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RESEARCH ARTICLE

Short-term prognostic implications of serum and urine neutrophil gelatinase-associated lipocalin in acute heart failure: findings from the AKINESIS study

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Aims

Kidney impairment has been associated with worse outcomes in acute heart failure (AHF), although recent studies challenge this association. Neutrophil gelatinase-associated lipocalin (NGAL) is a novel biomarker of kidney tubular injury. Its prognostic role in AHF has not been evaluated in large cohorts. The present study aimed to determine if serum NGAL (sNGAL) or urine NGAL (uNGAL) is superior to creatinine for predicting short-term outcomes in AHF.

Methods and results

The study was conducted in an international, multicentre, prospective cohort consisting of 927 patients with AHF. Admission and peak values of sNGAL, uNGAL and uNGAL/urine creatinine (uCr) ratio were compared to admission and peak serum creatinine (sCr). The composite endpoints were death, initiation of renal replacement therapy, heart failure (HF) readmission and any emergent HF-related outpatient visit within 30 and 60 days, respectively. The mean age of the cohort was 69 years and 62% were male. The median length of stay was 6 days. The composite endpoint occurred in 106 patients and 154 patients within 30 and 60 days, respectively. Serum NGAL was more predictive than uNGAL and the uNGAL/uCr ratio but was not superior to sCr (area under the curve [AUC]; admission sNGAL 0.61 [95% confidence interval (CI) 0.55–0.67] and 0.59 [95% CI 0.54–0.65], peak sNGAL 0.60 [95% CI 0.54–0.66] and 0.57 [95% CI 0.52–0.63], admission sCr 0.60 [95% CI 0.54–0.64] and 0.59 [95% CI 0.53–0.64] [area under the curve: admission sNGAL 0.61, 95% confidence interval (CI) 0.55–0.67, and 0.59, 95% CI 0.54–0.65; peak sNGAL: 0.60, 95% CI 0.54–0.66, and 0.57, 95% CI 0.52–0.63; admission sCr: 0.60, 95% CI 0.54–0.64, and 0.59, 95% CI 0.53–0.64, at 30 and 60 days, respectively], peak sCr 0.61 [95% CI 0.55–0.67] and 0.59 [95% CI 0.54–0.64] at 30 and 60 days, respectively). NGAL was not predictive of the composite endpoint in multivariate analysis.

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Conclusions Serum NGAL outperformed uNGAL but neither was superior to admission or peak sCr for predicting adverse events.

Keywords Acute heart failure • Kidney function • Biomarkers • Prognosis

Introduction

Renal impairment is commonly observed in heart failure (HF) as the pathophysiologies of the two organs are closely intertwined through numerous pathways.[1] A worsening in cardiac function is often associated with renal impairment that may lead to injury and dysfunction. Nearly half of patients with acute HF (AHF) have chronic kidney disease (CKD) and 27–40% of patients experience worsening renal function [WRF; a rise in serum creatinine (sCr) not definitively associated with kidney injury] during hospitalization, which has historically been associated with worse short- and long-term outcomes.[2] Recent studies have questioned the significance of WRF in AHF, suggesting most rises in sCr are haemodynamic or result from other confounding factors that influence prognosis.[3,4] Thus, the discriminating of situations in which WRF is clinically significant in AHF is a clinical dilemma.

Kidney function is often evaluated with sCr, which is frequently used to establish the estimated glomerular filtration rate (eGFR). However, sCr reflects kidney glomerular function and not necessarily tubular damage. In addition, sCr levels are influenced by several factors, including changes in volume status and medications, which can play important roles in AHF.[5,6] Furthermore, sCr has a delayed rise after an insult, suggesting admission values may not reflect kidney dysfunction in the early phase of AHF and can lead to a delayed diagnosis of deteriorating kidney function.[7] A paradigm shift in the understanding of acute kidney injury (AKI) has led to the evaluation of novel biomarkers that reflect tubular damage and not just glomerular function.[8] Whether novel injury biomarkers can improve prognostication of WRF is to be determined.

Neutrophil gelatinase-associated lipocalin (NGAL) is a novel kidney biomarker associated with kidney injury and is measurable in both serum and urine.[9] NGAL elevates promptly after tubular injury in several conditions, including cardiac surgery, contrast-induced nephropathy and critical illness, and is associated with WRF and worse outcomes.[10,11]

The Acute Kidney Injury N-GAL Evaluation of Symptomatic heart failure Study (AKINESIS) was an international, multicentre, prospective cohort study enrolling patients presenting with AHF.[12] Previous findings have shown that neither serum (sNGAL) nor urine (uNGAL) NGAL were superior to sCr for predicting WRF and the need for renal replacement therapy (RRT).[12,13] However, WRF defined by changes in sCr, an imperfect marker of kidney function, cannot uniformly be used as a surrogate for worse outcomes. The current analysis evaluated whether sNGAL or uNGAL is superior to sCr for predicting adverse short-term outcomes.

Methods

Study design

The design and background of AKINESIS have been reported previously.[12] Briefly, AKINESIS was an international, multicentre, prospective cohort study conducted at 16 sites in the USA and Europe, which enrolled patients with AHF and planned treatment with i.v. diuretics. The study was jointly sponsored by Abbott Laboratories (Chicago, IL, USA) and Alere, Inc. (San Diego, CA, USA). The principal investigators and sponsors designed and oversaw the trial. The study was approved by the institutional review board (IRB) at each institution. Written informed consent was obtained from all participants. A core data management facility collected data. The principal investigators had full access to the database. The investigators performed the statistical analysis.

Study population

Patients were required to be aged at least 18 years, to have presented to the emergency department or hospital with one or more symptoms or signs of AHF, and to have received or to be subject to planned treatment with i.v. diuretics. Exclusion criteria were: (i) acute coronary syndrome; (ii) current dialysis or planned initiation of dialysis; (iii) prior major organ transplant; (iv) participation in a drug treatment study within the past 30 days or previous enrolment in this study; (v) pregnancy, and (vi) status within a population determined to be vulnerable by an IRB (i.e. children, prisoners, cognitively impaired people).

Data collection

Data on patient demographics, medical history and medications prior to and during hospitalization were collected. Laboratory results for white blood cell count, haemoglobin, haematocrit, sodium, potassium, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, troponin and natriuretic peptides were collected on admission as ordered at the discretion of the treating physician.

Assessment of NGAL

Specimens for NGAL assessment were collected at six time-points if the participant remained hospitalized for the duration of collection times: (i) day of enrolment within 2 h of first diuretic dose; (ii) 2–6 h later; (iii) hospital day 1; (iv) hospital day 2; (v) hospital day 3, and (vi) the day of discharge or anticipated discharge. Rates of compliance with sample collection at the listed time-points were 98.3%, 92.0%, 94.7%, 96.7%, 94.5% and 65.4% for sNGAL and 89.2%, 84.3%, 87.9%, 93.9%, 92.8% and 58.4% for uNGAL, respectively. Specimens were frozen and shipped to the core laboratory for analysis with the Alere Triage® platform for sNGAL and ARCHITECT® platform (Abbott Laboratories) for uNGAL. The Triage® assay had lower and upper limits of detection

of 0 ng/mL and 1500 ng/mL, respectively, and the coefficient of variance was 3.1%. The ARCHITECT® assay had lower and upper limits of detection of 0.7 ng/mL and 1500 ng/mL, respectively, and the coefficient of variance was 2.1%.

The predictive ability of admission and peak values of sNGAL, uNGAL and uNGAL/urine creatinine (uCr) ratio was measured and compared to admission sCr and peak sCr. The peak NGAL value was defined as the highest value obtained at least a day prior to any adverse events as previously defined.[12,13]

Endpoints

The primary endpoints were the prognostic utility of NGAL for a composite of death, initiation of RRT including dialysis, ultrafiltration and haemofiltration, HF readmission, and any emergent HF-related outpatient visit to either an emergency department or a clinic with requirement for i.v. diuretics, at 30 and 60 days, respectively, after enrolment. Multivariate analysis was prespecified for 60-day outcomes to capture adequate clinical events; an exploratory post hoc analysis for 30-day events was also conducted. Additional post hoc analyses included the endpoints of death, HF hospitalization and a composite of these at 30 and 60 days, respectively; these are the outcomes most commonly reported in the literature. Outcomes were collected by telephone follow-up and/or medical record review.

Statistical analysis

Baseline characteristics are presented as means and standard deviations when normally distributed, medians with interquartile ranges (IQRs) when non-parametric, and percentages for categorical variables. If the status of a comorbidity was unknown, it was assumed not to be present. Baseline characteristics were compared between patients who did and did not reach the 60-day composite endpoint using Student's *t*-test, the chi-squared test and the Mann–Whitney U-test as appropriate. Baseline characteristics were also compared by tertiles of admission sNGAL, uNGAL and uNGAL/uCr values using analysis of variance (ANOVA), the chi-squared test and the Kruskal–Wallis test as appropriate. Independent predictors of log-transformed admission sNGAL, uNGAL and uNGAL/uCr values were examined by univariate and multivariate linear regression analysis. Receiver operating characteristic (ROC) curves were generated for 30- and 60-day endpoints to determine the area under the ROC curve (AUC) with 95% confidence intervals (CIs) for admission and peak sNGAL, uNGAL and uNGAL/uCr ratio, and admission and peak sCr. Cut-points for 80% sensitivity and 80% specificity were determined. A post hoc analysis was performed to evaluate AUCs in patients with admission eGFR of <60 mL/min/1.73 m^2 or ≥ 60 mL/min/1.73 m^2. Kaplan–Meier analysis was performed for the composite endpoint at 60 days stratifying by tertiles of NGAL. To explore the prognostic value of NGAL to the endpoints, Cox proportional hazard models were used with log-transformed NGAL (natural log) as a continuous predictor. Four multivariate models clustered by different baseline characteristics to avoid overfitting were constructed. In multivariate analysis, covariates were: (i) demographics: age, gender, race (non-White); (ii) medical history: CKD, hypertension, diabetes mellitus (DM), tobacco use, hypercholesterolaemia, sepsis, pneumonia, myocardial infarction, arrhythmias, anaemia, hyperthyroidism, coronary artery disease, percutaneous coronary intervention, coronary artery bypass grafting, chronic obstructive pulmonary disease, gastroesophageal reflux disease, peripheral arterial disease, dialysis, liver failure, cancer and stroke; (iii) concomitant medications given while hospitalized, including beta-blockers, vasodilators, non-steroidal anti-inflammatory agents, digoxin and potassium, and (iv) vital signs and laboratory results, including systolic blood pressure (SBP), heart rate, sodium, haemoglobin and sCr. These possible confounders were included in the model when the univariate analysis found them to be significant ($P < 0.05$). All statistical calculations were performed in R x64 3.4.1 for Windows (R Project for Statistical Computing, R Foundation, Vienna, Austria).

Results

Patient demographics

A total of 930 patients were enrolled from January 2011 to September 2013. Three patients were excluded (one did not meet all inclusion criteria, one met exclusion criteria, and one withdrew consent), leaving 927 patients for analysis. The mean age was 69 years and 62% were male. Of the entire cohort, 46% had a history of coronary artery disease, 81% had hypertension, 44% had DM and 26% had CKD. The median admission sCr was 105.2 μmol/L [(1.19 mg/dL), IQR 83.1–141.4 μmol/L (0.94–1.60 mg/dL)] and median eGFR was 57 mL/min/1.73m^2 (IQR 40–78 mL/min/1.73m^2). Median admission and peak values of NGAL were, respectively, 135.5 ng/mL (IQR 81.9–241.2 ng/mL) and 189.1 ng/mL (IQR 115.3–324.4 ng/mL) for sNGAL, 12.5 ng/mL (IQR 4.3–32.2 ng/mL) and 28.6 ng/mL (IQR 13.0–73.4 ng/mL) for uNGAL, and 26.8 μg/g (IQR 12.7–64.8 μg/g) and 43.6 μg/g (IQR 20.7–112.6 μg/g) for the uNGAL/uCr ratio.

Baseline characteristics divided by tertiles of admission sNGAL, uNGAL and uNGAL/uCr are displayed in online supplementary *Table S1*. The prevalence of a history of CKD increased across tertiles for all NGAL assessments; sCr also increased and eGFR declined across tertiles. The frequency of women increased across tertiles for uNGAL and uNGAL/uCr, but not for sNGAL. Age increased across sNGAL tertiles, but not uNGAL or uNGAL/uCr. History of anaemia also increased across sNGAL tertiles, but not across uNGAL or uNGAL/uCr tertiles, although admission haemoglobin values decreased across tertiles for all NGAL assessments.

Independent predictors of admission sNGAL, uNGAL and uNGAL/uCr are displayed in online supplementary *Table S2*. All NGAL assessments were associated with lower haemoglobin levels and higher admission sCr. The presence of DM was associated with sNGAL and uNGAL, but not with uNGAL/uCr, whereas gender and angiotensin receptor blocker use were associated with uNGAL and uNGAL/uCr, but not with sNGAL. White race and chronic obstructive pulmonary disease were associated only with sNGAL, and SBP was associated only with uNGAL/uCr.

Outcomes

At 30 days, the primary composite endpoint was found to have occurred in 106 patients (11%), and the composite of death or HF readmission was observed in 85 patients (9%); 43 patients had died, 4 patients had undergone RRT, 44 patients had been readmitted for HF and 19 patients had required emergent outpatient i.v. diuretics. At 60 days, the primary composite endpoint had occurred 154

Table 1 Baseline characteristics of patients with and without the composite endpoint at 60 days

	With events (n = 154)	Without events (n = 773)	P-value
Age, years, mean ± SD	70 ± 14.9	68 ± 13.6	0.147
Male sex, *n* (%)	93 (60)	484 (63)	0.668
White ethnicity, *n* (%)	93 (60)	489 (63)	0.561
Myocardial infarction, *n* (%)	48 (31)	207 (27)	0.310
Coronary artery disease, *n* (%)	73 (47)	354 (46)	0.782
Prior PCI, *n* (%)	40 (26)	169 (22)	0.313
Prior CABG, *n* (%)	31 (20)	127 (16)	0.318
Arrhythmia, *n* (%)	77 (50)	365 (47)	0.587
Hypertension, *n* (%)	123 (80)	625 (81)	0.865
Hyperlipidaemia, *n* (%)	90 (58)	396 (51)	0.122
Diabetes mellitus, *n* (%)	71 (46)	333 (43)	0.547
Cerebrovascular accident, *n* (%)	21 (14)	108 (14)	1.000
Peripheral arterial disease, *n* (%)	4 (2.6)	26 (3.4)	0.809
COPD, *n* (%)	50 (33)	192 (25)	0.062
Chronic kidney disease, *n* (%)	54 (35)	186 (24)	0.006
Anaemia, *n* (%)	47 (31)	161 (21)	0.012
Liver failure, *n* (%)	5 (3.2)	21 (2.7)	0.923
Tobacco use, *n* (%)	28 (18)	126 (16)	0.650
Cancer, *n* (%)	30 (20)	101 (13)	0.050
Systolic blood pressure, mmHg, mean ± SD	132 ± 29	141 ± 29	<0.001
Heart rate, b.p.m., mean ± SD	88 ± 22	82 ± 23	0.999
Sodium, mg/dL, mean ± SD	137 ± 6	138 ± 7	0.060
Haemoglobin, g/dL, mean ± SD	11.2 ± 2.8	11.7 ± 2.5	0.029
Admission creatinine, μmol/L, median (IQR)	121.1 (89.3–168.0)	103.4 (82.2–135.3)	0.001
eGFR, mL/min/1.73 m^2, median (IQR)	50 (32–70)	58 (42–79)	0.001
Admission sNGAL, ng/mL, median (IQR)	175.0 (103.4–331.9)	129.4 (79.9–222.0)	<0.001
Peak sNGAL, ng/mL, median (IQR)	234.8 (135.6–400.2)	184.2 (112.5–304.8)	0.004
Admission uNGAL, ng/mL, median (IQR)	16.8 (5.4–44.7)	12.0 (4.2–30.4)	0.023
Peak uNGAL, ng/mL, median (IQR)	34.1 (14.7–95.9)	28.0 (12.8–69.5)	0.194
Admission uNGAL/uCr ratio, μg/g, median (IQR)	33.1 (14.9–75.8)	25.6 (12.0– 63.1)	0.090
Peak uNGAL/uCr ratio, μg/g, median (IQR)	45.2 (21.5–120.4)	42.2 (20.6–104.9)	0.407
Medication prior to admission			
Beta-blockers, *n* (%)	102 (66)	549 (71)	0.276
ACE inhibitors, *n* (%)	59 (38)	346 (45)	0.166
Angiotensin receptor blockers, *n* (%)	31 (20)	144 (19)	0.747
Diuretics, *n* (%)	120 (78)	535 (69)	0.038
Antiarrhythmic agent, *n* (%)	18 (12)	124 (16)	0.212
Digoxin, *n* (%)	15 (9.7)	87 (11)	0.684
Medications during hospitalization			
Beta-blockers, *n* (%)	110 (71)	604 (78)	0.083
Vasodilator, *n* (%)	50 (33)	239 (31)	0.800
Digoxin, *n* (%)	25 (16)	112 (15)	0.674
Potassium, *n* (%)	62 (40)	291 (38)	0.612

ACE, angiotensin-converting enzyme; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; eGFR estimated glomerular filtration rate; IQR, interquartile range; NGAL, neutrophil gelatinase-associated lipocalin; PCI, percutaneous coronary intervention; SD, standard deviation; sNGAL, serum NGAL; uNGAL, urine NGAL; uCr, urine creatinine.

(17%) patients, and the composite of death or HF readmission was seen in 127 (14%); 65 patients had died, 5 patients had undergone RRT, 71 patients had been readmitted for HF and 26 patients had required emergent outpatient i.v. diuretics.

Table 1 shows the baseline characteristics of patients with and without the primary composite endpoint at 60 days. Patients with events more frequently had a history of CKD and anaemia, were more often treated with diuretics before admission, and had lower SBP, haemoglobin and eGFR and higher sCr at admission compared to those without events. Admission and peak sNGAL and admission uNGAL were significantly higher in patients with events.

Prognostic utility of NGAL

Figure 1 and *Table 2* show the 30-day ROC analyses, 80% sensitivity cut-points and 80% specificity cut-points for sNGAL, uNGAL, uNGAL/uCr ratio and admission and peak sCr for the primary

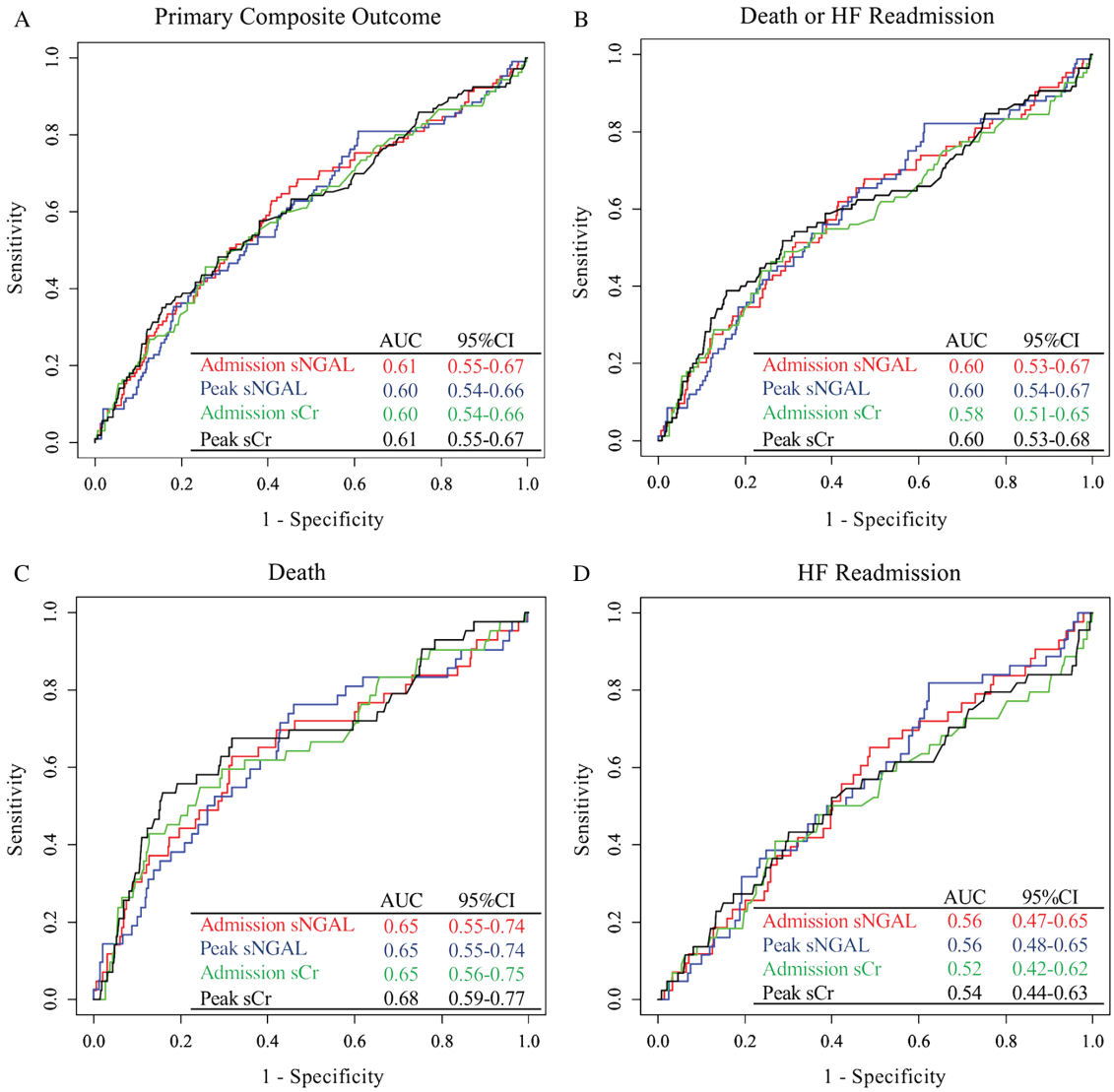

Figure 1 Receiver operating characteristic (ROC) curves for the primary outcome and components at 30 days: (*A*) the 30-day composite endpoint, (*B*) composite of death or heart failure (HF) readmission, (*C*) mortality, and (*D*) HF readmission. Each plot contains the ROC for admission serum neutrophil gelatinase-associated lipocalin (sNGAL), peak sNGAL and admission serum creatinine (sCr), and peak sCr, as well as areas under the ROC curve (AUCs) with 95% confidence interval (CI).

composite endpoint, the composite of death and HF readmission, death and HF readmission. Overall, the prognostic value of each was fair to poor; none of the biomarkers had an AUC close to 0.7 except peak sCr (AUC 0.68) for death at 30 days. Admission sNGAL, peak sNGAL, admission sCr and peak sCr had the highest AUCs for all outcomes and were similar to one another. Admission sNGAL and peak sNGAL showed the strongest prognostic ability for 30-day mortality (AUC for both: 0.65); however, this was not better than admission sCr (AUC 0.65) or peak sCr (AUC 0.68).

Findings were similar for events at 60 days although with overall numerically lower AUCs than outcomes at 30 days (online supplementary *Table S3*). Again, admission and peak sNGAL values had the highest AUCs and were similar to admission sCr and peak sCr AUC values. The AUC for admission uNGAL was closer to sNGAL, admission sCr and peak sCr in prognostic utility for the primary composite outcome and the composite of death and HF readmission and had the highest AUC of all biomarkers for HF readmission, although the overall AUC was poor at 0.56 and the 95% CI crossed 0.5.

Area under the curve values in patients with admission eGFR values of, respectively, <60 mL/min/1.73m^2 and ≥ 60 mL/min/1.73m^2 for 30-day and 60-day outcomes are displayed in *Table 3* and online supplementary *Table S4*, respectively. In patients with an eGFR of ≥ 60 mL/min/1.73m^2, AUCs for NGAL assessments predominately declined for all outcomes with the majority of values close to 0.50, which suggests that NGAL assessments have no predictive value

Table 2 Areas under the curve and 80% sensitivity and specificity cut-points for 30-day outcomes

	AUC	95% CI	80% sensitivity cut-point	80% specificity cut-point
Primary composite endpoint				
Admission sNGAL, ng/mL	0.61	0.55–0.67	84.7	269.0
Peak sNGAL, ng/mL	0.60	0.54–0.66	148.0	354.8
Admission uNGAL, ng/mL	0.55	0.49–0.62	3.6	39.8
Peak uNGAL, ng/mL	0.54	0.48–0.59	12.1	95.2
Admission uNGAL/uCr ratio, µg/g	0.55	0.48–0.61	13.5	82.7
Peak uNGAL/uCr ratio, µg/g	0.54	0.48–0.60	19.1	134.9
Admission creatinine, µmol/L	0.60	0.54–0.66	87.5	151.2
Peak creatinine, µmol/L	0.61	0.55–0.67	97.2	177.7
Composite death or HF readmission				
Admission sNGAL, ng/mL	0.60	0.53–0.67	83.9	276.2
Peak sNGAL, ng/mL	0.60	0.54–0.67	148.0	359.7
Admission uNGAL, ng/mL	0.54	0.47–0.61	3.7	41.1
Peak uNGAL, ng/mL	0.54	0.47–0.60	12.1	96.1
Admission uNGAL/uCr ratio, µg/g	0.54	0.47–0.61	13.5	83.8
Peak uNGAL/uCr ratio, µg/g	0.55	0.48–0.62	19.5	138.3
Admission creatinine, µmol/L	0.58	0.51–0.65	80.4	152.0
Peak creatinine, µmol/L	0.60	0.53–0.68	97.2	178.6
Death				
Admission sNGAL, ng/mL	0.65	0.55–0.74	87.2	277.8
Peak sNGAL, ng/mL	0.65	0.55–0.74	158.3	363.4
Admission uNGAL, ng/mL	0.60	0.51–0.69	5.8	41.3
Peak uNGAL, ng/mL	0.57	0.48–0.66	13.5	96.1
Admission uNGAL/uCr ratio, µg/g	0.58	0.49–0.67	14.8	82.8
Peak uNGAL/uCr ratio, µg/g	0.59	0.51–0.68	27.3	137.4
Admission creatinine, µmol/L	0.65	0.56–0.75	90.2	153.8
Peak creatinine, µmol/L	0.68	0.59–0.77	97.2	179.5
HF readmission				
Admission sNGAL, ng/mL	0.56	0.47–0.65	78.8	282.7
Peak sNGAL, ng/mL	0.56	0.48–0.65	148.0	368.5
Admission uNGAL, ng/mL	0.50	0.40–0.60	3.6	41.2
Peak uNGAL, ng/mL	0.49	0.40–0.59	11.0	98.1
Admission uNGAL/uCr ratio, µg/g	0.51	0.42–0.60	11.6	85.9
Peak uNGAL/uCr ratio, µg/g	0.52	0.42–0.61	17.5	142.5
Admission creatinine, µmol/L	0.52	0.42–0.62	69.8	155.6
Peak creatinine, µmol/L	0.54	0.44–0.63	88.4	186.5

AUC, area under the curve; CI, confidence interval; HF, heart failure; NGAL, neutrophil gelatinase-associated lipocalin; sNGAL, serum NGAL; uNGAL, urine NGAL; uCr, urine creatinine.

for 30-day events. In patients with an eGFR of <60 mL/min/1.73m^2, AUCs for NGAL assessments remained the same or were slightly higher for all outcomes at 30-days. These trends were similar for 60-day events (online supplementary *Table S4*).

Utility of NGAL in models

Figure 2 shows the Kaplan–Meier analysis for the primary composite endpoint (online supplementary *Table S5* displays tertile cut-points). Tertile 3 of admission sNGAL (>194.2 ng/mL) was associated with worse outcomes, whereas tertiles 1 (<97.0 ng/mL) and 2 (97.0–194.2 ng/mL) had similar incidences of outcomes (log-rank $P < 0.001$). Similarly, tertile 3 of peak sNGAL (>265.7 ng/mL) was associated with worse outcomes, whereas tertiles 1 (<138.2 ng/mL) and 2 (138.2–265.7 ng/mL) had similar incidences of outcomes (log-rank $P = 0.003$). Similar trends were observed for admission uNGAL, but were not statistically significant (log-rank $P = 0.073$). No significant difference was found between tertiles of peak uNGAL or uNGAL/uCr ratios.

Table 4 (and online supplementary *Table S6*) shows the multivariate Cox analysis for the relationship between log-transformed NGAL and outcomes. For 30-day outcomes, higher admission sNGAL was significantly associated with the primary composite endpoint, the composite of death or HF hospitalization, and death [*Table 4* multivariate model 4; adjusted hazard ratio (HR) 2.0, 95% CI 1.1–3.5, $P = 0.02$ for the composite endpoint; adjusted HR 2.0, 95% CI 1.0–4.0, $P = 0.04$ for the composite of death or HF readmission; adjusted HR 2.8, 95% CI 1.1–7.1, $P = 0.03$ for death]. Peak sNGAL was a significant predictor for mortality within

Table 3 Outcomes at 30 days in patients with an admission estimated glomerular filtration rate of <60 mL/min/1.73 m^2 or ≥60 mL/min/1.73 m^2

	eGFR <60 mL/min/1.73 m^2				eGFR ≥60 mL/min/1.73 m^2			
	AUC	95% CI	80% sensitivity cut-point	80% specificity cut-point	AUC	95% CI	80% sensitivity cut-point	80% specificity cut-point
Primary composite								
Admission sNGAL	0.61	0.54–0.68	153.3	396.5	0.49	0.39–0.6	63.9	161.4
Peak sNGAL	0.58	0.52–0.65	201.9	548.3	0.51	0.4–0.62	74.9	222.8
Admission uNGAL	0.56	0.48–0.64	5.5	62.8	0.52	0.42–0.62	3.2	26.1
Peak uNGAL	0.52	0.44–0.6	11.7	139.3	0.49	0.39–0.58	13.5	59.3
Admission uNGAL/uCr ratio	0.54	0.47–0.62	15.1	135.2	0.51	0.41–0.61	10.5	52.8
Peak uNGAL/uCr ratio	0.54	0.47–0.62	22.5	197.4	0.52	0.42–0.63	13.6	94.3
Admission creatinine	0.61	0.54–0.68	121.1	186.5	0.52	0.41–0.63	63.6	98.1
Peak creatinine	0.60	0.53–0.68	128.2	219.2	0.51	0.4–0.62	69.0	120.2
Death or heart failure readmission								
Admission sNGAL	0.62	0.54–0.69	159.2	396.5	0.51	0.4–0.62	63.9	164.1
Peak sNGAL	0.61	0.53–0.68	212.9	547.9	0.51	0.39–0.63	65.4	224.6
Admission uNGAL	0.56	0.47–0.65	6	63.1	0.54	0.43–0.66	3.2	26.1
Peak uNGAL	0.53	0.44–0.61	12.1	143.7	0.51	0.4–0.62	9	59.3
Admission uNGAL/uCr ratio	0.53	0.44–0.61	14.1	150.3	0.50	0.39–0.61	10.5	52.8
Peak uNGAL/uCr ratio	0.55	0.47–0.64	27.3	200.5	0.49	0.38–0.6	11.7	94.3
Admission creatinine	0.62	0.54–0.7	122.0	186.5	0.58	0.47–0.69	63.6	98.1
Peak creatinine	0.63	0.55–0.71	135.3	219.2	0.53	0.42–0.65	66.3	120.2
Death								
Admission sNGAL	0.64	0.54–0.75	194.4	389.4	0.48	0.3–0.66	64.1	163.6
Peak sNGAL	0.63	0.53–0.73	218	542.3	0.51	0.31–0.71	87.2	224.6
Admission uNGAL	0.60	0.5–0.7	11.5	65.2	0.53	0.34–0.71	3.3	25.6
Peak uNGAL	0.54	0.44–0.65	15.4	143.7	0.53	0.34–0.71	9	59.3
Admission uNGAL/uCr ratio	0.57	0.46–0.67	21.2	150.3	0.54	0.35–0.72	13.6	52
Peak uNGAL/uCr ratio	0.58	0.49–0.68	21.5	150.3	0.49	0.29–0.68	13.6	93.6
Admission creatinine	0.68	0.58–0.78	132.6	186.5	0.49	0.33–0.65	67.4	98.1
Peak creatinine	0.69	0.59–0.79	152.0	220.1	0.47	0.32–0.63	90.2	120.2
Heart failure readmission								

Table 3 (Continued)

	eGFR <60 mL/min/1.73 m²				eGFR ≥60 mL/min/1.73 m²			
	AUC	95% CI	80% sensitivity cut-point	80% specificity cut-point	AUC	95% CI	80% sensitivity cut-point	80% specificity cut-point
Primary composite								
Admission sNGAL	0.58	0.48–0.68	159.2	406.8	0.53	0.38–0.68	52.2	163.6
Peak sNGAL	0.57	0.47–0.67	207.4	548.3	0.51	0.36–0.66	65.4	224.6
Admission uNGAL	0.47	0.33–0.61	5.5	63.1	0.55	0.41–0.69	3.2	26.1
Peak uNGAL	0.49	0.36–0.62	11	144.5	0.50	0.37–0.63	14.3	59.3
Admission uNGAL/uCr ratio	0.50	0.36–0.64	13	151.3	0.52	0.38–0.66	10.5	52
Peak uNGAL/uCr ratio	0.53	0.4–0.66	19.1	200.5	0.49	0.36–0.63	11.7	95.1
Admission creatinine	0.56	0.45–0.67	121.1	188.3	0.63	0.47–0.78	53.0	98.1
Peak creatinine	0.57	0.45–0.68	135.3	221.9	0.58	0.41–0.74	64.5	120.2

AUC, area under the curve; CI, confidence interval; eGFR, estimated glomerular filtration rate; sNGAL, serum neutrophil gelatinase-associated lipocalin; uNGAL, urine neutrophil gelatinase-associated lipocalin.

30 days, but not for the composite outcomes. For 60-day outcomes, a higher admission sNGAL was initially associated with an increased risk for the primary composite outcome, the composite of death or HF readmission and death, but after multivariate adjustment including for sCr, sNGAL was no longer statistically significant in the final model (online supplementary *Table S6*; multivariate model 4, $P = 0.05$ for the primary composite endpoint, $P = 0.09$ for the composite of death or HF readmission, and $P = 0.06$ for death). None of the assessments of uNGAL were significant for the composite or individual outcomes.

Discussion

In this large, multicentre, international study, neither sNGAL nor uNGAL were superior to sCr for predicting the composite endpoint of death, initiation of RRT, HF readmission, or an emergent HF-related outpatient visit within 30 or 60 days. Findings were similar for post hoc analyses for death, HF readmission and the composite of these two outcomes. Admission and peak sNGAL had greater prognostic utility than uNGAL or the uNGAL/uCr ratio; however, prognostic utility overall was fair to poor with AUCs of <0.7. The prognostic ability of NGAL varied based on admission eGFR, a finding seen in prior analyses carried out in the context of AKINESIS.[12] The highest tertiles of admission and peak sNGAL were associated with higher incidences of the composite endpoint within 60 days. Serum NGAL but not uNGAL was significantly associated with worse 30-day outcomes independently of confounding factors, including admission sCr, in multivariate models. Similarly, the post hoc endpoints of death, HF readmission and the composite of these showed trends similar to those of the primary composite outcome.

In prior analyses of AKINESIS, neither sNGAL nor uNGAL were superior to sCr in predicting the development of WRF or adverse hospital outcomes.[12,13] Although WRF during AHF has been reported to predict worse outcomes, more recent studies have questioned the clinical significance of rises in sCr or declines in eGFR.[3,4] These studies have shown that the prognostic implications of WRF often depend on the context in which WRF occurs. WRF associated with decongestion has been shown to portend improved outcomes.[3,14] In these cases, WRF may represent a transient haemodynamic or functional change in sCr without actual kidney injury, and has been termed 'pseudo-WRF'.[7,15] The findings of AKINESIS and other studies support this as the scenario encountered more frequently in clinical practice as NGAL levels are not markedly above those of healthy volunteers.[12,13,16,17]

In some situations, kidney injury does appear to occur with WRF, and novel kidney injury biomarkers have been found to predict 'clinically significant WRF' (i.e. WRF occurring with an adverse clinical event), but this occurs in the minority of episodes of WRF.[18] Chen *et al*. showed urinary angiotensinogen predicted progressive WRF in patients with AHF; however, this scenario occurred in a minority of patients as only 213 (29%) of the 732 patients studied experienced WRF and only 50 (6.8%) had progressive WRF (23% of those with WRF).[19] Although kidney injury occurs in the minority of cases of WRF in AHF, injury may also occur without changes

Table 4 Univariable and multivariate Cox analysis for the 30-day endpoints

	Composite outcomes			Death or HF readmission			Death			HF hospitalization		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Admission sNGAL												
Univariate	2.7	1.6–4.4	<0.01	2.5	1.4–4.4	<0.01	4.0	1.9–8.5	<0.01	1.6	0.7–3.5	0.26
Multivariate 1		–		2.3	1.3–4.1	<0.01[h]	3.1	1.4–6.9	<0.01[a]		–	
Multivariate 2	2.2	1.3–3.8	<0.01[b]	2.0	1.1–3.7	0.03[i]	3.1	1.3–7.1	<0.01[c]		–	
Multivariate 3	2.7	1.7–4.5	<0.01[d]	2.6	1.5–4.5	<0.01[j]	4.2	1.9–8.9	<0.01[e]		–	
Multivariate 4	2.0	1.1–3.5	0.02[f]	2.0	1.0–4.0	0.04[k]	2.8	1.1–7.1	0.03[g]		–	
Peak sNGAL												
Univariate	2.4	1.4–4.1	<0.01	2.5	1.4–4.5	<0.01	3.9	1.8–8.7	<0.01	1.6	0.7–3.7	0.24
Multivariate 1		–		2.2	1.2–4.1	<0.01[h]	2.8	1.2–6.7	0.02		–	
Multivariate 2	1.9	1.1–3.4	0.02	1.9	1.0–3.7	0.04[i]	2.8	1.2–6.9	0.02		–	
Multivariate 3	2.6	1.5–4.4	<0.01	2.7	1.5–4.8	<0.01[j]	4.4	2.0–10.0	<0.01		–	
Multivariate 4	1.7	0.9–3.1	0.11	2.0	1.0–4.0	0.06[i]	2.7	1.0–7.2	0.05		–	
Admission uNGAL												
Univariate	1.4	1.0–1.8	0.04	1.3	1.0–1.9	0.08	1.8	1.1–2.8	0.02	1.1	0.7–1.8	0.67
Multivariate 1		–		1.3	1.0–1.8	0.10[h]	1.7	1.1–2.8	0.02[a]		–	
Multivariate 2	1.3	1.0–1.7	0.10[b]	1.2	0.9–1.7	0.20[i]	1.6	1.0–2.5	0.06[c]		–	
Multivariate 3	1.4	1.0–1.8	0.03[d]	1.4	1.0–1.9	0.07[j]	1.7	1.1–2.7	0.01[e]		–	
Multivariate 4	1.2	0.9–1.6	0.35[f]	1.2	0.9–1.7	0.29[k]	1.5	0.9–2.5	0.10[g]		–	
Peak uNGAL												
Univariate	1.3	0.9–1.8	0.13	1.3	0.9–1.8	0.19	1.5	0.9–2.6	0.10	1.1	0.7–1.8	0.70
Multivariate 1		–		1.2	0.8–1.8	0.32[h]	1.4	0.8–2.3	0.26		–	
Multivariate 2	1.2	0.9–1.7	0.22	1.2	0.8–1.7	0.34[i]	1.4	0.9–2.4	0.16		–	
Multivariate 3	1.3	0.9–1.8	0.11	1.3	0.9–1.9	0.15[j]	1.6	1.0–2.6	0.07		–	
Multivariate 4	1.1	0.8–1.6	0.45	1.2	0.8–1.8	0.36[k]	1.4	0.8–2.4	0.20		–	
Admission uNGAL/uCr ratio												
Univariate	1.3	1.0–1.8	0.09	1.3	0.9–1.8	0.20	1.5	0.9–2.5	0.09	1.1	0.7–1.9	0.66
Multivariate 1		–		1.2	0.8–1.8	0.34[h]	1.4	0.8–2.3	0.25[a]		–	
Multivariate 2	1.2	0.9–1.7	0.202[b]	1.2	0.8–1.7	0.43[i]	1.4	0.8–2.3	0.22[c]		–	
Multivariate 3	1.3	0.9–1.8	0.11[d]	1.2	0.9–1.8	0.24[j]	1.5	0.9–2.4	0.12[e]		–	
Multivariate 4	1.1	0.8–1.6	0.46[f]	1.2	0.8–1.7	0.42[k]	1.4	0.8–2.3	0.25[g]		–	
Peak uNGAL/uCr ratio												
Univariate	1.3	0.9–1.8	0.11	1.3	0.9–1.9	0.14	1.6	1.0–2.6	0.06	1.1	0.7–1.9	0.67
Multivariate 1		–		1.2	0.8–1.8	0.31[h]	1.3	0.8–2.3	0.30		–	
Multivariate 2	1.2	0.9–1.7	0.27	1.2	0.8–1.7	0.35[i]	1.4	0.9–2.4	0.15		–	
Multivariate 3	1.3	0.9–1.8	0.13	1.3	0.9–1.8	0.17[j]	1.6	1.0–2.5	0.07		–	
Multivariate 4	1.1	0.8–1.6	0.51	1.2	0.8–1.8	0.36[k]	1.4	0.9–2.5	0.17		–	

CI, confidence interval; HF, heart failure; HR, hazard ratio; NGAL, neutrophil gelatinase-associated lipocalin; sNGAL, serum NGAL; uNGAL, urine NGAL; uCr, urine creatinine. Multivariate 1, adjusted for demographics; Multivariate 2, adjusted for medical history; Multivariate 3, adjusted for medications administered in emergency department or upon hospital admission; Multivariate 4, adjusted for physical and laboratory test.

[a]Adjusted for age and race.
[b]Adjusted for chronic kidney disease and anaemia.
[c]Chronic kidney disease and anaemia.
[d]Beta-blocker given while admitted to the hospital.
[e]Beta-blocker given while admitted to the hospital.
[f]Systolic blood pressure, sodium, creatinine.
[g]Systolic blood pressure, sodium, haemoglobin and creatinine.
[h]Adjusted for age.
[i]History of chronic kidney disease, anaemia and cerebrovascular accident.
[j]Beta-blocker given while admitted to the hospital.
[k]Systolic blood pressure, sodium, haemoglobin and creatinine.

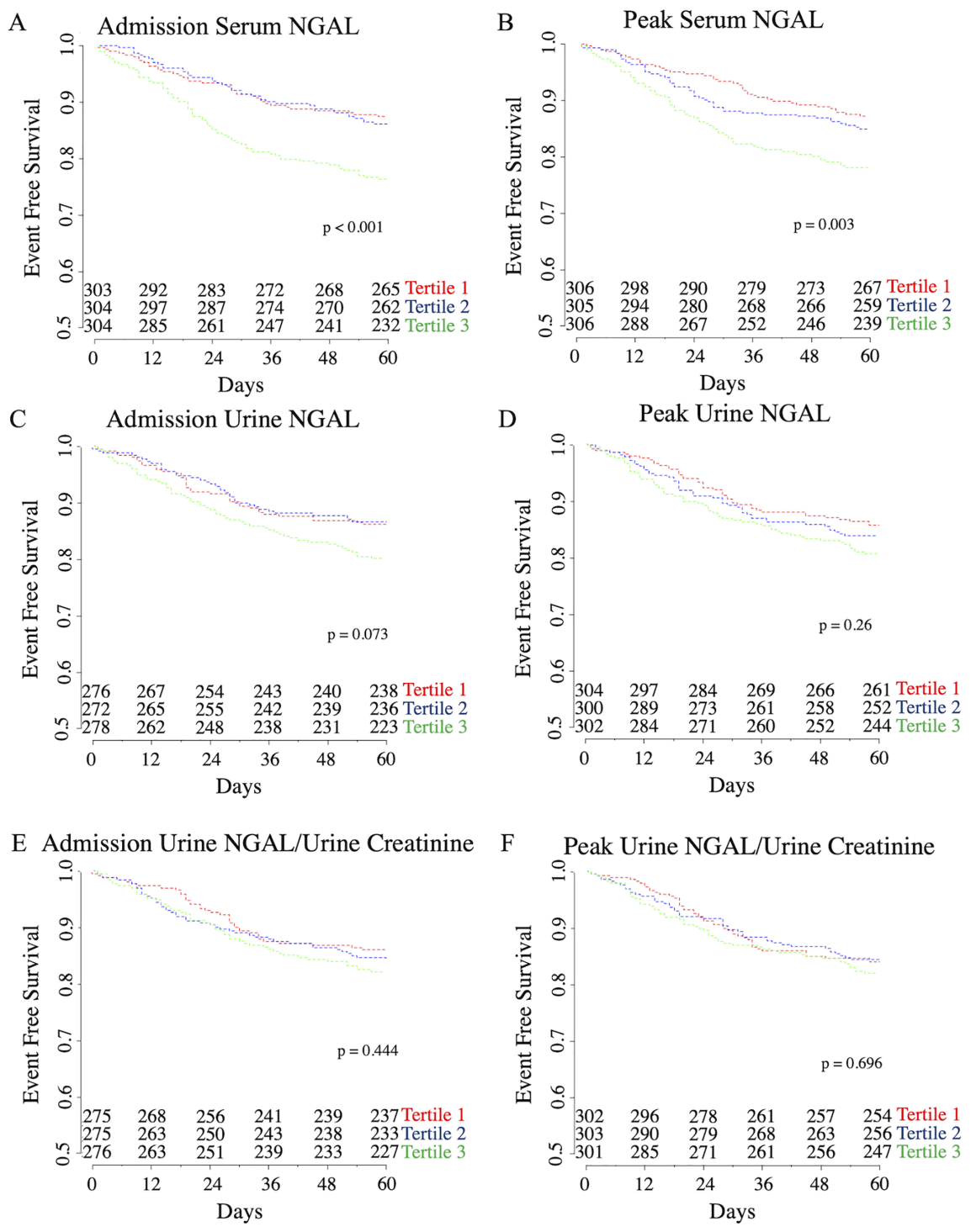

Figure 2 Kaplan–Meier analysis for the 60-day composite endpoint: (*A*) admission serum neutrophil gelatinase-associated lipocalin (sNGAL), (*B*) peak sNGAL, (*C*) admission urine NGAL (uNGAL), (*D*) peak uNGAL, (*E*) admission uNGAL/urine creatinine (uCr) ratio, and (*F*) peak uNGAL/uCr ratio. *P*-values refer to 60-day outcomes. Tertile 3 of admission and peak sNGAL were associated with worse outcomes, whereas tertiles 1 and 2 had similar incidences of outcomes (log-rank $P < 0.001$ and $P < 0.003$, respectively). Similar trends were observed with admission uNGAL, but were not statistically significant (log-rank $P = 0.073$).

in creatinine. A pooled data analysis of 2322 critically ill patients showed elevated levels of sNGAL and uNGAL without elevation in sCr were associated with RRT and in-hospital mortality.[20] Thus, it is essential to evaluate the prognostic implications of NGAL, or any novel kidney biomarker, for its association with clinical outcomes independent of the development of WRF. Theoretically, NGAL in AKINESIS could identify patients who had developed kidney injury and had an increased risk for adverse outcomes without concurrent WRF. However, this was not the case in the current analysis because kidney tubular injury appears to be an uncommon occurrence in AHF. Furthermore, whether NGAL has prognostic significance over other validated prognostic biomarkers of natriuretic peptides and high-sensitivity troponin in AHF remains to be determined. If natriuretic peptides and troponins had been measured on a standardized assay in AKINESIS, a head-to-head comparison might have shown NGAL to be outperformed by other biomarkers, thereby further diminishing NGAL's clinical utility.

AKINESIS is the first large-scale, multicentre, international study to prospectively investigate the prognostic values of sNGAL and uNGAL for short-term clinical outcomes. Although neither biomarker demonstrated strong prognostic utility, sNGAL was associated with poor prognosis. Urine NGAL is thought to be a more sensitive and specific marker of kidney tubular damage than sNGAL because its production is proximate to the site of injury, whereas sNGAL production can increase with systemic processes such as inflammation, which is well recognized as a pathophysiologic process in HF.[21] Thus, one might suspect that kidney injury detected with uNGAL would have significant prognostic implications, if kidney injury were occurring. This further supports a predominant lack of kidney injury with elevations in sCr from diuresis in AHF, but also questions the utility of sNGAL for the diagnosis of kidney injury.

Prior studies have shown conflicting results on the prognostic utility of uNGAL for short- and long-term outcomes. In a prospective cohort study of 399 patients presenting to the emergency department with AHF, elevated uNGAL measured 12–24 h after initial therapy was associated with increased risk for the composite outcome of death, HF hospitalization and other cardiovascular events at 30 days.[22] A study of 260 patients with AHF in whom uNGAL/uCr values were collected over 24 h showed those with uNGAL/uCr higher than the median of 32.5 μg/g were at increased risk for all-cause death, cardiovascular death and HF readmission.[23] Lastly, a study of 141 patients with AHF found that patients with uNGAL/uCr above the median for hospitalization had a higher rate of mortality and HF readmission at 180 days.[15] In contrast to these studies, a study of 83 patients with AHF did not find uNGAL or its ratio to uCr to predict mortality.[24] In a subanalysis of the ROSE-AHF trial, which included 283 AHF patients treated with aggressive diuretic therapy, Ahmad *et al*. found no relationship between WRF and uNGAL, N-acetyl-b-D-glucosaminidase (NAG), or kidney injury molecule 1 (KIM-1).[16] Additionally, baseline levels of uNGAL and KIM-1 were not associated with 180-day survival and higher baseline levels of NAG were associated with improved survival. Furthermore, increases in kidney injury markers were paradoxically associated with better outcomes. Similar results were recently reported when the same biomarkers were measured in 105 patients in the CARRESS-HF cohort, a population of patients already experiencing significant WRF.[25] Notably, patients who had a rise in injury biomarkers were more likely to have an improvement in creatinine at 60 days after enrolment. AKINESIS adds to these studies because its population represents the largest cohort studied and its results are in line with those of the latter studies in finding that uNGAL does not predict long-term adverse outcomes. The current findings and those of others suggest that acute tubular injury expressed by a rise in the urine biomarkers under current study does not have a significant impact on patients' outcomes or clinical course.

Although uNGAL was not prognostic, sNGAL was and has shown prognostic utility in other studies. Prior studies have reported the prognostic values of sNGAL in AHF patients mainly at hospital discharge. In the GALLANT study, sNGAL at discharge was predictive of 30-day all-cause mortality and HF readmission independently of sCr and eGFR.[26] In another study evaluating 562 AHF patients, sNGAL at discharge predicted mortality at 36 months independently of eGFR and cystatin C.[27] In the current analysis, higher levels of sNGAL on admission were predictive of worse short-term outcomes. However, sNGAL is not a kidney-specific biomarker because it is also produced in other tissues, including skin, lung and intestines, during systemic inflammation. NGAL is also expressed in failing myocardium and atherosclerotic plaques.[28] Therefore, sNGAL should probably be considered as a more sensitive and less specific comprehensive biomarker that is not specific to kidney tubular injury, but also reflects reduced glomerular filtration, systemic inflammation and a heightened cardiovascular risk.

The findings from AKINESIS are in line with those of the recent literature but the study represents one of the largest studies of cardiorenal biomarkers to include serial measurements and unique prognostic outcomes. Most prior studies of NGAL in AHF evaluated in-hospital outcomes, specifically changes in kidney function, and some evaluated outcomes at 30 days or over longer periods. The present analysis focused on the short-term (30- and 60-day) prognostic utility of NGAL for adverse outcomes as this time scale represents a period of heightened vulnerability to adverse outcomes if renal injury has occurred. In addition, beyond the outcomes of death and HF readmission, the present group evaluated outcomes of urgent i.v. diuretic therapy and RRT, relevant outcomes for renal injury that can potentially lead to the failure of oral diuretic therapy or injury severe enough to require RRT. The present study has confirmed an overall lack of substantial kidney tubular injury in AHF, which does not portend adverse outcomes. Additionally, it has again shown that the prognostic utility of NGAL seems to vary based on eGFR, a concept that generates hypotheses and warrants further investigation. Thus, the current findings provide improved insights into the short-term prognostic utility of NGAL in AHF.

Limitations

Although rates of sample collection on admission were good (98.3% for sNGAL and 89.2% for uNGAL), missing values at other collection times may have resulted in underestimations of the

prognostic implications of peak values. There is debate as to the absolute value of uNGAL and the uNGAL/uCr ratio may not be an adequate corrective measure. In the present study, the results were compared to those for sCr rather than for sCr clearance because increases in sCr are most commonly used to identify patients with AKI. This study did not use formalized enrolment criteria for diagnosing HF, such as the European Society of Cardiology or Framingham criteria, and thus the authors cannot state with certainty that all participants had AHF; however, the distribution and values of natriuretic peptides are within the range consistent with HF (Supplementary material online, *Figure S1*). Data on clinical events were collected from chart review or by telephone follow-up and were not centrally adjudicated. Outcomes were evaluated at 30 and 60 days and a longer follow-up may have yielded different results; however, it is likely that if kidney injury were to occur, it would influence events more proximate to the time of injury. Although the statistical analysis was performed in accordance with a prespecified plan, inadequate adjustment might occur in multivariate models. Lastly, many of the analyses were post hoc, but were performed to match more commonly reported outcomes in the literature.

Conclusions

Neither sNGAL nor uNGAL were more prognostic than admission sCr for the primary composite outcome of death, initiation of RRT, HF hospitalization and emergent HF-related outpatient visit. Overall, the prognostic utility of any of these biomarkers was poor. The assessment of NGAL does not add significant clinical value in AHF and tubular injury is infrequent in AHF.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Distributions of BNP and NT-proBNP in the total cohort.

Table S1. Whole cohort stratified by tertiles of amission serum neutrophil gelatinase-associated lipocalin (NGAL), urine NGAL, and urine NGAL/urine creatinine ratio.

Table S2. Independent predictors of admission serum neutrophil gelatinase-associated lipocalin (NGAL), admission urine NGAL, and admission urine NGAL/urine creatinine ratio.

Table S3. Areas under the curve and 80% sensitivity and specificity cut-points for 60-day outcomes.

Table S4. Outcomes at 60 days in patients with admission estimated glomerular filtration rate of <60 mL/min/1.73 m^2 or ≥ 60 mL/min/1.73 m^2.

Table S5. Tertile cut-points of neutrophil gelatinase-associated lipocalin.

Table S6. Univariable and multivariate Cox analysis for the 60-day endpoints.

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