

Clinical Investigation

Utility of Urine Neutrophil Gelatinase-Associated Lipocalin for Worsening Renal Function during Hospitalization for Acute Heart Failure: Primary Findings of the Urine N-gal Acute Kidney Injury N-gal Evaluation of Symptomatic Heart Failure Study (AKINESIS)

PATRICK T. MURRAY, MD,¹ NICHOLAS WETTERSTEN, MD,² DIRK J. VAN VELDHUISEN, MD,³ CHRISTIAN MUELLER, MD,⁴ GERASIMOS FILIPPATOS, MD,⁵ RICHARD NOWAK, MD,⁶ CHRISTOPHER HOGAN, MD,⁷ MICHAEL C. KONTOS, MD,⁸ CHAD M. CANNON, MD,⁹ GERHARD A. MÜELLER, MD, PhD,¹⁰ ROBERT BIRKHANN, MD,¹¹ YU HORIUCHI, MD,¹² PAUL CLOPTON, MS,¹² PAM TAUB, MD,² GARY M. VILKE, MD,¹³ OLGA BARNETT, MD,¹⁴ KENNETH MCDONALD, MD,^{1,15} NIALL MAHON, MD,^{1,16} JULIO NÚÑEZ, MD,¹⁷ CARLO BRIGUORI, MD, PhD,¹⁸ CLAUDIO PASSINO, MD,¹⁹ AND ALAN MAISEL, MD²

Dublin, Ireland; La Jolla, California; the Netherlands; Basel, Switzerland; Athens, Greece; Detroit, Michigan; Richmond, Virginia; Kansas City, Kansas; Göttingen, Germany; Brooklyn, New York; San Diego, California; Lviv, Ukraine; Madrid, Spain; and Naples, and Pisa, Italy

ABSTRACT

Background: Worsening renal function (WRF) during acute heart failure (AHF) occurs frequently and has been associated with adverse outcomes, though this association has been questioned. WRF is now evaluated by function and injury. We evaluated whether urine neutrophil gelatinase-associated lipocalin (uNGAL) is superior to creatinine for prediction and prognosis of WRF in patients with AHF.

Methods and Results: We performed a multicenter, international, prospective cohort of patients with AHF requiring IV diuretics. The primary outcome was whether uNGAL predicted development of WRF, defined as a sustained increase in creatinine of 0.5 mg/dL or $\geq 50\%$ above first value or initiation of renal replacement therapy, within the first 5 days. The main secondary outcome was a composite of in-hospital adverse events. We enrolled 927 patients (mean 68.5 years of age, 62% men). The primary outcome occurred in 72 patients (7.8%). The first, peak and the ratio of uNGAL to urine creatinine (area under curves (AUC) ≤ 0.613) did

From the ¹School of Medicine, University College Dublin, Dublin, Ireland; ²Division of Cardiovascular Medicine, University of California, La Jolla, California; ³Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; ⁴Department of Cardiology, University Hospital Basel, University of Basel, Basel, Switzerland; ⁵Department of Cardiology, Athens University Hospital Attikon, University of Athens, Athens, Greece; ⁶Department of Emergency Medicine, Henry Ford Hospital System, Detroit, Michigan; ⁷Division of Emergency Medicine and Acute Care Surgical Services, VCU Medical Center, Virginia Commonwealth University, Richmond, Virginia; ⁸Division of Cardiology, VCU Medical Center, Virginia Commonwealth University, Richmond, Virginia; ⁹Department of Emergency Medicine, University of Kansas Medical Center, Kansas City, Kansas; ¹⁰Department of Nephrology and Rheumatology, University Medical Center Göttingen, University of Göttingen, Göttingen, Germany; ¹¹Department of Emergency Medicine, New York Methodist, Brooklyn, New York; ¹²Division of Cardiovascular Medicine, Veterans Affairs Medical Center, San Diego, La Jolla, California; ¹³Department of Emergency Medicine, University of California, San Diego, California; ¹⁴Division of Cardiology, Danylo Halatsky Lviv National Medical University, Lviv, Ukraine; ¹⁵Department of Cardiology, St. Vincent's University Hospital, Dublin, Ireland; ¹⁶Department of Cardiology, Mater Misericordiae University Hospital, Dublin, Ireland; ¹⁷Department of Cardiology, Hospital Clínico Universitario de Valencia, INCLIVA, University of Valencia, Valencia, Spain & CIBER in Cardiovascular Diseases, Madrid, Spain; ¹⁸Department of Cardiology, Interventional Cardiology, Mediterranean Cardiocentro, Naples, Italy and ¹⁹Department of Cardiology and Cardiovascular Medicine, Fondazione Gabriele Monasterio, Pisa, Italy.

Manuscript received January 21, 2019; revised manuscript received May 6, 2019; revised manuscript accepted May 20, 2019.

Reprint requests: Patrick T. Murray, MD, University College Dublin Clinical Research Centre, UCD Catherine McAuley Education & Research Centre, Nelson Street, Dublin 7, Ireland. Tel: +353-1-7164504. E-mail: patrick.murray@ucd.ie

Funding: The study was funded by Abbott Laboratories (Chicago, Illinois) and Alere (San Diego, California). The sponsors assisted in the design of the study, the data management and the study's oversight. The sponsors did not participate in the analysis presented in this article or in the preparation, review or approval of the manuscript.

1071-9164/\$ - see front matter

© 2019 Elsevier Inc. All rights reserved.

<https://doi.org/10.1016/j.cardfail.2019.05.009>

not have diagnostic utility over the first creatinine (AUC 0.662). There were 235 adverse events in 144 patients. uNGAL did not predict (AUCs ≤ 0.647) adverse clinical events better than creatinine (AUC 0.695).

Conclusions: uNGAL was not superior to creatinine for predicting WRF or adverse in-hospital outcomes and cannot be recommended for WRF in AHF. (*J Cardiac Fail* 2019;25:654–665)

Key Words: Acute heart failure, worsening renal function, biomarker.

Introduction

Heart failure (HF) is a growing global health problem; more than 26 million individuals are afflicted worldwide.¹ The interplay between the heart and kidney in acute heart failure (AHF) has important clinical implications.² Between 20% and 40% of patients with AHF develop worsening renal function (WRF), which has been associated with worse outcomes.^{3–5} Recently, studies have challenged this association, showing no adverse effects of WRF.^{6,7} Additionally, the conceptual framework of acute kidney injury (AKI) has changed, with renal health being assessed by functional and injury biomarkers.⁸ The traditional renal biomarker is serum creatinine, a functional biomarker. However, creatinine has a delayed rise after insult, and elevations in AHF may not reflect tubular injury.^{9,10} Thus, novel biomarkers that detect WRF earlier and are associated with adverse outcomes are needed.¹¹

Neutrophil gelatinase-associated lipocalin (NGAL) is a small molecule of the lipocalin family of proteins. Found on the brush-border of renal tubular cells, serum and urine levels increase with acute ischemic or nephrotoxic injury.¹² Urine biomarkers may be more sensitive to changes in renal function and hemodynamic perturbation than serum creatinine.^{10,13} Urine NGAL (uNGAL) has been shown to predict AKI in patients undergoing cardiac surgery, presenting to the emergency department, receiving iodinated contrast, and during critical illness.^{14–16} We have shown that plasma NGAL (pNGAL) has limited diagnostic utility for WRF in AHF.¹⁷ Previous studies have conflicting results for uNGAL.^{18–24}

The Acute Kidney Injury N-gal Evaluation of Symptomatic heart failure Study (AKINESIS) is an international, multicenter, prospective cohort study enrolling patients presenting with AHF. Previously, we evaluated pNGAL for diagnosing WRF and the need for renal replacement therapy (RRT) in patients with AHF who were being treated with diuretics, and we tested the prognostic ability related to in-hospital outcomes. In this analysis, uNGAL is evaluated for in-hospital outcomes.

Materials and Methods

Study Design

The design and background of AKINESIS have been described.¹⁷ Briefly, AKINESIS was an international, multicenter prospective cohort study enrolling patients hospitalized with AHF and who were intended to be treated with diuretics. The study was jointly sponsored by Abbott Laboratories and Alere. The principal investigators and sponsors designed and oversaw the trial. The study was approved by the institutional

review board at each center. All participants provided written informed consent. Data were collated at a core data-management facility. The principal investigators had full access to the database and independently performed statistical analysis. Clinical trial registration: <https://clinicaltrials.gov/ct2/show/NCT01291836?term=AKINESIS&rank=1> (NCT01291836).

Participants

Patients ≥ 18 years of age presenting to the emergency department or hospital with AHF were screened for inclusion. Patients had to have 1 or more signs or symptoms of HF and to have been treated or with plan to treat with IV diuretics. Patients were excluded if 1) their symptoms were caused by acute coronary syndrome; 2) they were on dialysis or dialysis was planned during the current hospitalization; 3) they had had a major organ transplant; 4) they had participated in a drug-treatment study within the past 30 days or had already been enrolled in this study; or 5) they were pregnant or were part of a vulnerable population.

Data Collection

Demographics, vital signs, HF signs and symptoms, medications, and past medical histories were collected. At the discretion of the treating physicians, laboratory samples for white blood cell count, hemoglobin, hematocrit, sodium, potassium, blood urea nitrogen (BUN), creatinine, alanine aminotransferase, aspartate aminotransferase, troponin, and natriuretic peptides were collected on admission and discharge. They were analyzed in the respective treating facilities' clinical laboratories. Creatinine values were recorded for at least the first 7 days of hospitalization. In-hospital adverse outcomes were recorded.

NGAL Assessment

Specimens for NGAL assessment were collected at 6 time points of hospitalization during the collection period: day of enrollment within 2 hours of first IV diuretic dose; 2 to 6 hours later; hospital days 1, 2 and 3; and day of discharge or anticipated discharge. Compliance with collection was good, except for discharge, with 89%, 84%, 88%, 94%, 93%, and 58% of samples collected at the listed times. Urine specimens were frozen and shipped to the University College Dublin Clinical Research Centre Core Laboratory for analysis by the ARCHITECT platform (Abbott, Abbott Park, Illinois, United States). This assay has a coefficient of variance of $<10\%$, and in the study, the intra-assay coefficient of variance was 2.1%, and the inter-assay coefficient of variance was 1.0%.

Endpoints

The primary outcome was whether uNGAL predicted WRF or initiation of RRT within 5 days of hospitalization compared to the first measured serum creatinine level. WRF was defined as a sustained (2 or more consecutive days) increase in creatinine of ≥ 0.5 mg/dL (44.2 $\mu\text{mol/L}$) or $\geq 50\%$ of first creatinine value. Acute RRT modalities included dialysis, ultrafiltration and hemofiltration. The predictive ability of uNGAL for WRF and RRT were also assessed individually.

The main secondary outcome was uNGAL's ability to predict adverse in-hospital outcomes. These included severe WRF (sustained increase of $\geq 100\%$ of first creatinine value), initiation of RRT, death, nephrology consultation, and admission to the intensive care unit with mechanical ventilation, inotropic/vasopressor support or both.

A prespecified secondary analysis included if elevated uNGAL on admission predicted a greater decrease in estimated glomerular filtration rate (eGFR) at discharge in patients with admission eGFRs of < 60 mL/min/1.73 m². Alternative definitions of WRF analyzed included sustained increase of creatinine $\geq 50\%$ of first creatinine value (WRF2); nonsustained increase of creatinine of 0.5 mg/dL or $\geq 50\%$ of first creatinine value (WRF3); sustained increase of creatinine of 0.3 mg/dL or $\geq 50\%$ of first creatinine (WRF4); and nonsustained increase of creatinine of 0.3 mg/dL or $\geq 50\%$ of first creatinine value (WRF5); these definitions were to occur during the first 5 days as well as a nonsustained increase of creatinine of 0.3 mg/dL or $\geq 50\%$ of the first creatinine value at any time (WRF6). WRF3 and WRF4 definitions were pre specified, whereas WRF2, WRF5 and WRF6 were posthoc analyses.

The primary metric was peak uNGAL, and first uNGAL and first serum creatinine values were also analyzed. Additionally, uNGAL/urine creatinine (uCr) ratio was analyzed (units: micrograms uNGAL to grams creatinine). Ratios evaluated include first uNGAL/uCr, peak uNGAL/uCr and highest ratio (peak ratio) prior to the adverse outcome, as the last may differ from peak uNGAL/uCr. Peak uNGAL was defined as the highest value obtained at least 6 hours prior to the event, if the time was available, or at least a day prior to the event if only the date was available. If the event occurred on the first day, or the only available uNGAL value was within 6 hours of the event, that was used. If uNGAL was available only after the event occurred, it was not used. Peak values for an event were compared to peak values from all samples in patients without the outcome. The first uNGAL was the value obtained at the first collection time. The first creatinine was the first value obtained.

Statistical Analysis

Baseline characteristics are presented as means and standard deviations (SD) for normally distributed variables, medians with interquartile ranges (IQR) for non-normalized variables and percentages for categorical variables. If the status of a comorbidity was unknown, it was assumed to be not present. Characteristics of patients with and without the primary outcomes were compared using the Student t test, the

chi-square test and the Mann-Whitney U test, as appropriate. Quartiles of the first uNGAL and its ratio were compared by analysis of variance, chi-square test and Kruskal-Wallis test, as appropriate, with posthoc testing performed for significant difference with a Bonferroni correction. The distribution of uNGAL and its ratios were skewed and, thus, were log-transformed (base 10) for certain analyses.

Univariable logistic regression analysis was performed to explore the predictive value of uNGAL and the influence of covariates on the primary outcome. Variables with P values < 0.1 were retained for forward stepwise multivariate logistic regression analysis, and those with P values < 0.05 were retained for a multivariate model. The log-transformed values of peak and first uNGAL were added separately to the model, and the C-statistic was calculated with and without uNGAL. Receiver operating characteristic curves were generated to determine the AUC and 95% confidence intervals for peak uNGAL, first uNGAL, first creatinine, and the ratios for the primary and secondary outcomes. Cutpoints for 80% sensitivity and 80% specificity were determined. The net reclassification index (NRI) and integrated discrimination index (IDI) were calculated for the different uNGAL values. Time-to-event curves were made to display when the primary and combined secondary endpoints occurred. The Spearman correlation was used to assess whether there was a correlation between change in eGFR in patients with eGFRs < 60 at admission and the first uNGAL values. A P value < 0.05 was considered significant. All statistical calculations were performed with SPSS v 24 (SPSS, Chicago, Illinois, United States) and R project, v 3.5 (R foundation for statistical computing, Vienna, Austria).

Results

Patient Demographics

From January 2011 through September 2013, 930 patients were enrolled. Three patients were excluded (1 failed inclusion criteria, 1 met exclusion criteria, and 1 withdrew consent), leaving 927 patients. All patients had follow-up through hospitalization. Patients were hospitalized for a median of 5 days. The average age was 68.5 years; 62% were men and 67% were white (Table 1). Hypertension was common (81%); almost half had coronary artery disease (46%) and diabetes mellitus (44%), and 26% had chronic kidney disease (CKD). The median admission creatinine level was 1.19 mg/dL with a median eGFR of 57 mL/min/1.73 m². The median admission B-type natriuretic peptide (BNP) and N-terminal prohormone B-type natriuretic peptide values were 795 pg/dL and 3446 pg/dL, respectively. Of the patients, 70% were taking diuretics prior to admission, 70% were taking beta-blockers, and 63% were taking an angiotensin converting enzyme inhibitor or an angiotensin receptor blocker.

The median values and IQRs for first uNGAL, first uNGAL/uCr ratio, peak uNGAL, peak uNGAL ratio (ratio of peak uNGAL/uCr prior to event), and peak ratio (highest ratio of uNGAL/uCr prior to event) were 12.5 ng/mL (4.3–32.5 ng/mL), 26.8 $\mu\text{g/g}$ (12.6–65.1 $\mu\text{g/g}$), 29.6 ng/mL (13.9–77.9 ng/mL), 45.0 $\mu\text{g/g}$ (21.0–116.0 $\mu\text{g/g}$) for, and

Table 1. Baseline Characteristics of Study Patients and Comparison of Those With and Without Primary Outcome

	Total N = 927	Primary Outcome n = 72	No Primary Outcome n = 855
Age (n = 921)	68.5 (54.7–82.3)	70.8 (57.1–84.4)	68.3 (54.4–82.1)
Male (n = 927)	62%	53%	63%
White (n = 921)	67%	65%	67%
Systolic blood pressure (n = 927)	140 (111–169)	146 (116–175)	139 (110–168)
Diastolic blood pressure (n = 927)	80 (61–99)	82 (60–105)	80 (61–99)
Heart rate (n = 927)	88 (65–111)	87 (68–106)	88 (65–111)
Medical history			
Acute myocardial infarction (n = 927)	28%	29%	27%
Coronary artery disease (n = 927)	46%	54%	45%
Prior PCI (n = 927)	23%	26%	22%
Prior CABG (n = 927)	17%	25%	16%
Arrhythmia (n = 927)	48%	44%	48%
Hypertension (n = 927)	81%	88%	80%
Hyperlipidemia (n = 927)	52%	53%	52%
Diabetes* (n = 927)	44%	58%	42%
Cerebrovascular accident (n = 927)	14%	8%	14%
Peripheral arterial disease (n = 927)	3%	3%	3%
COPD (n = 927)	26%	24%	26%
Chronic kidney disease** (n = 927)	26%	46%	24%
Anemia (n = 927)	22%	29%	22%
Liver failure (n = 927)	3%	6%	3%
Tobacco use (n = 927)	17%	13%	17%
Cancer (n = 927)	14%	14%	14%
Prior medications			
β-blockers (n = 927)	70%	74%	70%
ACE inhibitors (n = 927)	44%	39%	44%
Angiotensin receptor blockers (n = 927)	19%	26%	18%
Diuretics (n = 927)	71%	68%	71%
Antiarrhythmics (n = 927)	15%	18%	15%
Digoxin (n = 927)	11%	7%	11%
Labs			
Sodium (mg/dL) (n = 921)	139 (136–141)	139 (136–142)	139 (136–141)
Creatinine (mg/dL)** (n = 927)	1.19 (0.94–1.6)	1.60 (1.02–2.46)	1.17 (0.93–1.55)
eGFR** (n = 927)	57 (40–78)	38 (25–66)	58 (42–79)
Hemoglobin (g/dL)** (n = 924)	11.8 (10.1–13.2)	10.4 (8.7–12.4)	11.9 (10.3–13.3)
First uNGAL (ng/mL)* (n = 826)	12.5 (4.3–32.5)	22.5 (7.9–55.5)	11.9 (4.2–31.1)
First uNGAL/uCr (ug/g)* (n = 826)	26.8 (12.6–65.1)	48.5 (16.3–133.7)	25.1 (12.2–61.6)
Peak uNGAL (ng/mL) (n = 907)	29.6 (13.9–77.9)	27.3 (12.9–98.3)	29.7 (13.9–76.4)
Peak uNGAL/uCr (ug/g) (n = 907)	45 (21–116)	42.2 (22.6–125.4)	45 (21–115.8)
Peak ratio (ug/g) (n = 907)	45.5 (21.8–116)	54 (28.4–138.3)	45 (21–115.8)
BUN (mg/dL)** (n = 918)	24 (17–36)	34 (21–61)	23 (16–35)
BNP (ng/L) (n = 529)	795 (337–1486)	810 (501–1915)	793 (328–1479)
NT-proBNP (ng/L) (n = 316)	3446 (1552–7376)	4814 (2389–7567)	3221 (1507–7360)
Troponin T (ng/mL)* (n = 343)	0.029 (0.015–0.056)	0.045 (0.024–0.080)	0.027 (0.014–0.051)
Troponin I (ng/mL) (n = 479)	0.04 (0.02–0.07)	0.04 (0.025–0.115)	0.04 (0.02–0.066)

BNP, B-type natriuretic peptide; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; NTproBNP, N-terminal prohormone B-type natriuretic peptide; PCI, percutaneous intervention; uNGAL, urine neutrophil gelatinase associated lipocalin; uNGAL/uCr, urine neutrophil gelatinase associated lipocalin/urine creatinine.

*P value <0.05.

**P value <0.005.

45.5 μg/g (21.8–116 μg/g), respectively. The distribution of characteristics by quartiles of first uNGAL and its ratio are displayed in Supplementary Table 1 and Supplementary Table 2. Significant differences in patient characteristics between quartiles of first uNGAL included gender, history of myocardial infarction, percutaneous coronary intervention, CKD, systolic blood pressure at admission, heart rate at admission, creatinine at admission, eGFR at admission, hemoglobin at admission, BUN at admission and BNP at admission (Supplementary Table 1A and 1B). Significant differences in outcomes between quartiles of first uNGAL included RRT at 5 days, the composite secondary outcome, nephrology consults, inotropic support, and RRT at 30 days. Significant differences in patient characteristics between

quartiles of first uNGAL/uCr ratio included age, gender, history of arrhythmia, CKD, anemia, beta-blocker use, admission creatinine, admission eGFR, admission hemoglobin, and admission BUN (Supplementary Table 2A and 2B). Significant differences in outcomes between quartiles of first uNGAL/uCr ratio included RRT at 5 days, the composite secondary outcome, nephrology consults, and RRT at 30 days.

Primary Outcome

Of the patients, 72 (7.8%) experienced the primary outcome (Figure 1A), with 66 (7.1%) having WRF and 11 (1.2%) requiring RRT. Patients with and without the primary outcome were similar, except that there was a

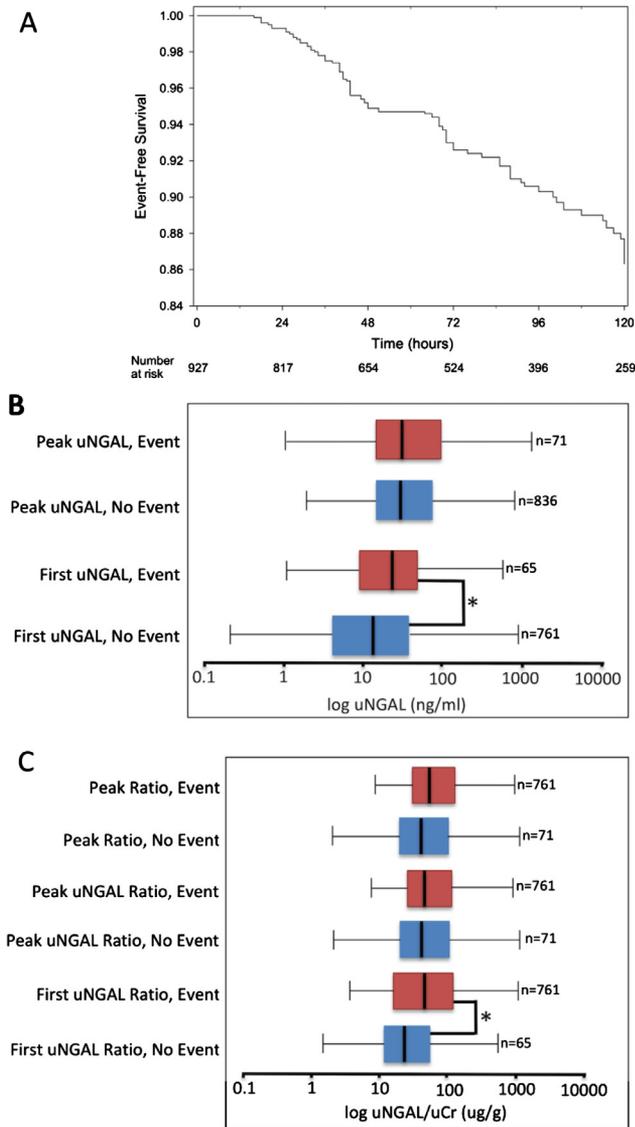


Fig. 1. uNGAL values for primary outcome. A: Time to event curve for the occurrence of the primary outcome. B: Log peak and first uNGAL values in those with and without the primary outcome. C: Ratio uNGAL values in those with and without the primary outcome. * $p < 0.05$; uNGAL = urine neutrophil gelatinase-associated lipocalin.

significantly higher prevalence of diabetes and CKD, higher admission creatinine and BUN levels and lower eGFR and hemoglobin levels in patients with the primary outcome (Table 1). Troponin T was significantly higher in patients with the primary outcome, but troponin I was not.

Peak uNGAL was available for 907 patients, and first uNGAL was available for 826 patients. Peak values occurred an average of 1.5 days (SD 1.1 days) and peak ratios an average of 1.7 days (SD 1.2 days) prior to the primary outcome. Only first uNGAL and its ratio were significantly different between patients with and without events (Figure 1, B and C).

The means for creatinine, log uNGAL and log uNGAL/uCr on each day for patients with and without the primary outcome are shown in Figure 2. Patients with and without the primary outcome showed a trend of increasing uNGAL

values with each subsequent hospital day, while only those with the primary outcome had higher uNGAL/uCr values with each subsequent hospital day after hospital day 1. The mean for log uNGAL in those with the primary outcome was significantly higher on days 0 and 3 compared to those without the primary outcome. The mean for log uNGAL/uCr was significantly higher on all days in patients with the primary outcome. Additionally, we examined the percent change of log uNGAL and log uNGAL/uCr for hospital days 1 through 3 compared to the admission value in those with and without the primary outcome (Supplementary Figure 1). Log uNGAL values increased by a greater percent in those without the primary outcome than in those with the primary outcome initially but showed a similar percentage of increase at hospital day 3. Log uNGAL/uCr values did not change markedly in those without the primary outcome, while they initially declined before increasing to 10% increase on hospital day 3 in those with the primary outcome.

There were 71 peak values with and 836 peak values without the primary outcome. The AUC, 80% sensitivities and 80% specificities for peak uNGAL, peak uNGAL ratio (ratio of peak uNGAL/uCr prior to event), peak ratio (highest ratio of uNGAL/uCr prior to event), and creatinine are displayed in Figure 3A and Table 2A. None of the peak values had predictive AUCs, whereas creatinine was predictive, with a fair AUC of 0.66.

There were 65 first values with and 761 first values without the primary outcome. The AUC, 80% sensitivities and 80% specificities for first uNGAL, first uNGAL ratio and creatinine are displayed in Figure 3B and Table 2A. The first values and creatinine were prognostic for the primary outcome; however, AUCs ranged only from 0.61 to 0.65. Only peak uNGAL ratio improved the NRI, but none of the uNGAL values improved the IDI (Supplementary Table 3).

Variables significant in univariable analysis included gender, prior coronary artery bypass grafting, presence of CKD, presence of diabetes mellitus, use of an angiotensin receptor blocker prior to admission, eGFR, admission hemoglobin, BUN, and systolic blood pressure. After forward stepwise multivariate analysis, only systolic blood pressure, hemoglobin, BUN, and CKD retained significance (Supplementary Table 4A). Since only first uNGAL and its ratio were significant in ROC analysis, these were entered into the model. Neither first uNGAL ($P=0.052$) nor its ratio ($P=0.195$) was significant in the model (Supplementary Table 4B).

For the individual outcomes of WRF and RRT, the AUCs for peak uNGAL, peak uNGAL ratio, peak ratio, first uNGAL, and first uNGAL ratio are displayed in Table 3. For WRF, only first uNGAL and its ratio had a significant AUC, but it was lower than that of creatinine. For RRT, all measurements had significant AUCs, but they were lower than those of creatinine.

Secondary Outcomes

A total of 235 adverse outcomes occurred in 144 patients (Figure 4A). When patients experienced multiple adverse

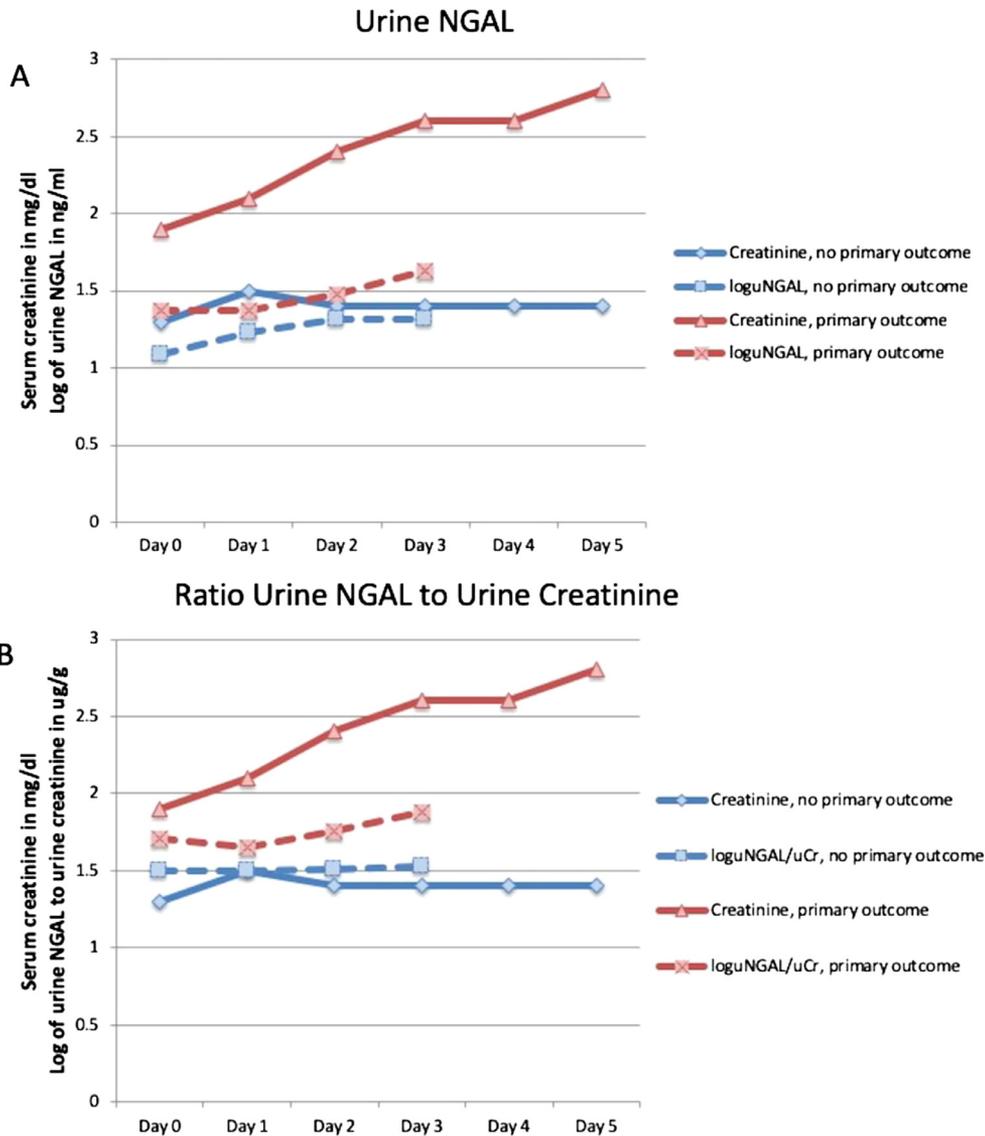


Fig. 2. Mean values of creatinine and uNGAL in patients with and without primary outcome. Daily means of log uNGAL (A) and uNGAL/uCr (B) with mean creatinine in patients with and without the primary outcome. uNGAL = urine neutrophil gelatinase-associated lipocalin; uCr = urine creatinine.

outcomes that were part of the composite secondary outcome, the earliest adverse outcome was used to determine the peak uNGAL value. Medians and IQRs for patients with and without the composite secondary outcomes for peak, first and ratios of uNGAL are shown in Table 4. uNGAL values were significantly higher in patients with and without an event, except for peak uNGAL ratio. Individual adverse outcomes were nephrology consultation (n = 59), inotropic support (n = 51), ventilator support (n = 43), death (n = 29), RRT (n = 18), severe WRF (n = 18), and combined ventilator/inotropic support (n = 17).

There were 143 peak values with and 764 peak values without the secondary composite outcome. The AUC, 80% sensitivities and 80% specificities for peak uNGAL, peak uNGAL, peak ratio, and creatinine are displayed in Figure 4B and Table 2B. Only peak ratio was predictive of

the secondary outcome but showed a poor AUC of 0.58. Creatinine was predictive, with a good AUC of 0.70.

There were 126 first values with and 700 first values without the secondary composite outcome. The AUC, 80% sensitivities and 80% specificities for first uNGAL, first uNGAL ratio and creatinine are displayed in Figure 4C and Table 2B. First uNGAL and its ratio had fair AUCs of 0.65 and 0.61, respectively. Creatinine also had a fair and numerically higher AUC (0.69). None of the uNGAL values improved the NRI or IDI (Supplementary Table 3).

For alternative definitions of WRF, there were 38 (4.1%), 112 (12.1%), 128 (13.8%), 222 (23.9%), and 284 (30.6%) events for WRF2, WRF3, WRF4, WRF5, and WRF6, respectively. The AUC for first uNGAL and its ratio performed the best among all uNGAL measurements, but no AUC was

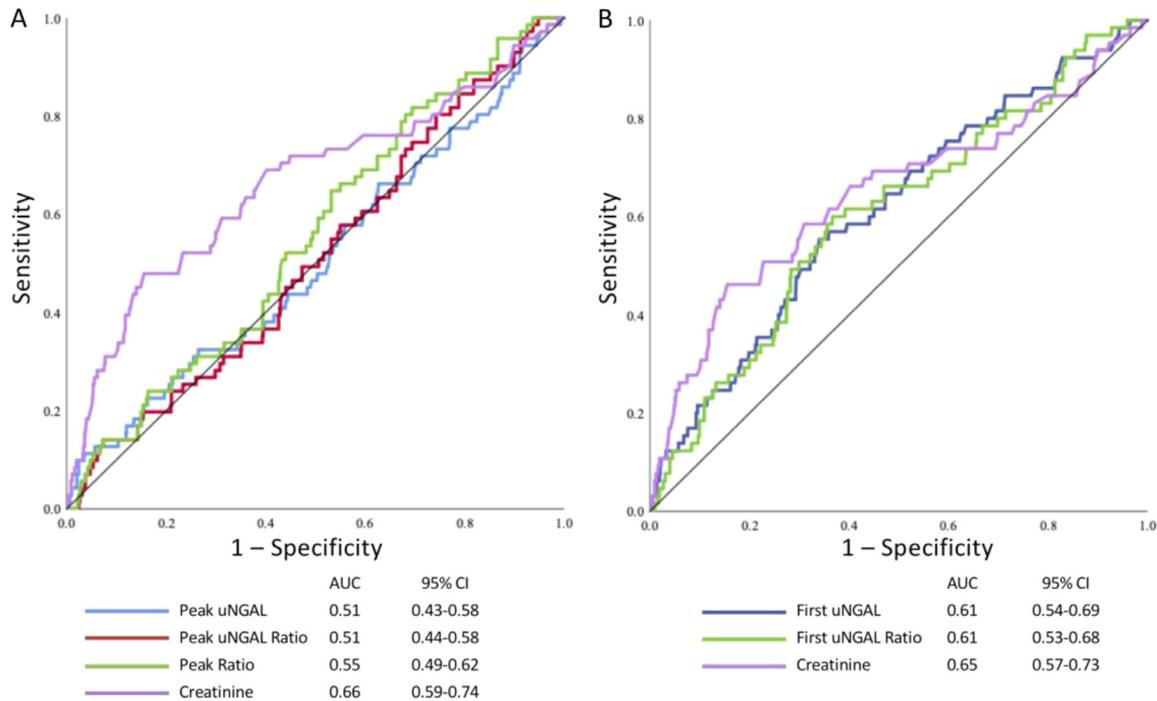


Fig. 3. ROC curves for the primary outcome. ROC curves for primary outcome (A) with peak uNGAL and first uNGAL (B). AUCs with 95% confidence intervals (CI) are below. uNGAL = urine neutrophil gelatinase-associated lipocalin; uCr = urine creatinine

Table 2A. AUCs, 95% CIs, 80% Sensitivity, and 80% Specificity Values for the Primary Outcome

	AUC	95% CI	<i>P</i> value	80% Sensitivity	80% Specificity
Peak uNGAL (ng/mL)	0.51	0.43–0.58	0.58	9.8	101.1
Peak uNGAL ratio (ug/g)	0.51	0.44–0.58	0.52	21.3	150.0
Peak ratio (ug/g)	0.55	0.49–0.62	0.07	26.2	150.0
Creatinine (peaks)	0.66	0.59–0.74	<0.01		
First uNGAL (ng/mL)	0.61	0.54–0.69	<0.01	5.8	39.0
First uNGAL ratio (ug/g)	0.61	0.53–0.68	<0.01	14.5	79.8
Creatinine (first)	0.65	0.57–0.73	<0.01		

Table 2B. AUCs, 95% CIs, 80% Sensitivity, and 80% Specificity Values for the Secondary Composite Outcome

	AUC	95% CI	<i>P</i> value	80% Sensitivity	80% Specificity
Peak uNGAL (ng/mL)	0.56	0.51–0.62	0.02	12.8	88.3
Peak uNGAL ratio (ug/g)	0.54	0.49–0.60	0.11	18.5	132.0
Peak ratio (ug/g)	0.58	0.52–0.63	<0.01	20.5	132.0
Creatinine (peaks)	0.70	0.64–0.75	<0.01		
First uNGAL (ng/mL)	0.65	0.59–0.70	<0.01	6.0	36.2
First uNGAL ratio (ug/g)	0.61	0.55–0.66	<0.01	14.5	76.3
Creatinine (first)	0.69	0.63–0.74	<0.01		

AUC, area under the curve; CI, confidence interval; uNGAL, urine neutrophil gelatinase associated lipocalin.

better than 0.6, and none was higher than that of creatinine (Table 5). Only peak uNGAL ratio improved the NRI and IDI for WRF3 definition as well as the IDI for WRF5 and WRF6 definitions, while no other uNGAL value improved the NRI or IDI for the differing definitions of WRF (Supplementary Table 3). A Spearman correlation showed that neither first uNGAL ($P = 0.495$) nor the first uNGAL/uCr ratio

($P = 0.105$) correlated with change in eGFR in patients with eGFR levels <60 mL/min/1.73 m² at admission.

Discussion

We showed that neither uNGAL nor its ratio to uCr predicted WRF and/or the need for RRT in patients with AHF

Table 3. AUCs and 95% CIs for Worsening Renal Function and Renal Replacement Therapy

	Worsening Renal Function n = 66			Renal Replacement Therapy n = 11		
	AUC	95% CI	P value	AUC	95% CI	P value
Peak uNGAL	0.49	0.41–0.57	0.82	0.75	0.62–0.88	0.02
Peak uNGAL ratio	0.50	0.43–0.57	0.97	0.73	0.58–0.89	0.02
Peak ratio	0.54	0.47–0.61	0.28	0.77	0.65–0.89	0.01
First uNGAL	0.59	0.52–0.67	0.02	0.8	0.67–0.92	<0.01
First uNGAL ratio	0.60	0.52–0.67	0.02	0.76	0.61–0.90	<0.01
Creatinine	0.64	0.56–0.72	<0.01	0.88	0.76–1.0	<0.01

AUC, area under the curve; CI, confidence interval; uNGAL, urine neutrophil gelatinase associated lipocalin.

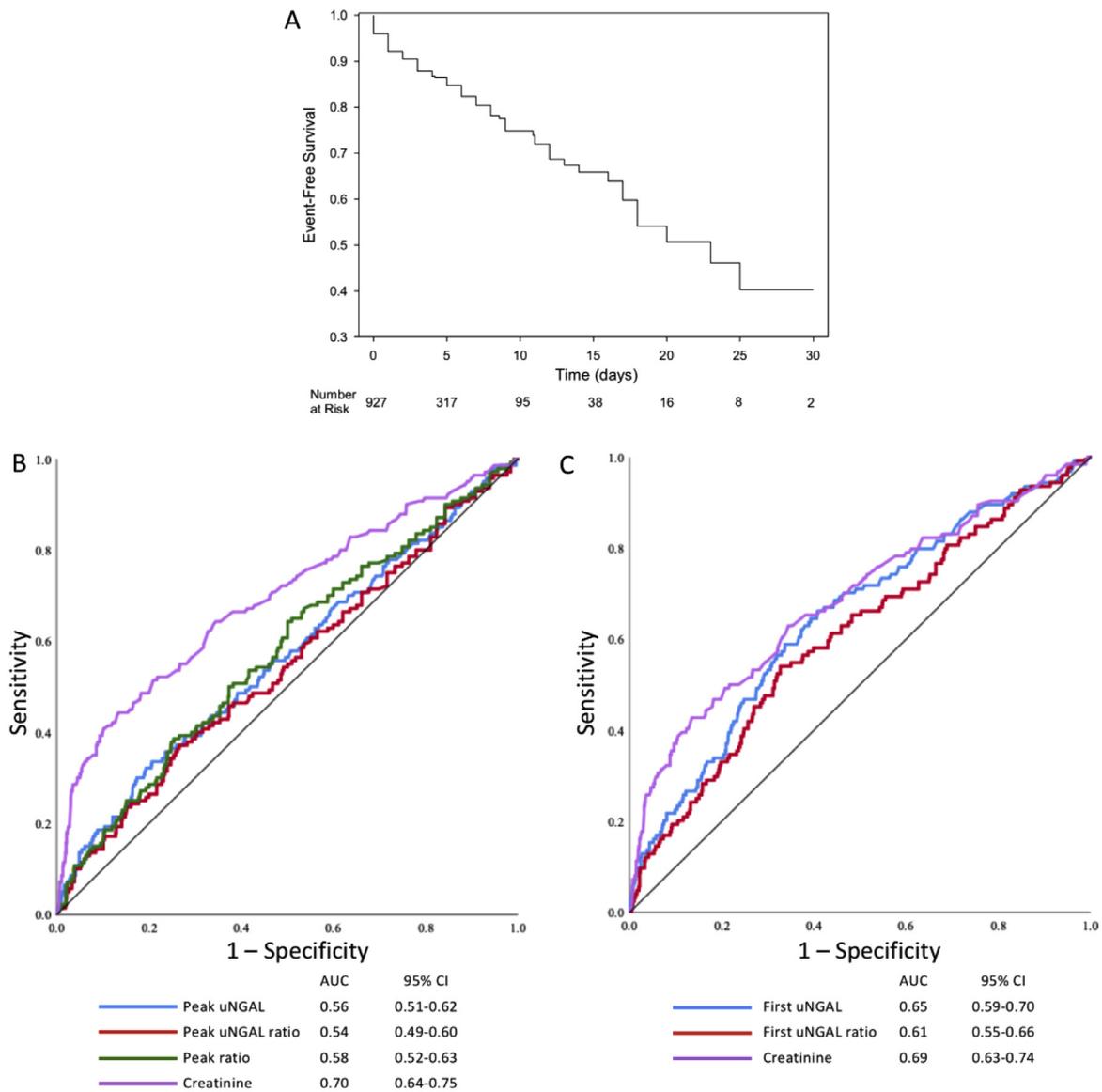


Fig. 4. Outcomes for in-hospital adverse events A: Time to event curve for the occurrence of the secondary outcome. B: ROC curves for composite secondary outcome with peak uNGAL. C: ROC curves for composite secondary outcome with first uNGAL. AUCs with 95% confidence intervals (CI) are below. AUC = area under curve; CI = Confidence Interval; uNGAL = urine neutrophil gelatinase-associated lipocalin.

Table 4. Medians and IQRs for Patients With and Without the Composite Secondary Outcome

	Without Event n = 783		With Event n = 144		P value
	Median	IQR	Median	IQR	
Peak uNGAL (ng/mL)	27.6	13.2–69.7	36.0	15.4–115.5	0.017
Peak uNGAL ratio (ug/g)	42.0	20.3–102.0	47.1	22.0–162.0	0.116
Peak ratio (ug/g)	42.0	20.3–102.0	61.0	27.9–177.3	0.005
First uNGAL (ng/mL)	10.9	4.0–27.6	24.4	8.2–64.1	<0.001
First uNGAL ratio (ug/g)	24.3	11.8–58.3	44.7	15.5–117.0	<0.001

IQR, interquartile range; uNGAL, urine neutrophil gelatinase associated lipocalin.

receiving diuretics better than did serum creatinine level at admission. uNGAL did not predict adverse events during hospitalization better than serum creatinine. Analyses of uNGAL with differing definitions of WRF or deterioration in eGFR did not have favorable results. These findings apply only to in-hospital events and may not reflect the potential utility of uNGAL for post discharge or long-term outcomes.

Increased morbidity and mortality due to WRF during AHF have been described.^{3,5} The pathophysiologic mechanisms between the heart and kidney are not fully understood and are an active area of research.² Since the inception of AKINESIS, the conceptual framework of WRF in AHF has evolved. In 2013, a new definition of AKI incorporated novel tubular injury biomarkers.^{8,5} Creatinine, the traditional biomarker for diagnosing AKI, reflects predominantly glomerular filtration.²⁵ Its shortcomings include missing tubular injury without change in filtration (called subclinical AKI); it rises from hemodynamic changes in filtration without injury and delayed rise during injury.²⁶ Novel tubular injury biomarkers such as NGAL could fill these gaps.²⁶

Studies have found uNGAL to predict WRF and adverse outcomes, whereas other studies have not.^{19–23} Nakada et al found uNGAL/uCr above the median of 32.5 $\mu\text{g/g}$ predicted WRF and increased mortality, cardiovascular death, and HF readmission; however, they measured uNGAL over 24 hours from admission.¹⁹ Collins et al found admission uNGAL predicted development of WRF in univariate but not multivariate analysis.²⁰ Other studies found either a weak association or no association with WRF.^{21,23} Ahmad et al showed no association between urine tubular injury biomarkers (including uNGAL) and WRF and, paradoxically, improved outcomes with elevations in biomarkers.²⁴ They noted a lack of marked tubular injury in cases of WRF. Of these, AKINESIS has the largest cohort and found no association between uNGAL and WRF. Thus, unlike other scenarios, uNGAL is not useful for diagnosing AKI/WRF during hospitalizations for AHF. This highlights the importance of testing novel biomarkers specifically in AHF. Our analysis, though, cannot conclude that uNGAL is not useful for long-term prognosis because prior studies, such as that of Nakada et al, have found its utility for long-term prognosis.

These studies and AKINESIS evaluated uNGAL's ability (an injury biomarker) to predict changes in creatinine or eGFR (functional biomarkers); however, this might be the

wrong measure for assessing tubular injury. Other measures, such as diuretic efficiency, natriuresis or acid-base balance, may better track the pathophysiologic processes of tubular dysfunction. A rise in NGAL without functional change has been shown to portend adverse outcomes.^{15,27}

Yet uNGAL did not predict in-hospital adverse outcomes better than creatinine in AKINESIS, probably because AHF lacks marked renal injury. Values for uNGAL and its ratio were higher in those with events, but not greatly higher. The median first uNGAL was less than the 95th percentile found in healthy subjects, while its ratio to uCr was more than double the 95th percentile.²⁸ Peak values did not differ between those with and without events. Values of uNGAL in AKINESIS are similar to those found in other studies (Supplementary Table 5). Additionally, patients without events showed increases in uNGAL values during treatment, similar to the findings of Ahmad et al, while those with events did not show much change in uNGAL values from admission to peak.²⁴ This supports the conclusion that most patients with AHF do not experience significant tubular injury during treatment for AHF, and most creatinine changes are hemodynamic in nature. However, the findings of this analysis are limited to in-hospital events, and the evaluation of post hospital outcomes may show that kidney injury is more prevalent and has long-term implications for patients' prognoses.

Recent studies have shown that not all episodes of WRF in AHF are created equal, and the context of WRF often determines its significance.^{6,7} WRF with effective decongestion, reflected by decreasing natriuretic peptide, has neutral or positive outcomes.^{6,29} This has been called pseudo-WRF (acute worsening of creatinine with no long-term functional change). Alternatively, WRF with ineffective diuresis and decongestion is identified as true-WRF and is linked to poor outcomes.⁴ Thus, combined analysis of decongestion and serum creatinine may improve evaluation of WRF. There may be a role for tubular injury biomarkers with renal and cardiac functional biomarkers (such as BNP) to discriminate true- vs pseudo-WRF.³⁰

With these considerations and other untested, novel tubular injury biomarkers, further research is needed. Studies have found tubular injury biomarkers are prognostic when assessing the trend at the time of injury or in the context of "clinically significant WRF," which fits the current understanding of WRF in AHF but needs validation by larger

Table 5. AUCs and 95% CIs for uNGAL and Serum Creatinine With Different Definitions of Worsening Renal Function

	WRF2			WRF3			WRF4			WRF5			WRF6		
	AUC	95% CI	P value	AUC	95% CI	p-value									
First uNGAL	0.47	0.37–0.57	0.51	0.56	0.50–0.62	0.05	0.60	0.54–0.66	<0.01	0.59	0.54–0.63	<0.01	0.56	0.52–0.60	<0.01
First uNGAL ratio	0.48	0.38–0.58	0.70	0.56	0.50–0.62	0.06	0.60	0.54–0.65	<0.01	0.58	0.54–0.63	<0.01	0.57	0.53–0.62	<0.01
Peak uNGAL	0.40	0.31–0.50	0.05	0.48	0.43–0.54	0.58	0.52	0.46–0.58	0.49	0.51	0.47–0.56	0.62	0.52	0.48–0.56	0.36
Peak uNGAL ratio	0.42	0.33–0.51	0.09	0.46	0.41–0.52	0.19	0.50	0.44–0.55	0.92	0.49	0.45–0.54	0.76	0.50	0.46–0.54	0.98
Peak uRatio	0.47	0.39–0.56	0.57	0.50	0.45–0.56	0.94	0.55	0.50–0.60	0.08	0.53	0.49–0.57	0.19	0.55	0.51–0.59	0.03
Creatinine	0.44	0.35–0.54	0.06	0.57	0.51–0.63	0.03	0.60	0.54–0.65	<0.01	0.60	0.55–0.64	<0.01	0.59	0.55–0.63	<0.01

AUC, area under the curve; CI, confidence interval; uNGAL, urine neutrophil gelatinase associated lipocalin; WRF, worsening renal function.

studies.^{20,30,31} However, our findings and those of others should temper excitement about novel tubular injury biomarkers in AHF.

Limitations

AKINESIS was designed prior to the paradigm shifts discussed, and the pre specified analysis may not reflect current concepts of WRF in AHF. Different definitions of WRF and outcomes could have been used; however, analysis using alternative WRF definitions did not improve uNGAL’s diagnostic utility. The WRF definition used tried to capture renal function changes that would be associated with injury. Subjects had higher values of uNGAL but, in fitting with current research, they were not markedly elevated or predictive of worse outcomes. Although in-hospital outcomes were closely monitored, events were not centrally adjudicated. This study lacks post hospital and long-term outcomes for which uNGAL may be clinically useful.

Additionally, often more than 10% of samples were missing at collection times, especially at admission, which could potentially exclude important outcomes or bias the results of this study. Samples were collected after diuretic therapy had been administered so as to avoid delaying treatment, but this may have influenced NGAL levels. Ratios to uCr were used to correct for this possibility, but that may not have been sufficient. Samples were collected only early during the hospitalizations, as the intent was to assess development of early WRF, but this may have limited prognostic ability for in-hospital outcomes. The first measured creatinine levels were used as the baseline for assessment of WRF, but that may not reflect patients’ true baseline creatinine levels. Patients may have been experiencing WRF on admission.

Conclusions

In conclusion, uNGAL is not useful for detection of WRF or need for RRT in patients admitted with AHF who are treated with diuretics. uNGAL does not predict adverse in-hospital outcomes better than creatinine.

Acknowledgments

We give special thanks to Dr. Peter Doran for his work in organizing and processing the samples of AKINESIS as the Director of the University College Dublin Clinical Research Centre.

Conflicts of interest: P.T.M has received research funding from Abbott Laboratories and Alere; C.M. has previously received grant funding and other support from Abbott Laboratories and Alere and has received research support and speaker/consulting honoraria from several diagnostic companies; G.F. has served as a committee member for trials sponsored by Bayer, Novartis, Servier, and Medtronic; C.M.C. Institution has received research support from Abbott Laboratories and Alere; R.B. has received grant

funding from Alere; A.M. previously received grant funding from Abbott Laboratories and Alere; all other authors declare no conflicts of interest.

Statement of Authorship

Category 1

(a) Conception and Design

Maisel and Murray

(b) Acquisition of Data

Murray, Van Veldhuisen, C. Mueller, Filippatos, Nowak, Hogan, Kontos, Cannon, G. Mueller, Birkhan, Taub, Vilke, McDonal, Mahon, Nunez, Briguori, Passino, Maisel

(c) Analysis and Interpretation of Data

Clopton, Wettersten, Horiuchi

Category 2

(a) Drafting the Article

Wettersten, Horiuchi, Murray, Maisel

(b) Revising It for Intellectual Content

All authors

Category 3

(a) Final Approval of the Completed Article

All authors

Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.cardfail.2019.05.009](https://doi.org/10.1016/j.cardfail.2019.05.009).

References

- Ponikowski P, Anker SD, AlHabib KF, Cowie MR, Force TL, Hu S, et al. Heart failure: preventing disease and death worldwide. *ESC Heart Fail* 2014;1:4–25.
- Ronco C, Cicoira M, McCullough PA. Cardiorenal syndrome type 1: pathophysiological crosstalk leading to combined heart and kidney dysfunction in the setting of acutely decompensated heart failure. *J Am Coll Cardiol* 2012;60:1031–42.
- Damman K, Valente MA, Voors AA, O'Connor CM, van Veldhuisen DJ, Hillege HL. Renal impairment, worsening renal function, and outcome in patients with heart failure: an updated meta-analysis. *Eur Heart J* 2014;35:455–69.
- van Veldhuisen DJ, Ruilope LM, Maisel AS, Damman K. Biomarkers of renal injury and function: diagnostic, prognostic and therapeutic implications in heart failure. *Eur Heart J* 2016;37:2577–85.
- Gottlieb SS, Abraham W, Butler J, Forman DE, Loh E, Massie BM, et al. The prognostic importance of different definitions of worsening renal function in congestive heart failure. *J Card Fail* 2002;8:136–41.
- Metra M, Davison B, Bettari L, Sun H, Edwards C, Lazzarini V, et al. Is worsening renal function an ominous prognostic sign in patients with acute heart failure? The role of congestion and its interaction with renal function. *Circ Heart Fail* 2012;5:54–62.
- Brisco MA, Zile MR, Hanberg JS, Wilson FP, Parikh CR, Coca SG, et al. Relevance of changes in serum creatinine during a heart failure trial of decongestive strategies: insights from the DOSE trial. *J Card Fail* 2016;22:753–60.
- McCullough PA, Bouchard J, Waikar SS, Siew ED, Endre ZH, Goldstein SL, et al. Implementation of novel biomarkers in the diagnosis, prognosis, and management of acute kidney injury: executive summary from the tenth consensus conference of the Acute Dialysis Quality Initiative (ADQI). *Contrib Nephrol* 2013;182:5–12.
- Damman K, Testani JM. The kidney in heart failure: an update. *Eur Heart J* 2015;36:1437–44.
- Huen SC, Parikh CR. Molecular phenotyping of clinical AKI with novel urinary biomarkers. *Am J Physiol Renal Physiol* 2015;309:F406–13.
- Murray PT, Mehta RL, Shaw A, Ronco C, Endre Z, Kellum JA, et al. Potential use of biomarkers in acute kidney injury: report and summary of recommendations from the 10th Acute Dialysis Quality Initiative consensus conference. *Kidney Int* 2014;85:513–21.
- Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis* 2008;52:595–605.
- Damman K, Ng Kam Chuen MJ, MacFadyen RJ, Lip GY, Gaze D, Collinson PO, et al. Volume status and diuretic therapy in systolic heart failure and the detection of early abnormalities in renal and tubular function. *J Am Coll Cardiol* 2011;57:2233–41.
- Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A, Group NM-aI. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009;54:1012–24.
- Nickolas TL, Schmidt-Ott KM, Canetta P, Forster C, Singer E, Sise M, et al. Diagnostic and prognostic stratification in the emergency department using urinary biomarkers of nephron damage: a multicenter prospective cohort study. *J Am Coll Cardiol* 2012;59:246–55.
- Endre ZH, Pickering JW, Walker RJ, Devarajan P, Edelstein CL, Bonventre JV, et al. Improved performance of urinary biomarkers of acute kidney injury in the critically ill by stratification for injury duration and baseline renal function. *Kidney Int* 2011;79:1119–30.
- Maisel AS, Wettersten N, van Veldhuisen DJ, Mueller C, Filippatos G, Nowak R, et al. Neutrophil gelatinase-associated lipocalin for acute kidney injury during acute heart failure hospitalizations: The AKINESIS Study. *J Am Coll Cardiol* 2016;68:1420–31.
- Soyler C, Tanriover MD, Ascioğlu S, Aksu NM, Arici M. Urine neutrophil gelatinase-associated lipocalin levels predict acute kidney injury in acute decompensated heart failure patients. *Ren Fail* 2015;37:772–6.
- Nakada Y, Kawakami R, Matsui M, Ueda T, Nakano T, Takitsume A, et al. Prognostic value of urinary neutrophil gelatinase-associated lipocalin on the first day of admission for adverse events in patients with acute decompensated heart failure. *J Am Heart Assoc* 2017;6. <https://doi.org/10.1161/JAHA.116.004582>.
- Collins SP, Hart KW, Lindsell CJ, Fermann GJ, Weintraub NL, Miller KF, et al. Elevated urinary neutrophil gelatinase-associated lipocalin after acute heart failure treatment is associated with worsening renal function and adverse events. *Eur J Heart Fail* 2012;14:1020–9.
- Dupont M, Shrestha K, Singh D, Awad A, Kovach C, Scarpino M, et al. Lack of significant renal tubular injury despite acute kidney injury in acute decompensated heart failure. *Eur J Heart Fail* 2012;14:597–604.
- Shrestha K, Shao Z, Singh D, Dupont M, Tang WH. Relation of systemic and urinary neutrophil gelatinase-associated lipocalin levels to different aspects of impaired renal function in patients with acute decompensated heart failure. *Am J Cardiol* 2012;110:1329–35.

23. Verbrugge FH, Dupont M, Shao Z, Shrestha K, Singh D, Finucan M, et al. Novel urinary biomarkers in detecting acute kidney injury, persistent renal impairment, and all-cause mortality following decongestive therapy in acute decompensated heart failure. *J Card Fail* 2013;19:621–8.
24. Ahmad T, Jackson K, Rao VS, Tang WHW, Brisco-Bacik MA, Chen HH, et al. Worsening renal function in acute heart failure patients undergoing aggressive diuresis is not associated with tubular injury. *Circulation* 2018;137(19):2016–28.
25. McCullough PA, Shaw AD, Haase M, Bouchard J, Waikar SS, Siew ED, et al. Diagnosis of acute kidney injury using functional and injury biomarkers: workgroup statements from the tenth Acute Dialysis Quality Initiative Consensus Conference. *Contrib Nephrol* 2013;182:13–29.
26. Cruz DN, Bagshaw SM, Maisel A, Lewington A, Thadhani R, Chakravarthi R, et al. Use of biomarkers to assess prognosis and guide management of patients with acute kidney injury. *Contrib Nephrol* 2013;182:45–64.
27. Basu RK, Wong HR, Krawczeski CD, Wheeler DS, Manning PB, Chawla LS, et al. Combining functional and tubular damage biomarkers improves diagnostic precision for acute kidney injury after cardiac surgery. *J Am Coll Cardiol* 2014;64:2753–62.
28. Cullen MR, Murray PT, Fitzgibbon MC. Establishment of a reference interval for urinary neutrophil gelatinase-associated lipocalin. *Ann Clin Biochem* 2012;49:190–3.
29. Salah K, Kok WE, Eurlings LW, Bettencourt P, Pimenta JM, Metra M, et al. Competing risk of cardiac status and renal function during hospitalization for acute decompensated heart failure. *JACC Heart Fail* 2015;3:751–61.
30. Sokolski M, Zymlinski R, Biegus J, Siwolowski P, Nawrocka-Millward S, Todd J, et al. Urinary levels of novel kidney biomarkers and risk of true worsening renal function and mortality in patients with acute heart failure. *Eur J Heart Fail* 2017;19:760–7.
31. Chen C, Yang X, Lei Y, Zha Y, Liu H, Ma C, et al. Urinary biomarkers at the time of AKI Diagnosis as predictors of progression of AKI among patients with acute cardiorenal syndrome. *Clin J Am Soc Nephrol* 2016;11:1536–44.