of NGAL. We used the terms NGAL(−) or NGAL(+) according to study-specific NGAL cutoff for optimal AKI prediction and the terms sCREA(−) or sCREA(+) according to consensus diagnostic increases in serum creatinine defining AKI. A priori-defined outcomes included need for renal replacement therapy (primary endpoint), hospital mortality, their combination, and duration of stay in intensive care and in-hospital.

Results Of study patients, 1,296 (55.8%) were NGAL(−)/sCREA(−), 445 (19.2%) were NGAL(+)/sCREA(−), 107 (4.6%) were NGAL(−)/sCREA(+), and 474 (20.4%) were NGAL(+)/sCREA(+). According to the 4 study groups, there was a stepwise increase in subsequent renal replacement therapy initiation—NGAL(−)/sCREA(−): 0.0015% versus NGAL(+)/sCREA(−): 2.5% (odds ratio: 16.4, 95% confidence interval: 3.6 to 76.9, $p < 0.001$), NGAL(−)/sCREA(+): 7.5%, and NGAL(+)/sCREA(+): 8.0%, respectively, hospital mortality (4.8%, 12.4%, 8.4%, 14.7%, respectively) and their combination (4-group comparisons: all $p < 0.001$). There was a similar and consistent progressive increase in median number of intensive care and in-hospital days with increasing biomarker positivity: NGAL(−)/sCREA(−): 4.2 and 8.8 days; NGAL(+)/sCREA(−): 7.1 and 17.0 days; NGAL(−)/sCREA(+): 6.5 and 17.8 days; NGAL(+)/sCREA(+): 9.0 and 21.9 days; 4-group comparisons: $p = 0.003$ and $p = 0.040$, respectively. Urine and plasma NGAL indicated a similar outcome pattern.

Conclusions In the absence of diagnostic increases in serum creatinine, NGAL detects patients with likely subclinical AKI who have an increased risk of adverse outcomes. The concept and definition of AKI might need re-assessment. (J Am Coll Cardiol 2011;57:1752–61) © 2011 by the American College of Cardiology Foundation

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The current concept and diagnosis of acute kidney injury (AKI) are mainly based on diagnostic increases in serum creatinine indicating loss of excretory renal function. Acute kidney injury is then classified according to either the renal risk (R), injury (I), failure (F), loss of renal function (L), end stage renal disease (E) classification (RIFLE) (1) or AKI Network consensus criteria (2). Although such diagnosis of AKI is of prognostic relevance (3,4), it is delayed by 24 to 72 h compared with diagnosis by means of novel renal biomarkers of tubular injury like neutrophil gelatinase-associated lipocalin (NGAL) (5–7).

Neutrophil gelatinase-associated lipocalin fulfills many characteristics for an ideal biomarker for AKI. It was discovered with unbiased transcriptomic approaches (8,9); it is rapidly induced and released from the injured distal nephron in experimental models and human disease (5,9,10); its urine and plasma concentrations increase proportionally to severity and duration of renal injury (9,11,12); its concentration rapidly decreases with attenuation of renal injury (13); and it is readily and easily measured in plasma (11) and urine (12). Finally, NGAL seems to play a key role in early AKI and local iron transport (10,14,15), providing biologic plausibility for its use as an AKI biomarker.

Several studies have found NGAL useful compared with early measurements of serum creatinine in AKI (6,7,16,17). In response to renal injury, increases in NGAL levels predict AKI 24 to 72 h before diagnostic creatinine increases (5–7,11,12) and are of prognostic value (18). Despite the aforementioned characteristics, the ability of NGAL to predict the development of AKI seems imperfect (19,20). However, the predictive value of NGAL improves with increasing RIFLE class of AKI (21), suggesting that limitations in its accuracy reflect the use of an imperfect test (serum creatinine) as study endpoint. Serum creatinine requires several hours to days to accumulate, it increases in serum only after 50% or more of renal function is lost, and its concentration is affected by multiple confounding factors (22). Accordingly, we hypothesized that, without diagnostic increases in serum creatinine, NGAL(+) patients might have likely subclinical AKI and therefore carry a worse prognosis—as indicated by the need for renal replacement therapy (RRT) and other patient-centered outcomes—than NGAL(−) patients. We tested this hypothesis by analyzing pooled data from multiple centers and determined the clinical outcomes of subjects classified according to their NGAL and serum creatinine concentrations.

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Methods

Abbreviations and Acronyms

AKI = acute kidney injury
ICU = intensive care unit
NGAL = neutrophil gelatinase-associated lipocalin
RIFLE = renal risk, injury, failure, loss of renal function, end stage renal disease classification
RRT = renal replacement therapy
sCREA = serum creatinine

We performed a summary patient-level analysis with summarized data from each study (see data collection form in the Online Appendix). For this purpose, we used data from a previously described multicenter data pool created to study the predictive value of NGAL for AKI defined by increases in serum creatinine (18). This pool was extended by newly identified clinical studies on NGAL as biomarker of AKI. Individual patient data were not available. Figure 1 displays the results of the search and data acquisition strategies.

Data pool creation. Two investigators (A.H.F. and P.R.M.) independently searched PUBMED, EMBASE, CENTRAL, and abstracts submitted to the Congress of the American Society of Nephrology. They identified relevant articles or abstracts and independently screened studies for inclusion (Fig. 1). Selection was restricted to published prospective cohort studies in humans investigating the diagnostic and prognostic ability of NGAL for AKI, need for renal replacement therapy, and in-hospital mortality. Each independent biomarker study was approved by a local institutional review board, and all participants gave written informed consent. All studies originally were designed to examine the diagnostic accuracy of NGAL level, prospectively enrolled consecutive patients, and had clearly defined enrollment and exclusion criteria, and laboratory/research personnel and clinicians were blinded. All studies enrolled representative patient cohorts who might receive the test in clinical practice. Included studies had similar sample collection and processing and provided full datasets.

NGAL measurement and AKI definition. For the purpose of this study, we included urine and plasma NGAL values and reported those in a combined fashion and separately. We asked each author to use the measurement performed at least 24 to 48 h before the diagnosis of AKI when NGAL was measured more than once or, when no serum creatinine increase occurred, to use NGAL measured at intensive care unit (ICU) admission or 2 to 6 h after any new renal insult. We defined AKI according to the RIFLE classification (1) as an increase in serum creatinine ≥50% from baseline to peak value within 7 days of admission to the ICU on the basis of daily serum creatinine measurement (RIFLE class R or worse). We chose RIFLE over the AKI Network classification (2) because of its greater sensitivity (23). Baseline serum creatinine was defined as the concentration obtained at outpatient departments or at hospital admission in cardiac surgery patients or at ICU admission in critically ill patients when previous values were not available. Chronic kidney disease was defined as an estimated glomer-

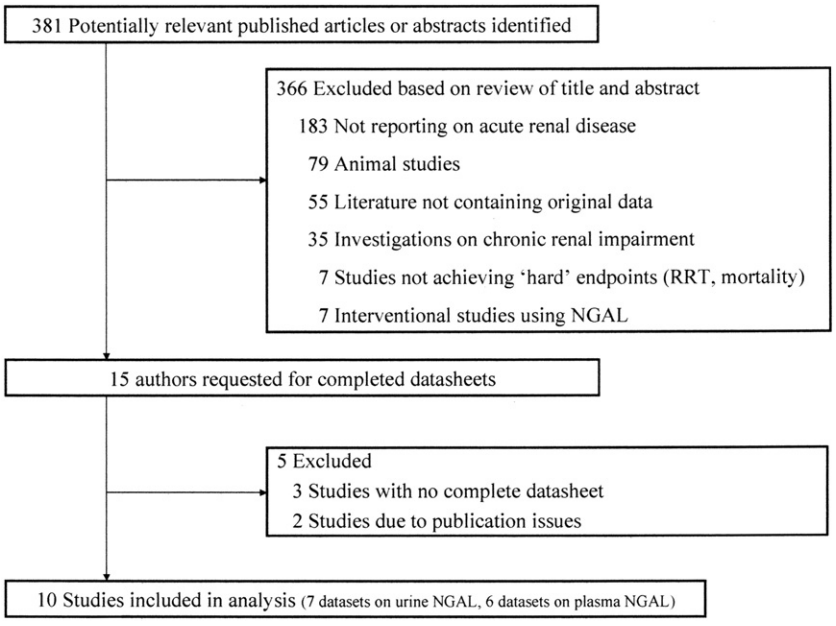

Figure 1 **Flow of Study Selection**

NGAL = neutrophil gelatinase-associated lipocalin; RRT = renal replacement therapy.

ular filtration rate <60 ml/min/1.73 m^2 calculated with the simplified Modification of Diet in Renal Disease study formula (24) in adults or the simplified Schwartz formula in children (25). Renal replacement therapy (RRT) was initiated on the basis of center- and physician-specific practice, and no patient received RRT for nonrenal indications.

We applied the term "NGAL(−)" or "NGAL(+)" to indicate the absence or presence of tubular injury, as defined by each study-specific NGAL cutoff value for the optimal combination of sensitivity and specificity for AKI prediction (18). The early "research-based" urine or plasma NGAL enzyme-linked immunosorbent assays were internally valid, but their cutoff values were not transferable to other enzyme-linked immunosorbent assays or immunoblots used. We used the term serum creatinine "sCREA(−)" or "sCREA(+)" to indicate the absence or presence of manifest AKI as defined by RIFLE criteria.

Patients were classified as follows: 1) NGAL(−)/sCREA(−); 2) NGAL(+)/sCREA(−); 3) NGAL(−)/sCREA(+); and 4) NGAL(+)/sCREA(+).

Patient outcomes. For the present study, we invited each investigator to analyze data from their individual patient cohort and return a specifically designed data collection form (Online Appendix) recording demographic data, information on comorbidities, and a priori-defined patient outcomes including renal replacement therapy initiation (primary endpoint), in-hospital mortality, the combination of both and the length of ICU and hospital stay and according to the NGAL of patients and serum creatinine states as defined in the preceding text.

Statistical analysis. All analyses followed a preset statistical analysis plan, which included the aforementioned a priori-defined hypothesis. Data were tested on normality with histograms. When data were normally distributed, analysis of variance was used to compare numerical data of patients according to the defined groups. Otherwise, nonparametric testing was used (Kruskal-Wallis test for 4-group comparison, Mann-Whitney test for 2-group comparison). Fisher exact test or the chi-square test was applied for comparison of categorical values as appropriate. Analysis was repeated after weighing of the study endpoints according to the relative sample size of each study. We used SPSS (version 16.0, SPSS, Inc., Chicago, Illinois). A 2-sided p value <0.05 was considered to be statistically significant.

Results

Ten (6,7,11,12,19,20,26–29) of 15 authors contacted returned complete datasets. The reasons for exclusion are reported in Figure 1.

We obtained data on 2,322 critically ill patients from America, Europe, and Australia (Table 1), with the majority having cardiorenal syndrome (30). The incidence of AKI ranged from 15% to 49%. Characteristics and baseline peak NGAL levels of patients are shown in Table 2. The majority of patients were NGAL(−)/sCREA(−), whereas 25% of patients developed AKI on the basis of the RIFLE definition, 3% required renal replacement therapy, and 8% died in-hospital. In patients with diabetes, NGAL(+)/sCREA(−) status was less common than NGAL(−)/

Table 1 Characteristics of Studies

First Author (Ref. #)	Population Type	AKI Etiology	AKI Incidence	No. of Patients*		NGAL Measurement,† h
				Urine NGAL	Plasma NGAL	
Dent et al. (11)	Children	Cardiac surgery	36%	—	125	48
Bennett et al. (12)	Children	Cardiac surgery	49%	194	—	48
Krawczeski et al. (29)	Children	Cardiac surgery	35%	189	358	48
Zappitelli et al. (27)	Children	Critically ill	30%	33	—	36
Cruz et al. (6)	Adults	Critically ill	28%	—	279	24
Haase-Fielitz et al. (7)	Adults	Cardiac surgery	23%	—	100	48
Koyner et al. (26)	Adults	Cardiac surgery	16%	52	51	36
Wagener et al. (19)	Adults	Cardiac surgery	15%	383	—	36
Martensson et al. (28)	Adults	Critically ill	34%	64	64	48
Siew et al. (20)	Adults	Critically ill	19%	430	—	48

*With neutrophil gelatinase-associated lipocalin (NGAL) measured at least 24 to 48 h before the diagnosis of acute kidney injury (AKI) or at intensive care unit admission several hours after new renal insult and excluding patients with urinary tract infection. †Preceding the diagnosis of AKI on the basis of renal risk, injury, failure, loss of renal function, or end stage renal disease classification class R or worse (1).

sCREA(+). Chronic kidney disease was similarly common among NGAL(−)/sCREA(−) (11.0%) and NGAL(+)/sCREA(−) (10.6%) patients.

Figure 2A shows the proportion of patients in each study according to NGAL/sCREA status, with approximately 20% of all patients being NGAL(+)/sCREA(−). Overall, 43% of patients diagnosed with AKI by means of NGAL would have been classified as non-AKI using creatinine criteria alone (Fig. 2B).

Patient outcomes. Treatment with RRT increased from 0.0015% in biomarker-negative patients to 2.5% in NGAL(+)/sCREA(−) patients, 7.5% in NGAL(−)/sCREA(+) patients, and 8.0% in NGAL(+)/sCREA(+) patients (4-group comparison: $p < 0.001$) (Fig. 3). Specifically, more patients with NGAL(+)/sCREA(−) status needed RRT initiation than patients with NGAL(−)/sCREA(−) status (odds ratio: 16.4, 95% confidence interval: 3.6 to 76.9, $p < 0.001$). Additional endpoints comparing these patient groups are shown in Table 3.

Hospital mortality doubled from biomarker negative patients to NGAL(+)/sCREA(−) status and more than tripled in patients positive for both biomarkers (4-group comparison: $p < 0.001$) (Fig. 3). More NGAL(+)/sCREA(−) patients (69 of 445; 15.5%) developed the composite endpoint of RRT initiation or in-hospital mortality when compared with biomarker negative patients (63 of 1,296; 4.9%) ($p < 0.001$). A greater proportion of NGAL(+)/sCREA(+) patients (84 of 474; 17.7%) reached the combined endpoint of RRT initiation and in-hospital mortality compared with patients negative for both biomarkers (odds ratio: 4.2, 95% confidence interval: 3.0 to 6.0, $p < 0.001$).

The NGAL(+)/sCREA(−) patients had a >70% longer stay in the ICU compared with patients negative for both biomarkers ($p = 0.026$) (Fig. 4A). There was a gradual increase in median length of stay in the ICU with the shortest duration (in days, median [25th to 75th percentile]) for NGAL(−)/sCREA(−) patients (4.2 [2.2 to 6.4]), low intermediate duration for NGAL(−)/sCREA(+) patients (6.5 [3.0 to 11.7]), high intermediate duration for NGAL(+)/sCREA(−) patients (7.1 [5.4 to 10.3]), and longest duration for NGAL(+)/sCREA(+) patients (9.0 [8.0 to 14.0]) ($p = 0.003$; 4-group comparison).

The NGAL(+)/sCREA(−) patients spent twice as long in hospital compared with patients negative for both renal biomarkers ($p = 0.16$) (Fig. 4B). The shortest hospital stay (in days, median [25th to 75th percentile]) was for NGAL(−)/sCREA(−) patients (8.8 [7.7 to 19.0]) with low intermediate values for NGAL(+)/sCREA(−) patients (17.0 [8.4 to 24.2]), high intermediate values for NGAL(−)/sCREA(+) patients (17.8 [5.1 to 26.4]), and

Table 2 Baseline Characteristics of Patients

	NGAL(−)/sCREA(−)	NGAL(+)/sCREA(−)	NGAL(−)/sCREA(+)	NGAL(+)/sCREA(+)	p Value
n	1,296 (55.8%)	445 (19.2%)	107 (4.6%)	474 (20.4%)	—
Age, yrs	47.2 (4.5–61.6)	53.5 (3.6–60.7)	59.0 (4.4–67.3)	50.4 (3.6–66.8)	0.89
Female	475 (36.7%)	181 (40.7%)	42 (39.3%)	210 (44.3%)	0.028
Chronic kidney disease*	143 (11.0%)	47 (10.6%)	16 (15.0%)	81 (17.1%)	0.030
Diabetes mellitus	113 (8.7%)	78 (17.5%)	28 (26.2%)	29 (6.1%)	<0.001
Congestive heart failure	67 (5.2%)	51 (11.5%)	10 (9.4%)	14 (3.0%)	<0.001
Peak NGAL, ng/ml	59 (20–97)	213 (117–1,124)	69 (21–118)	354 (208–1,888)	<0.001

n = 2,322. Values given as n (%) or median (25th to 75th percentiles). *As defined by estimated glomerular filtration rate <60 ml/min/1.73 m^2 with the modification of diet in renal disease study formula (24,25).

NGAL = neutrophil gelatinase-associated lipocalin; sCREA = serum creatinine.

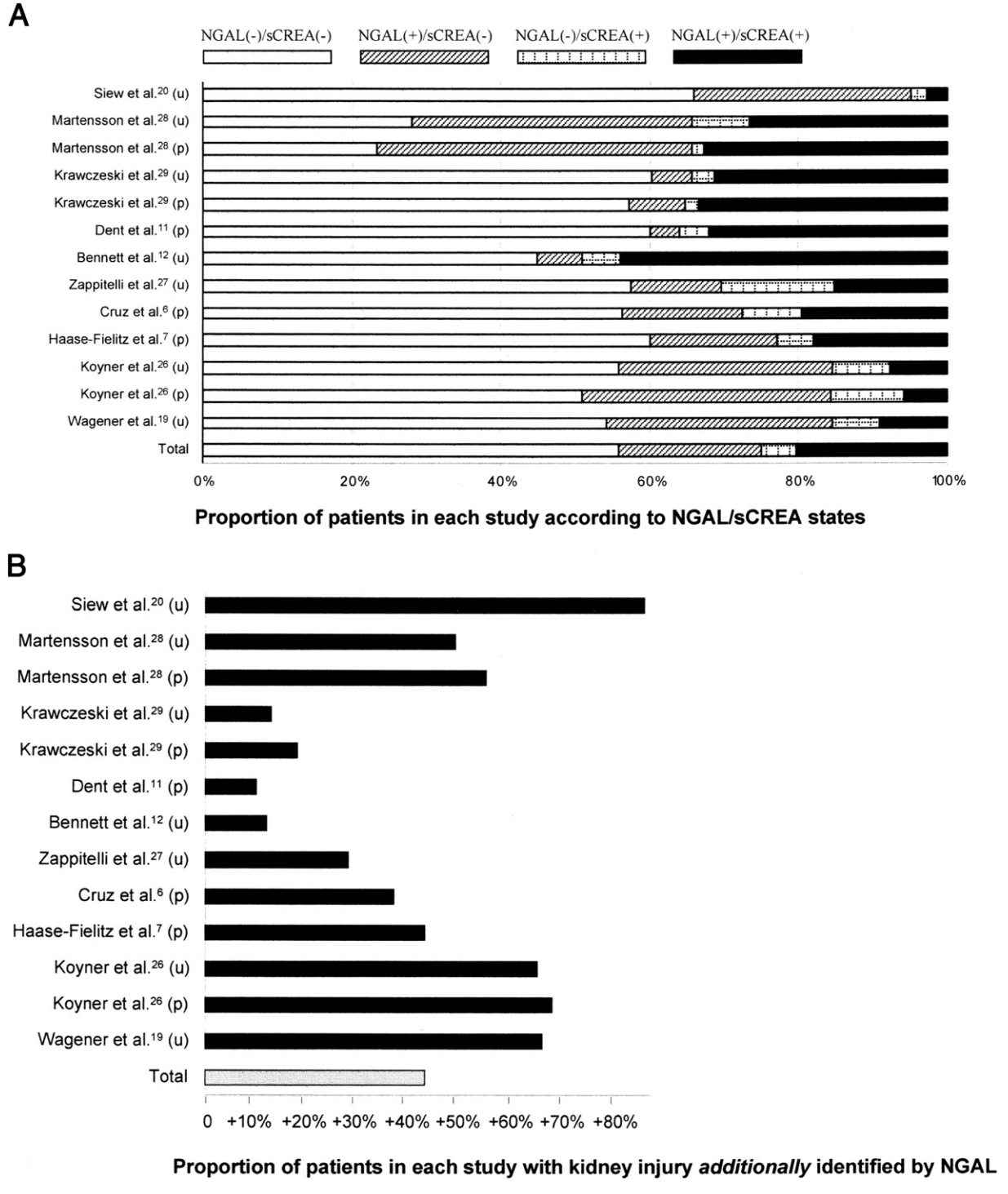

Figure 2 **Proportions of Patients**

(A) Proportion of patients according to biomarker states. **(B)** Proportion of neutrophil gelatinase-associated lipocalin (NGAL) (+)/serum creatinine (sCREA) (−) patients in relation to the proportion of patients diagnosed to have acute kidney injury by conventional creatinine-based criteria (renal risk, injury, failure, loss of renal function, end stage renal disease classification (1) and NGAL positivity. The formula used was:

$$\frac{(\text{NGAL}+/\text{sCREA}-) = \text{renal impairment identified by NGAL}}{(\text{NGAL}+/\text{sCREA}-) + (\text{NGAL}-/\text{sCREA}+) + (\text{NGAL}+/\text{sCREA}+) = \text{all renal impairment}}$$

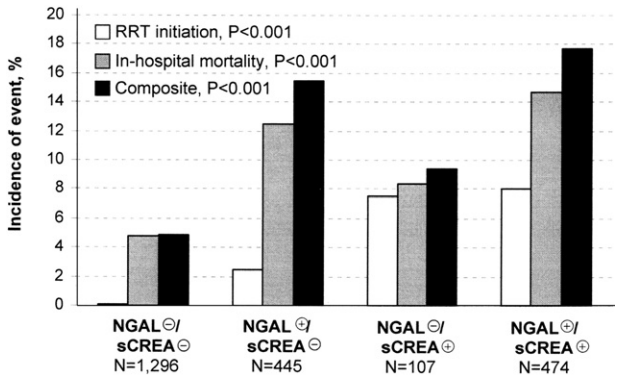

Figure 3 Incidence of Events

Incidence of RRT initiation, in-hospital morality, and a combination of both according to NGAL and sCREA. There was a stepwise increase in all outcomes. Abbreviations as in Figures 1 and 2.

longest stay for NGAL(+)/sCREA(+) patients (21.9 [15.8 to 29.9]) (p = 0.040; 4-group comparison).

Table 4 presents outcomes according to urine versus plasma NGAL, confirming a similar outcome pattern independent of the biological material used to measure NGAL.

Discussion

Key study findings. We conducted a multicenter analysis of pooled data to explore the prognostic value of AKI detected by NGAL. We hypothesized that, without diagnostic increase in serum creatinine, NGAL(+) patients might have likely subclinical AKI and carry a worse prognosis than NGAL(−) patients. We found that a positive NGAL finding carried a similar risk of adverse outcome than a positive creatinine finding. We also found that NGAL(+)/sCREA(−) tests identified approximately 40% more AKI cases than sCREA(+) alone and that these patients were at greater risk of longer ICU and hospital stay, RRT, and death compared with control subjects. As expected, NGAL(+)/sCREA(+) patients had the greatest risk of adverse outcomes. A smaller group of patients were NGAL(−)/sCREA(+), implying loss of renal function without evidence of acute tubular injury. Outcome of these patients was intermediate in severity. Finally, NGAL in the urine or plasma showed a similar pattern for the outcomes assessed.

Table 3 Value of NGAL Complements Prognosis in Patients Without Diagnostic Creatinine Increase

Outcome	NGAL−/sCREA− vs. NGAL+/sCREA−	p Value
Need for RRT initiation	16.4 (3.6–76.9)	0.001
In-hospital mortality	2.8 (1.9–4.1)	0.001
Need for RRT initiation/ in-hospital mortality	3.6 (2.5–5.2)	0.001
ICU stay, days*	2.9	0.026
Hospital stay, days*	8.2	0.16

Values given as odds ratio (95% confidence interval) or n. *Median difference.
CI = confidence interval; ICU = intensive care unit; OR = odds ratio; RRT = renal replacement therapy; other abbreviations as in Table 2.

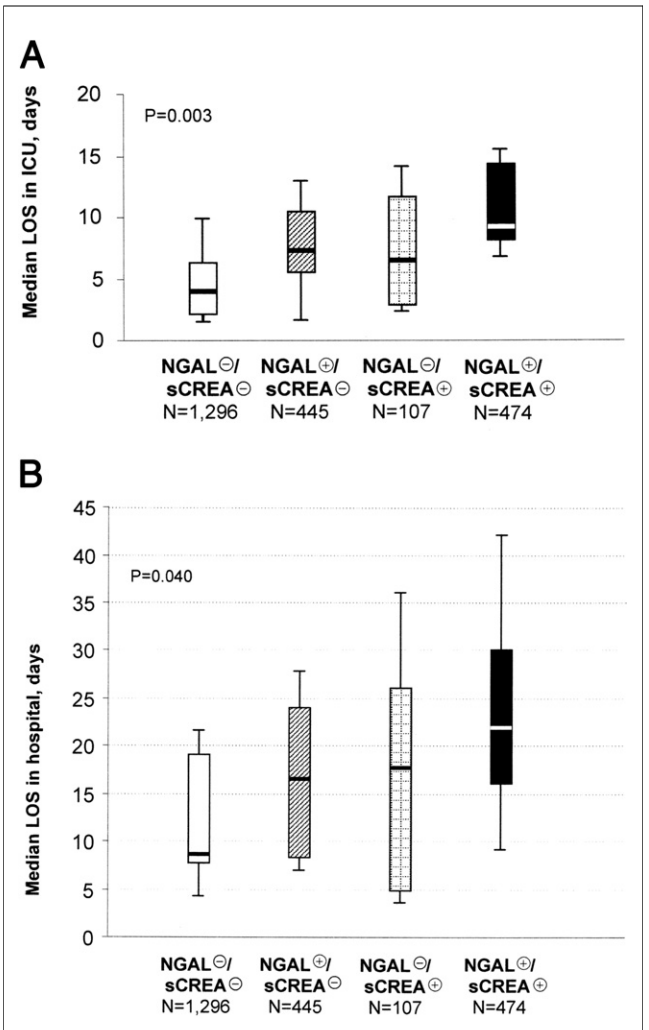

Figure 4 Median LOS in ICU

Length of stay (LOS) stratified by NGAL and sCREA in the intensive care unit (ICU) **(A)** and in-hospital **(B)**. There was a stepwise increase in median LOS in ICU and in-hospital. Abbreviations as in Figures 1 and 2.

Relation to previous studies. AKI might affect 20% to 30% of hospitalized patients; it carries significant costs and is independently associated with increased morbidity and mortality (31,32). Our study is consistent with other reports on the prognostic importance of serum creatinine increase (31,32). Similarly, NGAL has been repeatedly shown to predict the need for RRT and mortality in AKI (7,12,16). However, previous studies have also shown that NGAL failed to reliably predict changes in serum creatinine, even though its predictive value improved with increasing AKI severity (21).

Until now, the aforementioned findings have been interpreted as reflecting the shortcomings of NGAL as a biomarker

Table 4 Outcome According to Urine NGAL Versus Plasma NGAL

	NGAL(−)/sCREA(−)	NGAL(+)/sCREA(−)	NGAL(−)/sCREA(+)	NGAL(+)/sCREA(+)
Urine NGAL (n = 1,345)	758 (56.4%)	307 (22.8%)	63 (4.7%)	217 (16.1%)
Need for RRT initiation	2 (0.003%)	9 (2.9%)	2 (3.2%)	16 (7.4%)
In-hospital mortality	35 (4.6%)	37 (12.1%)	3 (4.8%)	30 (13.8%)
ICU stay, days	4.2 (2.1–8.5)	7.6 (5.7–14.5)	6.5 (3.0–11.6)	10.4 (7.7–14.8)
Hospital stay, days	8.8 (7.2–18.8)	17.0 (8.2–24.6)	18.3 (5.2–36.0)	21.8 (11.3–24.1)
Plasma NGAL (n = 977)	538 (55.1%)	138 (14.1%)	44 (4.5%)	257 (26.3%)
Need for RRT initiation	0 (0%)	2 (1.5%)	6 (13.6%)	22 (8.6%)
In-hospital mortality	27 (5.0%)	18 (13.0%)	6 (13.6%)	40 (15.6%)
ICU stay, days	4.0 (2.2–5.4)	6.5 (4.3–9.0)	5.1 (3.0–12.0)	8.6 (8.0–11.9)
Hospital stay, days	9.7 (7.3–20.3)	14.6 (8.1–22.7)	11.1 (4.5–21.1)	25.5 (18.0–33.9)

Values given as n (%) or median (25th to 75th percentiles).
Abbreviations as in Tables 2 and 3.

(20). An alternative explanation, however, is that the limitation lies with serum creatinine as the diagnostic standard and the different nature of the signal provided. Thus, NGAL indicates tubular injury that precedes renal functional loss by several days, and serum creatinine indicates subsequent loss of renal excretory function. Our findings suggest that a state of AKI likely exists when NGAL is increased, independent of serum creatinine increases.

Previous work supports the biological plausibility of the aforementioned notion and suggests that serum creatinine is a delayed, low-sensitivity, and could be a misleading biomarker of AKI (5,7), affected by many confounding factors (22). In this regard, several studies (33,34,35) suggest an analogy between the troponin/creatine kinase and the NGAL/creatinine relationship with a novel, more-sensitive biomarker identifying previously undetected organ injury. This increased diagnostic sensitivity of troponin is clinically relevant (33) and, in the field of cardiology, has altered the definition, diagnosis, and management of acute myocardial infarction. This concept might similarly apply to NGAL.

Significance of study findings. Potential explanations of study findings are given in Table 5. Our study suggests that acute tubular damage might occur without detectable loss of excretory function (and vice versa) and might predict worse clinical outcome and that NGAL and serum creatinine reflect distinct pathophysiological events. A changed view of AKI might change clinical practice and its treatment. Detection of elevated NGAL might enable more rapid conventional interventions or introduction of novel therapies to prevent or effectively treat such otherwise undetected AKI (30). Novel renal biomarkers might facilitate standardization of early diagnosis and treatment. By contrast, a normal NGAL result might inform clinical decision-making and lead to improved use of hospital resources.

For the first time, we identified a substantial group of patients who do not fulfill current creatinine-based consensus criteria for AKI yet are likely to have acute tubular injury. We further demonstrated that these patients have a higher risk of adverse outcomes, including death. These patients might benefit from medical attention. Such medical attention might carry a greater likelihood of success, because changes in NGAL levels are rapid (hours) and changes in serum creatinine slow (days). Patients with AKI might, by analogy with acute myocardial infarction (34), now receive early intervention (13,36,37). In addition, our findings suggest that the definition of AKI should be refined, potentially by the application of criteria that include novel renal biomarkers such as NGAL.

Because NGAL-positive AKI—with or without the development of sCREA(+)—is associated with poor patient outcomes, serum creatinine fails to identify likely AKI in some patients who are at increased risk of death. This observation does not imply that serum creatinine should be discarded as a marker of AKI. In fact, in most study patients, subclinical tubular injury preceded detectable decreases of renal function; and when both occurred

Table 5 Possible Combinations of NGAL and sCREA Status

Diagnostic Increase of NGAL (Subclinical AKI, Days Before Diagnostic Serum Creatinine Increase)	Diagnostic Increase of sCREA (Manifest AKI)	Patient Outcomes*	Potential Explanations of Study Findings
Positive	Positive	Worse	Subclinical AKI, manifest AKI
Positive	Negative	Worse	Subclinical AKI
Negative	Positive	Worse	False negative NGAL/glomerular impairment without tubular injury (pre-renal azotemia)/NGAL negative because measurement was days before serum creatinine increase
Negative	Negative	Better	No subclinical AKI, no manifest AKI

AKI as defined by the renal risk, injury, failure, loss of renal function, end stage renal disease classification criteria (1), or AKI Network classification (2). *e.g., need for RRT initiation; mortality, death during RRT, prolonged length of stay in the ICU or in the hospital.
Abbreviations as in Tables 1, 2, and 3.

together, clinical outcomes were worse. Thus, knowledge of NGAL levels modifies prognostic assessment not only in patients without an increase in serum creatinine but also in patients with creatinine-based consensus criteria for AKI. In these patients, however, AKI could only be diagnosed late, making any treatment less likely to succeed.

There might be alternative explanations for the NGAL(+)/sCREA(−) syndrome: Some of these patients might develop loss of renal function after the consensus period of 7 days, and NGAL identified these patients more than 1 week before such loss of function. A small number of patients might have died soon after NGAL testing, possibly too early to manifest sCREA(+) AKI. Finally, in a minority of patients, NGAL might have simply acted as a marker of inflammation, 1 of the most important risk factors for AKI.

We also identified a very small subgroup of patients with no biomarker evidence of tubular injury but loss of excretory function: NGAL(−)/sCREA(+), 4.6%. Because NGAL, in contrast to serum creatinine, was not consistently serially measured, this observation might represent a false negative finding. However, "pre-renal azotemia" (loss of function without tubular injury) might also explain such findings (38). Therefore, if NGAL was truly negative, these patients might represent a subgroup currently referred to as having pre-renal azotemia (39). In our study, NGAL(−)/sCREA(+) status was more common in elderly and diabetic patients and was still associated with a 2-fold increase in risk of developing the composite outcome of death and renal replacement therapy, compared with patients negative for both biomarkers (NGAL−/sCREA−). This is consistent with recent reports associating even transient azotemia with a greater risk of death (40). Neutrophil gelatinase-associated lipocalin might now help identify some of these patients.

Although it is known that NGAL concentration is somewhat increased in patients with diabetic nephropathy (41–43), there is no information on acute NGAL responsiveness. By contrast, it remains unknown whether patients in the NGAL(−)/sCREA(+) subgroup did not have active tubular damage, because no biopsies were performed. However, there is evidence from animal experiments that pre-renal azotemia does not translate into tubular damage or detection of NGAL in the urine or plasma (44,45).

Finally, NGAL detected approximately 40% of patients with probable AKI who were missed by consensus criteria. This proportion is similar to that identified by troponin in subjects with myocardial injury missed by conventional biomarkers (30).

This study cannot determine the source of NGAL, but the kidney should be the major source of NGAL. If plasma NGAL was produced outside the kidney and then filtered, the proximal renal tubules would completely reabsorb NGAL with no or minimal urine NGAL levels (10). Also, the decrease in glomerular filtration rate seen in AKI would decrease NGAL clearance, resulting in decreased urine NGAL and increased plasma NGAL. Therefore, NGAL measured in the urine should either result largely from injured and not reabsorbing proximal tubules or arise from the injured distal nephron. Indeed, experimental evidence supports this view that NGAL in plasma might predominantly arise from the injured thick ascending tubules and the collecting ducts via back-leak from injured renal tissue—again reflecting renal damage. Neutrophil gelatinase-associated lipocalin should be considered as a vigorous outcome marker in AKI, on the basis of these results and our study findings with urine and plasma NGAL that indicated a very similar patient outcome pattern.

Study strengths and limitations. This study has several strengths. It is multicenter and involved more than 2,000 patients at risk of AKI; it used data collected independently in different countries, assessed patients with diverse conditions, and used different commercially available assays. However, it is retrospective in design, with all of the inherent imperfections of such studies, and because no individual patient data were obtained, meaningful meta-regression analysis was not possible. Our results might be affected by selection on the basis of voluntary data contribution. The direction and the magnitude of this potential bias is unknown. Nonetheless, the findings show strength of association, temporality, consistency, biological plausibility and gradient, coherence with previous studies, and are analogous to other fields of biomarker investigations. These features make it probable that the findings reflect a biological phenomenon (46). Future prospective studies should confirm histopathological agreement of NGAL with tubular injury, shown in animal experiments, and explore the prognostic value of other structural AKI biomarkers beyond NGAL (47), independent of serum creatinine. More important, they should test whether NGAL-based early diagnosis of AKI leads to the more successful and timelier deployment of therapies that, until now, could only be delivered late in the course of AKI (48,49) and improved outcomes.

Conclusions

The study findings show that NGAL complements serum creatinine in AKI diagnosis and prognosis. In a significant proportion of patients at renal risk, acute tubular damage might occur without loss of excretory function. Such NGAL(+) patients are at greater risk of adverse outcomes, including death and renal replacement therapy, both in the presence or absence of an increase in serum creatinine. These patients might be reasonably classified as having AKI, even though they do not fulfill current AKI consensus criteria. Their detection and the size of their cohort justify re-assessment of the concept and definition of AKI.

Author Disclosures

Dr. Haase is a fellow of the Alexander von Humboldt-Foundation, Bonn, Germany; and has received an honorarium for speaking from Abbott Diagnostics and Biosite, Inc.

Dr. Devarajan has served as a consultant to and on the Speakers' Bureaus of Abbott Diagnostics and Biosite, Inc.; and has a pending patent application on NGAL as a biomarker of acute kidney injury. Dr. Haase-Fielitz is a grant recipient of the Jackstädt Foundation, Essen, Germany; both nonprofit foundations. Dr. Mertens is funded by DFG grant SFB 854TP1. Dr. Bellomo has acted as a paid consultant to Abbott Diagnostics and Biosite, Inc. Dr. Cruz has received honoraria for speaking from Alere (formerly Biosite, Inc.). Dr. Koyner is supported via K23DK081616 and has received research support from Abbott Laboratories. Dr. Murray has received research support from Abbott Diagnostics and Alere. Both companies are involved in the development of neutrophil gelatinase-associated lipocalin assays to be applied in clinical practice. Dr. Per Venge is the owner of a world wide granted patent of measuring HNL/NGAL in human disease and in the United States for measuring inflammation licensed to Phadia AB and Abbott; and owns shares in Diagnostics Development. Dr. Ikizler has received grant/research support from Amgen, Fresenius Medical Care, Satellite Health, the National Institutes of Health, NovoNordisk, Medical Nutrition Therapy, and the Maine Medical Center; has served as a consultant to Amgen, Abbott Renal Care, Abbott Nutrition, Orthobiotech, NovoNordisk, Renal Advantage, Inc., Roche Diagnostics, and Fresenius Medical Care; has served on the Speakers' Bureaus of Amgen and Abbott Renal Care; and is a board member of SatelliteHealth, the American Board of Internal Medicine, and the Journal of the American Society of Nephrology. Dr. Mertens is funded by DFG grant SFB 854TP1. All other authors have reported that they have no relationships to disclose.

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Key Words: acute kidney injury (AKI) ■ biomarker ■ creatinine ■ mortality ■ neutrophil gelatinase-associated lipocalin (NGAL) ■ renal replacement therapy (RRT).

APPENDIX

For the data collection form, please see the online version of this article.