**The Nature and Discriminatory Value of Urinary Neutrophil Gelatinase-Associated Lipocalin in
Critically Ill Patients at Risk of Acute Kidney Injury**

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Take Home Message

The nature and source of urinary NGAL in ICU patients with oliguria and SIRS are complex and challenge current views of NGAL as a uniform biomarker. Clinical and biochemical phenotypes based on different molecular forms (heterodimeric, homodimeric and monomeric) of NGAL exist and even after taking such molecular subtypes into account, NGAL is an overall poor predictor of patient and renal outcomes in critically ill patients at risk of acute kidney injury

Tweet

There are at least 3 forms of urinary NGAL of complex source and in disagreement with our current views of NGAL

Abstract

Background: Different molecular forms of urinary NGAL have recently been discovered. We aimed to explore the nature, source and discriminatory value of urinary neutrophil gelatinase associated lipocalin (NGAL) in ICU patients.

Methods: We simultaneously measured pNGAL, uNGAL, and estimated monomeric and homodimeric urinary NGAL contribution using Western-blotting validated ELISAs (uNGAL_{E1} and uNGAL_{E2}) and their calculated ratio in 102 patients with the systemic inflammatory response syndrome and oliguria, and/or a creatinine rise of > 25 µmol/L.

Measurements and Main Results: Bland-Altman analysis demonstrated that, despite correlating well ($r=0.988$), uNGAL and uNGAL_{E1} were clinically distinct, lacking both accuracy and precision (bias: 266.23, 95% CI: 82.03 – 450.44ng/mg creatinine; limits of agreement: - 1573.86 — 2106.32 ng/mg creatinine). At best, urinary forms of NGAL are fair (AUROC ≤ 0.799) predictors of renal or patient outcome; most perform significantly worse. The 44 patients with a primarily monomeric source of urinary NGAL had higher pNGAL (118.5 ng/ml vs. 72.5 ng/ml, $p < 0.001$), remaining significant following Bonferroni correction.

Conclusions: Urinary NGAL is not a useful predictor of outcome in this ICU population. Urinary NGAL patterns may predict distinct clinical phenotypes. The nature and source of urinary NGAL are complex and challenge the utility of NGAL as a uniform biomarker.

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Key words: acute kidney injury, oliguria, critical illness, intensive care, biomarker, systemic inflammatory response syndrome

Introduction

Neutrophil Gelatinase-Associated Lipocalin/Human Neutrophil Lipocalin (NGAL/HNL) is a potentially useful biomarker for Acute Kidney Injury (AKI) [1-4], and proprietary assays are now available [5-9]. NGAL was first identified in specific granules of human neutrophils [10], but has subsequently been found in a variety of cells [11, 12]. Its production is upregulated by oxidative stress, malignancy, infection, ischaemia and inflammation [1, 11, 13-16]

More recently, different molecular forms of NGAL have been identified. Neutrophils produce a unique 45-kD homodimer. In contrast, renal tubular epithelial cells do not produce the homodimer; instead they produce a 135-kD heterodimer covalently conjugated with gelatinase. Both cell types also produce a 25-kD monomer (Figure 1). Renal tubular epithelial cells are believed to be the primary source of the monomer in the urine [17, 18].

Most of plasma NGAL appears to come from circulating neutrophils. NGAL measured in the urine could be composed, in part, of NGAL secreted by renal tubular cells and/or local neutrophil release, as well as filtered plasma NGAL. Unfortunately, commercially available immunoassays appear to measure NGAL utilizing different monoclonal and polyclonal antibody preparations [7-9, 19-21]. These have differential performance in the estimation of NGAL [5] and different epitope affinity [22]. These characteristics make the identification of the nature of measured NGAL problematic.

Western blotting has recently demonstrated that the urinary ratio of two different ELISAs (ELISA-1 and ELISA-2) can identify the likeliest source of NGAL measured in the urine. Similar to

commercially available assays, ELISA-1 measures monomeric, homodimeric and heterodimeric urinary NGAL. ELISA-2 measures homodimeric urinary NGAL (Figure 1). In patients with a demonstrated urinary NGAL ELISA-1:ELISA-2 ratio greater than 50, Western blotting confirmed that the measured urinary NGAL is monomeric in nature (AUROC of 0.92) [23]. In patients with a ratio of less than 50, the major contributor to urinary NGAL is likely to be the homodimeric form [23]. These assays and their ratio can help determine the likely source of urinary NGAL.

Accordingly, we conducted a prospective observational study targeting those critically ill patients most at risk of developing AKI in whom a predictive test offering biological insight into the source of NGAL and its relationship to subsequent AKI may be of greatest utility.

We hypothesised that (1) NGAL would be detectable in the urine and plasma of such patients using commercial assays and the research ELISAs; (2) that patients with a urinary NGAL ELISA-1:ELISA-2 ratio > 50 would be at greater risk of AKI; and (3) that there would be a distinct clinical phenotype in patients with an ELISA-1:ELISA-2 ratio > 50.

Materials and Methods

Study design

We conducted a prospective observational study of critically ill patients at risk of acute kidney injury (AKI). The Human Research Ethics Committee at the Austin Hospital, Melbourne,

approved the study prior to commencement with waiver for consent (HREC approval number H2011/04219).

Between the 1st of September 2010 and the 17th of November 2010, we enrolled all patients within 48 hours of ICU admission who met the inclusion criteria. In these patients, we simultaneously used commercial assays to measure plasma (pNGAL) and urinary NGAL (uNGAL), and research assays ELISA-1 (measuring all forms of NGAL) and ELISA-2 (relatively specific to homodimeric NGAL) to identify the likely source of urinary NGAL.

Inclusion criteria were: age >18 years, the presence of the systemic inflammatory response syndrome (SIRS) and the presence of oliguria, or a 25 $\mu\text{mol/l}$ increase from baseline in their serum creatinine, or both. SIRS was defined according to consensus guidelines [24]. Oliguria was defined as a urine output of less than 0.5ml/kg/hr for two or more consecutive hours. Baseline creatinine was defined as the lowest creatinine measured in the six months preceding ICU admission. All creatinine measurements were converted into estimations of glomerular filtration rate using the Modification of Diet in Renal Disease calculation; in the absence of true baseline creatinine in patients with no history of renal dysfunction a glomerular filtration rate of 75 ml/min/1.73 m² was assigned as previously described [25]. AKI was defined according to RIFLE criteria [26]. Diagnoses and co-morbid illnesses were as defined according to the APACHE III system [27].

Study protocol

Patients were identified by daily surveillance. We collected clinical, demographic, physiologic and laboratory data and outcomes. Urine and blood for NGAL analysis were obtained for each patient on enrolment only. Serum creatinine as measured by point-of-care testing [28] at study inclusion was also documented.

Urine samples were stored at -70°C for batched analysis. uNGAL was measured by a chemiluminescent microparticle assay using the ARCHITECT platform (Abbott Diagnostics Inc., Abbott Park, IL), as previously described [9]. Urinary NGAL measurements were expressed as ng/mg creatinine, to standardize and correct for changes in urine concentration [3].

ELISA-1 was performed utilising monoclonal antibody clones m763/764, while ELISA-2 utilised monoclonal antibody clones m763/765, as previously described [17, 22, 23]. ELISA-1:ELISA-2 ratios were calculated as previously described [23].

Blood samples for pNGAL were collected in EDTA anti-coagulated tubes and analysed immediately using the Triage NGAL Test (Alere, San Diego, CA), a point- of-care, fluorescence immunoassay for quantitative measurement of pNGAL, as previously described [8].

Statistical analysis

Statistical analyses were performed with SPSS version 20 (IBM, North Castle, NY). Non-normally distributed continuous data are reported as medians with either inter-quartile (IQR) or total range and compared using the Mann-Whitney U test, Kruskal-Wallis test, or Friedman test where appropriate. The Bonferroni correction was used to account for multiple testing. Categorical data are reported as proportions and compared using the Fisher exact test. The

relationship between variables was assessed using Spearman's rank correlation co-efficient. We used the following values to describe the strength of correlation: >0.9 very strong, 0.68 – 0.9 strong, 0.3 – 0.67 moderate, <0.3 weak [29]. The Bland-Altman technique was used to compare methods [30-32]. The individual measures and the ratio of the different forms of NGAL were assessed for their ability to predict AKI, RRT initiation and mortality using the area under the receiver-operating characteristic (AUROC) curve. We used the following values to describe AUROCs: 0.90–1.0 excellent, 0.80–0.89 good, 0.70–0.79 fair, 0.60–0.69 poor and 0.50– 0.59 no useful performance [33]. A p-value of <0.05 was considered statistically significant.

Results

We studied 102 patients with a median age of 66 (53 – 75) years and median APACHE III score was 63.5 (46 - 78); 73.53% of patients were mechanically ventilated and 6.87% eventually required continuous renal replacement therapy (CRRT) (Table 1). Baseline and worst creatinine and GFR values are presented in Table 1. Of note, 54 (52.95%) of patients had RIFLE-R or greater AKI on study admission (Table 1).

The median time to enrolment from ICU admission was 0.69 (0.49 – 1.27) days. At enrolment, median pNGAL was 97 (60 – 260.25) ng/ml. Median uNGAL was 82.09 (27.18 – 413.52) ng/mg creatinine. The median urinary NGAL reported by ELISA-1 (uNGAL_{E1}) and the median urinary NGAL reported by ELISA-2 (uNGAL_{E2}) are presented in Table 1. Overall, 44 (43.14%) of the 102 patients had a uNGAL_{E1}: uNGAL_{E2} ratio > 50.

Correlations

There was a moderate correlation between pNGAL and uNGAL_{E1} and between pNGAL and uNGAL; there was no significant correlation between pNGAL and uNGAL_{E2}. There was a very strong correlation between uNGAL and uNGAL_{E1} (Figure E1, Online Data Supplement), but markedly weaker correlations between uNGAL_{E2} and uNGAL or between those reported by uNGAL_{E1} and uNGAL_{E2} (Table E1, Online Data Supplement).

Method Comparison Analysis

Using Bland-Altman analysis (Figure 2), the mean bias between commercial uNGAL and uNGAL_{E1} was 266.23 (95% confidence interval, 82.03 — 450.44) ng/mg creatinine, with a standard deviation of 937.82 ng/mg of creatinine. The limits of agreement varied from -1573.86 (95% CI: -1415.07 — -1731.79) ng/mg of creatinine to 2106.32 (95% CI: 1947.53 — 2265.11) ng/mg of creatinine (Figure 2).

Bland-Altman analysis was repeated after natural logarithmic transformation of the variables (See Figure E2 in the online data supplement) [30, 32]. While this removed the systematic bias, 95% of the values of uNGAL were still within 0.86 – 2.8 times the value of uNGAL_{E1}.

Acute Kidney Injury, renal replacement therapy and death

Overall, 72 of the 102 patients developed AKI compared to baseline. Of these, 7 (6.87%) received RRT (Table 1). No measure or derivation of NGAL was a useful predictor of hospital or intensive care mortality. NGAL measured by ELISA-2 was a poor predictor of any outcome (Table 2). The uNGAL_{E1}:uNGAL_{E2} ratio was not a useful predictor of outcome. pNGAL demonstrated good ability to predict RIFLE-F AKI, and was a fair predictor of the need for CRRT

No urinary measure of NGAL was a useful predictor of the development of AKI (Table 2). Both uNGAL and uNGAL_{E1} were fair predictors of the development of RIFLE-F AKI, but did not predict the need for CRRT, with 95% Confidence Intervals of the AUROC inclusive of 0.5.

Patient stratification

Overall, 44 (43.14%) of the 102 patients had uNGAL_{E1}:uNGAL_{E2} ratios > 50. These patients trended towards increased rates of diagnoses of sepsis or acute liver failure, but lower rates of admission following an emergency call (Table 3). They trended towards higher baseline creatinine and lower minimum MDRD derived GFR. More of these patients developed RIFLE-I AKI. The two groups demonstrated clear NGAL and uNGAL_{E1}:uNGAL_{E2} ratio separation (Table 3). Only the differences in NGAL measurement remained significant after Bonferroni correction. When stratified for the presence of AKI on study recruitment, patient groups had the expected differences in serum creatinine, GFR and the presence of AKI, as well as a significant difference in pNGAL (median pNGAL 64 ng/ml in patients without AKI, and 143.5 ng/ml in those with, $p < 0.001$). These differences retained significance after Bonferroni correction (Table E3, online data supplement).

Discussion

Statement of key findings

We assessed the relationships between pNGAL, uNGAL, uNGAL_{E1} and uNGAL_{E2}, whose ratio can determine the likely nature and source of urinary NGAL. We investigated their ability to predict the development of AKI, the need for CRRT and ICU and in-hospital mortality in undifferentiated critically ill admissions to the ICU, and found a lack of clinically acceptable performance. No urinary measure of NGAL was found, in this patient population, to be suitable for clinical use in a pragmatic fashion in the ICU. We found that plasma and urinary measurements of NGAL had limited correlation. We further found a strong relationship between uNGAL and uNGAL_{E1}, consistent with the observation that both assays measure all of the urinary forms of NGAL. Despite this strong relationship, the limits of agreement were too wide to permit interchange of measurement clinically.

In this population, both uNGAL and uNGAL_{E1} were poorly predictive of AKI, the need for CRRT and mortality. In contrast, pNGAL, which is thought to be primarily homodimeric neutrophil NGAL [23], showed fair discrimination for predicting CRRT, and good discrimination for RIFLE-F AKI. However, urinary NGAL measured by uNGAL_{E2} had no significant correlation with pNGAL, implying that is unlikely to represent a renally filtered pNGAL.

Finally, the ratios of pNGAL to urinary measures, and the uNGAL_{E1}:uNGAL_{E2} ratio, were not useful predictors of any outcome, although patients with uNGAL_{E1}:uNGAL_{E2} greater than 50 (monomeric NGAL of renal origin) were more likely to have septic AKI, liver failure and elevated pNGAL levels.

Relationship with previous studies

NGAL has been reported to be a good to excellent predictor of renal outcomes in highly specific groups of patients [1, 3, 9, 33-38], confirmed on meta-analysis [39]. However, the actual nature of the NGAL being measured with commercial assays remains unclear [5], particularly given the presence of different molecular forms of NGAL whose proportions seem to change with time [17, 22, 23].

In such ICU patients, uNGAL has demonstrated variable ability to predict AKI [40-47] and CRRT [40, 41, 43, 47]. NGAL was a poor indicator of subsequent hospital mortality [40, 41, 43] with only a single Japanese study finding it a useful only at a non-standardized point of follow-up [42]. In contrast, pNGAL poorly predicts mortality [40, 41, 48], but is a fair to excellent predictor of AKI [40, 41, 44, 45, 48, 49] and a fair to good predictor of CRRT use [40, 41, 48, 49]. In an undifferentiated ICU population, pNGAL and uNGAL demonstrated similarly poor predictive value [47].

The plasma:urinary ratios of NGAL and the temporal changes in ratio in septic and non-septic patients have been described previously, though with no comment on predictive ability [40]. In an undifferentiated ICU population, pNGAL and uNGAL demonstrated similarly poor predictive value [47].

Despite the existence of the RIFLE criteria [26] the definitions of AKI differ throughout the literature, making direct comparison even more problematic [50]. It is also difficult to compare studies using different commercial NGAL assays; the lack of agreement between such assays has been clearly demonstrated [5]. Different commercial assays utilise different mono- and polyclonal antibody combinations, have different epitope specificities, and detect different

proportions of the various molecular forms of urinary NGAL [5, 17, 22]. In addition, different calibrators are used by different companies, adding a systematic bias to any direct comparisons [19, 22, 51].

Study significance

Our study is the first to investigate the predictive value of three different types of urinary NGAL measurement simultaneously in ICU patients at risk of AKI; the first to directly compare the measurement of urinary NGAL by commercial assay and by techniques with relative specificity for homodimeric NGAL; the first to look at plasma:urinary ratios of the different urinary forms and to demonstrate biochemical differences and a trend towards differences in clinical phenotype in critically ill patients stratified by $\text{uNGAL}_{\text{E1}}:\text{uNGAL}_{\text{E2}}$ ratio. Finally, it is the first to assess the clinical utility of these laboratory techniques in a population of patients with a phenotype that justifies a high index of suspicion of likely subsequent AKI. It is in this very population that a test which can predict which patients will go on to develop AKI requiring CRRT and/or achieve successful independent resolution of their AKI would appear most useful.

Our findings fully challenge the current simplistic views of NGAL biology.

First, homodimeric uNGAL_{E2} is a uniformly poor predictor of patient centred outcomes in this population. Given the dissociation between pNGAL and uNGAL_{E2} , and given that pNGAL is mostly neutrophil-derived homodimeric NGAL, uNGAL_{E2} is unlikely to come from filtered pNGAL. Thus, its release may not be as indicative of tubular stress as the release of the

monomeric form. This notion is reinforced by the greater median pNGAL and smaller median uNGAL_{E2} results reported in patients with uNGAL_{E1}:uNGAL_{E2} ratios > 50.

Second, commercial uNGAL assays measure mostly monomeric NGAL, but also detect a variable amount of heterodimeric and homodimeric NGAL too. Therefore, the quantification of urinary NGAL remains challenging as uNGAL_{E1} and uNGAL cannot be used interchangeably.

Third, patients can be stratified by uNGAL_{E1}:uNGAL_{E2} ratio with significant differences in biochemical and clinical characteristics. Patient with ratios greater than 50 have significantly higher pNGAL measurements, and a higher incidence of sepsis and acute liver failure.

Neutrophil activation in sepsis [7] and liver disease could account for the high levels of pNGAL demonstrated in these patients.

Finally, our findings imply that widespread use of NGAL in ICU patients at potential risk of AKI is premature and should wait for a better understand of the biology of this biomarker.

Limitations of this study

This is a single centre study and its findings may not apply to other centres. However, our ICU has all the typical features of an academic ICU within a tertiary hospital of a developed country suggesting a degree of external validity. We made no correction for hypotension, inotropes, the fluid loading or the use of furosemide. Yet, to be useful in ICU patients, modern biomarkers must be able to perform well in a heterogeneous population irrespective of such confounders. The use of changes in serum creatinine to judge the value of biomarkers such as NGAL has been

called into question [3, 52, 53]. This is why we also sought to relate NGAL values to need for CRRT or mortality. For both, performance was poor.

We measured NGAL at only a single time point, up to 48 hours after admission to ICU. The temporal release profile of the different forms of NGAL is unclear [17, 22, 23]. The most impressive discriminatory abilities of NGAL seem to be when measured within hours of the insult [1, 9, 35, 36, 39]. It is possible that the renal insult in some of these patients occurred after ICU admission. However, the median time to peak creatinine observed was 1.06 days post-ICU admission, making this unlikely. Indeed, the movement of biomarkers from experimental to clinical practice means that they must be robust enough to account for the variability that exists between the onset of critical illness and the presentation to intensive care.

By including those patients with a 25 $\mu\text{mol/l}$ increase in serum creatinine we are including a population of patients who may have had an insult 12-24 hours previously that had technically resulted in KDIGO AKI [54]. Though including such patients may introduce selection bias, given their increased risk of developing AKI and dying in ICU [55-57], these are precisely the patients in whom prediction may be most useful.

The inter-relationship between uNGAL and the different molecular forms of NGAL in the urine remains poorly understood. The relative contribution of renally-filtered pNGAL to urinary NGAL levels, or the back-leak of monomeric, renal-specific NGAL released into the plasma by tubular damage, is unclear, as are the temporal relations of release. Heterodimeric NGAL is present in the urine in only very small quantities [23], is difficult to quantify, and has not been directly

measured in this study. The ability of heterodimeric NGAL to predict AKI and outcome remains unknown. In addition, uNGAL_{E2} appears to only measure a portion of the homodimeric NGAL present in the urine, possibly due to differences in epitope availability between the monomeric and homodimeric forms [23]. Recent studies have begun to examine these relationships [22, 23], but not yet in sufficient detail to allow conclusions to be drawn in the chaotic and confounding environment of the ICU. It is likely that differential patterns of biomarker release will emerge and therefore require specific assessment [34].

Conclusions

In a high-risk population, both plasma and urinary NGAL may represent NGAL release from a broad range of cells and have limited predictive value for mortality, the need for CRRT or the development of AKI. Moreover, pNGAL may be of greater value as a biomarker of AKI than uNGAL, or any of the specific urinary forms of NGAL. Finally, differences in patient demographics and biochemical parameters can be demonstrated when urinary NGAL is primarily monomeric or homodimeric in nature. Despite the increasing availability of commercial assays, this suggests the need for further investigations to define the natural history of the release of the different forms of NGAL in ICU patients with SIRS and early renal dysfunction before NGAL can be used to identify critically ill patients who will develop severe AKI or the need for CRRT.

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Author Contributions

Conception and design: NJG, AGS, RB.

Data collection and methodology: NJG, AGS, LP, HY, GME, SX, PV.

Analysis and interpretation: NJG, RB, PV, SX.

Drafting the manuscript for important intellectual content: NJG, RB, PV, SX

Manuscript review: NJG, AGS, RB, PV, SX, LP, HY, GME.

Conflict of Interest

SX holds patents with, and receives royalties from, Diagnostics Development. PV holds patents with, and stock in, P&M Venge AB. He has received royalties from Phadia. The remaining authors declare that they have no conflict of interest

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Glossary

NGAL:	Neutrophil gelatinase-associated lipocalin, also known as human neutrophil lipocalin HNL). Released from multiple tissues. Present in plasma and urine.
Monomeric NGAL:	A 25-kD monomeric form of NGAL produced by both neutrophils and renal epithelium.
Homodimeric NGAL:	A 45-kD homodimeric form of NGAL produced by neutrophils.
Heterodimeric NGAL:	A 135-kD heterodimeric form of NGAL, covalently conjugated with gelatinase, produced by renal epithelium.
pNGAL:	NGAL measured in the plasma using the Triage Point-of-Care NGAL test (Alere).
uNGAL:	NGAL measured in the urine using the ARCHITECT platform (Abbott).
uNGAL_{E1}:	NGAL in the urine measured by a research assay utilising monoclonal antibody clones m763/m764. Detects a proportion of all forms of NGAL in the urine.
uNGAL_{E2}:	NGAL in the urine measured by a research assay utilising monoclonal antibody clones m763/m765. Detects a fraction of homodimeric NGAL in the urine.
uNGAL_{E1}:uNGAL_{E2} ratio:	A ratio of NGAL detected in the urine by the research assays > 50 distinguishes monomeric from homodimeric NGAL with an AUROC of 0.92.

Figure 1

Figure 1. A urine sample may consist of a mixture of the three identified forms of NGAL – monomeric, homodimeric and heterodimeric. Different assays measure different forms and different proportions of the total quantity of each form. Western blotting and an ELISA-1:ELISA-2 ratio allow the identification of the source of the majority of the NGAL in the sample (Adapted from [17, 22, 23]).

TABLE 1: Patient demographic, biochemical and clinical features at enrolment and subsequent outcomes

Characteristic	Median (IQR) / Value (%)
Number of Patients	102
Age (years)	66.55 (52.93 — 74.78)
Male sex	56 (54.91%)
ICU Admission Characteristics	
APACHE 3	66.55 (52.93 — 74.78)
Ventilated (IPPV) during ICU admission	75 (73.53%)
CRRT during ICU admission	7 (6.87%)
IABP during ICU admission	3 (2.95%)
Study inclusion criteria	
Oliguria alone	83 (81.38%)
Creatinine rise alone	81 (79.42%)
Oliguria and creatinine rise	62 (60.79%)
Pre-existing APACHE III co-morbidities	
Cardiac	4 (3.93%)
Respiratory	3 (2.95%)
Liver Disease	11 (10.79%)
Immunosuppression	9 (8.83%)
Malignancy	6 (5.89%)
Admission following	
Respiratory Arrest	5 (4.91%)
Cardiac Arrest	8 (7.85%)
Emergency Call	27 (26.48%)
Admission diagnosis	
Sepsis	21 (20.59%)
Stroke	8 (7.85%)
Malignancy	8 (7.85%)
Liver failure/Transplantation	9 (8.83%)
Post-Cardiac Surgery	17 (16.67%)
Outcomes	
Duration of ICU stay (Days)	2.78 (1.66 — 5.71)
Duration of hospital stay (Days)	15.64 (8.2 — 30.71)
ICU Mortality	12 (11.77%)
Hospital Mortality	15 (14.71%)

Measures of Renal Function

Number of patients with true baseline creatinine (%)	86 (84.32%)
Baseline creatinine (micromol/l)	82 (64.25 — 112)
Baseline GFR (MDRD) (ml/min/1.73 m ²)	75 (61.92 — 98.81)
Peak POC creatinine (micromol/l)	126 (87.25 — 195.75)
GFR nadir (MDRD) (ml/min/1.73 m ²)	44.18 (27.29 — 65.73)
Time from ICU admission to maximal AKI (Days)	1.06 (0.47 — 1.75)
Time from study admission to maximal AKI (Days)	0.22 (-0.21 — 0.96)
Patients requiring CRRT during ICU admission (%)	7 (6.9)
Time from ICU admission to CRRT (hours)	47.99 (23.5 - 244.25)
Time from study admission to CRRT (hours)	33.46 (7.37 - 224.23)

RIFLE AKI Class on Study Admission

No AKI	48 (47.05%)
RIFLE-R AKI or greater	54 (52.95%)
RIFLE-I AKI or greater	28 (27.46%)
RIFLE-F AKI or greater	4 (3.93%)

Peak RIFLE AKI Class

No AKI	30 (29.41%)
RIFLE-R AKI or greater	72 (70.59%)
RIFLE-I AKI or greater	39 (38.24%)
RIFLE-F AKI or greater	8 (7.85%)

Urinary Creatinine Measurements

Urinary Creatinine (mmol/l)	7.65 (3.33 — 10.63)
Urinary Creatinine (mg/ml)	0.87 (0.38 — 1.2)

NGAL Measurements

pNGAL (ng/ml)	97 (60 — 260.25)
uNGAL (ng/ml)	75.75 (22.93 — 274.55)
uNGAL _{E1} (ng/ml)	39.8 (14.83 — 178.43)
uNGAL _{E2} (ng/ml)	1.94 (0.68 — 4.61)
Corrected uNGAL (ng/mg Creatinine)	82.09 (27.18 — 413.52)
Corrected uNGAL _{E1} (ng/mg Creatinine)	48.24 (17.32 — 308.01)
Corrected uNGAL _{E2} (ng/mg Creatinine)	2.17 (1.01 — 5.8)

NGAL Ratios

pNGAL:uNGAL, corrected	1.11 (0.34 — 2.98)
pNGAL:uNGAL _{E1} corrected	2.11 (0.5 — 5.28)
pNGAL:uNGAL _{E2} , corrected	46.02 (21.57 — 220.65)
uNGAL:uNGAL _{E1}	1.6 (1.31 — 1.91)
uNGAL:uNGAL _{E2}	38.11 (7.95 — 227.52)

All values presented as medians with interquartile range (IQR) or as absolute number with percentage in brackets. IPPV - intermittent positive pressure ventilation; NIV - non-invasive ventilation; CRRT - Continuous Renal Replacement Therapy; IABP - intra-aortic balloon pump; POC: Point of Care; IQR: Interquartile Range; GFR: Glomerular Filtration Rate; MDRD: Modification of Diet in Renal Disease. pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2.

TABLE 2: VALUE OF NGAL MEASUREMENT IN PREDICTING AKI, NEED FOR CRRT AND MORTALITY

	Area Under the Receiver Operator Curve (95% CI)				
	Any AKI	RIFLE - F	CRRT	Death in ICU	Death in Hospital
pNGAL	0.606 (0.491 — 0.722)	0.827 (0.646 — 1)	0.78 (0.579 — 0.982)	0.43 (0.252 — 0.608)	0.424 (0.271 — 0.578)
uNGAL*	0.55 (0.418 — 0.683)	0.754 (0.548 — 0.96)	0.705 (0.49 — 0.92)	0.372 (0.234 — 0.51)	0.389 (0.258 — 0.519)
uNGAL_{E1}*	0.553 (0.421 — 0.685)	0.757 (0.553 — 0.96)	0.699 (0.478 — 0.921)	0.381 (0.245 — 0.518)	0.392 (0.262 — 0.521)
uNGAL_{E2}*	0.48 (0.359 — 0.6)	0.581 (0.353 — 0.81)	0.531 (0.321 — 0.741)	0.486 (0.331 — 0.64)	0.461 (0.323 — 0.599)
pNGAL:uNGAL*	0.523 (0.392 — 0.654)	0.356 (0.15 — 0.562)	0.375 (0.18 — 0.57)	0.661 (0.5 — 0.822)	0.633 (0.484 — 0.783)
pNGAL:uNGAL_{E1}*	0.515 (0.386 — 0.644)	0.347 (0.147 — 0.547)	0.392 (0.177 — 0.608)	0.658 (0.495 — 0.822)	0.636 (0.483 — 0.789)
pNGAL:uNGAL_{E2}*	0.553 (0.433 — 0.673)	0.525 (0.295 — 0.755)	0.629 (0.415 — 0.842)	0.504 (0.34 — 0.667)	0.509 (0.368 — 0.65)
uNGAL:uNGAL_{E1}	0.432 (0.307 — 0.558)	0.417 (0.227 — 0.607)	0.552 (0.311 — 0.793)	0.486 (0.347 — 0.624)	0.525 (0.395 — 0.655)
uNGAL:uNGAL_{E2}	0.53 (0.408 — 0.653)	0.576 (0.343 — 0.809)	0.609 (0.412 — 0.806)	0.456 (0.301 — 0.612)	0.481 (0.346 — 0.617)
uNGAL_{E1}:uNGAL_{E2}	0.531 (0.409 — 0.654)	0.584 (0.36 — 0.807)	0.612 (0.427 — 0.797)	0.454 (0.294 — 0.614)	0.482 (0.342 — 0.622)

*pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2; AKI: Acute Kidney Injury; CRRT: Continuous Renal Replacement Therapy. *Urinary measures are corrected by urinary creatinine excretion.*

TABLE 3: Biochemical, demographic and clinical features of patients stratified by urinary ELISA-1:ELISA-2 positivity.

	uNGALE1:uNGALE2 < 50	uNGALE1:uNGALE2 > 50	Univariate Comparison
N	58	44	
Age (years)	67.91 (53.86 — 74.78)	63.07 (48.57 — 74.06)	p = 0.274
Sex (Male)	29 (50%)	27 (61.37%)	p = 0.316
Apache 3 Score	61 (44.25 — 74.25)	68 (56.25 — 78.25)	p = 0.074
Diagnoses			
Sepsis	7 (12.07%)	14 (31.82%)	p = 0.025
Stroke	6 (10.35%)	2 (4.55%)	p = 0.461
Cardiopulmonary bypass	12 (20.69%)	5 (11.37%)	p = 0.286
Malignancy	6 (10.35%)	2 (4.55%)	p = 0.461
Acute Liver Failure	2 (3.45%)	7 (15.91%)	p = 0.037
Mechanical ventilation	45 (77.59%)	30 (68.19%)	p = 0.366
Admitted following			
Respiratory Arrest	3 (5.18%)	2 (4.55%)	p = 1
Cardiac Arrest	3 (5.18%)	5 (11.37%)	p = 0.286
Emergency/MET call	21 (36.21%)	6 (13.64%)	p = 0.013
Comorbidities			
Immunosuppressed	5 (8.63%)	4 (9.1%)	p = 1
Chronic Liver Disease	3 (5.18%)	8 (18.19%)	p = 0.052
Malignancy	3 (5.18%)	3 (6.82%)	p = 1
Respiratory	1 (1.73%)	2 (4.55%)	p = 0.576
Cardiovascular	4 (6.9%)	0 (0%)	p = 0.132
Renal Function			
Baseline Creatinine (micromol/l)	77 (62.5 — 97)	103 (67.75 — 132.25)	p = 0.02
Baseline GFR (ml/min/1.73 m2)	77.45 (64.81 — 101.83)	75 (58.96 — 88.88)	p = 0.242
Maximum Creatinine (micromol/l)	104.5 (78.25 — 164.25)	148.5 (99.25 — 215.25)	p = 0.025
Minimum GFR (ml/min/1.73 m2)	49.61 (32.63 — 72.82)	36.57 (24.93 — 55.72)	p = 0.026
Time: ICU Admission to Peak POC Creatinine (D)	0.96 (0.5 — 1.75)	1.13 (0.44 — 1.81)	p = 0.981
Time :Study Admission to Peak POC Creatinine (D)	0.19 (-0.21 — 0.89)	0.24 (-0.21 — 1.13)	p = 0.927
AKI on Study Admission	29 (50%)	25 (56.82%)	p = 0.551

	ELISA-1:ELISA-2 > 50	ELISA-1:ELISA-2 < 50	Univariate Comparison
Biomarkers			
pNGAL	72.5 (60 — 121)	188.5 (68.5 — 426.75)	p < 0.001*
uNGAL (ng/ml)	50.55 (20.7 — 93.95)	311.3 (41.8 — 1011.1)	p < 0.001*
uNGAL _{E1} (ng/ml)	28.85 (12.93 — 60.08)	189.3 (21.2 — 650.25)	p = 0.001*
uNGAL _{E2} (ng/ml)	3.38 (1.74 — 9.03)	0.75 (0.15 — 2)	p < 0.001*
uNGAL _{E1} :uNGAL _{E2}	6.68 (4.34 — 14.56)	179.36 (87.82 — 915.22)	p < 0.001*
Outcomes			
Hospital Mortality	10 (17.25%)	5 (11.37%)	p = 0.574
ICU Mortality	8 (13.8%)	4 (9.1%)	p = 0.547
Length of ICU Stay (days)	2.74 (1.46 — 4.78)	3.36 (2.03 — 7.8)	p = 0.052
Length of Hospital Stay (days)	13.77 (7.23 — 24.16)	18.47 (9.21 — 44.14)	p = 0.094
RIFLE-R or more	39 (67.25%)	33 (75%)	p = 0.511
RIFLE-I or more	19 (32.76%)	20 (45.46%)	p = 0.221
RIFLE-F alone	3 (5.18%)	5 (11.37%)	p = 0.286
CRRT	3 (5.18%)	4 (9.1%)	p = 0.461

*All values presented as medians with interquartile range (IQR), or as absolute number with percentage in brackets. GFR: glomerular filtration rate; pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2; SIRS: systemic inflammatory response syndrome; AKI: acute kidney injury; CRRT: chronic renal replacement therapy. * indicates significance retained following Bonferroni correction for multiple univariate analyses.*

Figure 2: BLAND-ALTMAN PLOT COMPARING COMMERCIAL AND ELISA-1 URINARY NGAL
MEASUREMENT

**The Nature and Discriminatory Value of Urinary Neutrophil Gelatinase-Associated Lipocalin in
Critically Ill Patients at Risk of Acute Kidney Injury**

Online Data Supplement

Neil Glassford, Antoine Schneider, Shengyuan Xu, Glenn Eastwood, Helen Young, Leah Peck, Per
Venge, Rinaldo Bellomo

Online Data Supplement

Materials and Methods – Additional Information

The ELISA-1 and ELISA-2 assays used a similar technique. A 96-well microtitre plate was coated with a monoclonal antibody (Mab 763) against human HNL/NGAL. Samples and standards were incubated for 60min at room temperature. Subsequently, a biotinylated mouse monoclonal antibody (Mab 764 for ELISA-1 or 765 for ELISA-2) against human HNL/NGAL was added and incubated at room temperature for 60 min, followed by a diluted streptavidin conjugated horseradish peroxidase (30 min at room temperature). The enzyme reaction was visualized by using 3, 3', 5, 5'-tetramethylbenzidine solution as substrate at room temperature for 15 minutes and the reaction was stopped by adding 1M H₂SO₄. Absorbance was read at 450nm in a microplate reader.

TABLE E1: CORRELATION BETWEEN MEASURES OF NGAL

Spearman's Rank Correlation Between Measures of NGAL

	Rho	p
pNGAL:uNGAL, corrected	0.587	<0.001
pNGAL: uNGAL _{E1} , corrected	0.569	<0.001
pNGAL: uNGAL _{E2} , corrected	0.110	0.271
uNGAL:uNGAL _{E1} , corrected	0.988	<0.001
uNGAL:uNGAL _{E2} , corrected	0.406	<0.001
uNGAL _{E1} :uNGAL _{E2} , corrected	0.466	<0.001

pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2.

Figure E1: THE CORRELATION BETWEEN NGAL MEASURED BY COMMERCIAL ASSAY AND BY ELISA-1.

Figure E2: BLAND-ALTMAN PLOT COMPARING NATURAL LOGARITHMIC TRANSFORMATIONS OF
COMMERCIAL AND ELISA-1 URINARY NGAL MEASUREMENT

TABLE E2: Biochemical, demographic and clinical features of patients stratified by admission diagnosis of sepsis.

	Diagnosis of Sepsis		Univariate Comparison
	Yes	No	
N	21	81	
Age (years)	63.12 (41.58 — 71.63)	67.18 (53.89 — 75.02)	p = 0.213
Sex	9 (42.86%)	47 (58.03%)	p = 0.23
Apache 3 Score	66 (54 — 83)	63 (46 — 76)	p = 0.706
Diagnoses			
Stroke	0 (0%)	8 (9.88%)	p = 0.201
Cardiopulmonary bypass	0 (0%)	17 (20.99%)	p = 0.02
Malignancy	0 (0%)	8 (9.88%)	p = 0.201
Acute Liver Failure	0 (0%)	9 (11.12%)	p = 0.198
Mechanical ventilation	9 (42.86%)	66 (81.49%)	p = 0.001*
Admitted following			
Respiratory Arrest	0 (0%)	5 (6.18%)	p = 0.581
Cardiac Arrest	1 (4.77%)	7 (8.65%)	p = 1
Emergency/MET call	6 (28.58%)	21 (25.93%)	p = 0.787
Comorbidities			
Immunosuppressed	3 (14.29%)	6 (7.41%)	p = 0.386
Chronic Liver Disease	1 (4.77%)	10 (12.35%)	p = 0.452
Malignancy	1 (4.77%)	5 (6.18%)	p = 1
Respiratory	2 (9.53%)	1 (1.24%)	p = 0.107
Cardiovascular	0 (0%)	4 (4.94%)	p = 0.578
Renal Function			
Baseline Creatinine (micromol/l)	86 (69 — 117)	81 (64 — 112)	p = 0.554
Baseline GFR (ml/min/1.73 m2)	66.58 (56.18 — 99.55)	75 (64.69 — 97.08)	p = 0.223
Maximum Creatinine (micromol/l)	111 (78 — 175)	126 (89 — 200)	p = 0.471
Minimum GFR (ml/min/1.73 m2)	43.86 (33.13 — 64.62)	44.51 (26.66 — 65.77)	p = 0.617
Time from ICU Admission to Peak POC Creatinine (D)	1.35 (0.24 — 3.13)	1.04 (0.49 — 1.61)	p = 0.688
Time from Study Admission to Peak POC Creatinine (D)	0 (-0.4 — 2.28)	0.25 (-0.18 — 0.83)	p = 0.833
RIFLE-R or greater AKI on study admission	10 (47.62%)	44 (54.33%)	p = 0.63
RIFLE-I or greater AKI on study admission	7 (33.34%)	21 (25.93%)	p = 0.585
RIFLE-F or greater AKI on study admission	0 (0%)	4 (4.94%)	p = 0.578

	Diagnosis of Sepsis		Univariate Comparison
	Yes	No	
Biomarkers			
pNGAL	130 (60 — 327)	82 (60 — 238)	p = 0.428
uNGAL (ng/ml)	329.7 (90.7 — 868.2)	58.7 (22.2 — 138)	p = 0.008
uNGALE1 (ng/ml)	221 (66.2 — 598)	32.7 (14.2 — 96.2)	p = 0.01
uNGALE2 (ng/ml)	1.07 (0.7 — 2.49)	2.22 (0.67 — 4.92)	p = 0.253
uNGALE1:uNGALE2	125.81 (9.87 — 666.04)	15.93 (5.76 — 110)	p = 0.01
Outcomes			
Hospital Mortality	2 (9.53%)	13 (16.05%)	p = 0.73
ICU Mortality	1 (4.77%)	11 (13.59%)	p = 0.451
Length of ICU Stay (days)	3.98 (2.23 — 13.74)	2.71 (1.61 — 4.96)	p = 0.083
Length of Hospital Stay (days)	16.65 (8.24 — 50.22)	15.24 (8.01 — 29.19)	p = 0.335
RIFLE-R or more	12 (57.15%)	60 (74.08%)	p = 0.178
RIFLE-I or more	8 (38.1%)	31 (38.28%)	p = 1
RIFLE-F alone	0 (0%)	8 (9.88%)	p = 0.201
CRRT	1 (4.77%)	6 (7.41%)	p = 1

*All values presented as medians with interquartile range (IQR), or as absolute number with percentage in brackets. GFR: glomerular filtration rate; pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2; SIRS: systemic inflammatory response syndrome; AKI: acute kidney injury; CRRT: chronic renal replacement therapy. * indicates significance retained following Bonferroni correction for multiple univariate analyses.*

TABLE E3: Biochemical, demographic and clinical features of patients stratified by presence of AKI on admission.

	AKI Status on Study Admission		Univariate Comparison
	No	Yes	
N	48	54	
Age (years)	63.09 (46.36 — 72.95)	68.31 (58.67 — 75.43)	p = 0.09
Sex (Male)	20 (41.67%)	36 (66.67%)	p = 0.17
Apache 3 Score	20 (41.67%)	36 (66.67%)	p = 0.17
Diagnoses			
Sepsis	11 (22.92%)	10 (18.52%)	p = 0.63
Stroke	4 (8.34%)	4 (7.41%)	p = 1
Cardiopulmonary bypass	8 (16.67%)	9 (16.67%)	p = 1
Malignancy	4 (8.34%)	4 (7.41%)	p = 1
Acute Liver Failure	3 (6.25%)	6 (11.12%)	p = 0.495
Mechanical ventilation	34 (70.84%)	41 (75.93%)	p = 0.655
Admitted following			
Respiratory Arrest	2 (4.17%)	3 (5.56%)	p = 1
Cardiac Arrest	3 (6.25%)	5 (9.26%)	p = 0.719
Emergency/MET call	13 (27.09%)	14 (25.93%)	p = 1
Comorbidities			
Chronic Liver Disease	4 (8.34%)	7 (12.97%)	p = 0.534
Respiratory	2 (4.17%)	1 (1.86%)	p = 0.6
Cardiovascular	0 (0%)	4 (7.41%)	p = 0.12
Renal Function			
Baseline Creatinine (micromol/l)	74.5 (64 — 106.25)	96.5 (65.25 — 130)	p = 0.1
Baseline GFR (ml/min/1.73 m ²)	74.57 (57.49 — 100.2)	75 (66.08 — 95.95)	p = 0.279
Study Inclusion Creatinine (micromol/l)	72.5 (57.75 — 94.5)	150 (125.25 — 183.25)	p < 0.001*
Study Inclusion GFR (ml/min/1.73 m ²)	77.7 (54.95 — 103.74)	36.34 (29.72 — 49.48)	p < 0.001*
Maximum Creatinine (micromol/l)	95.5 (68.5 — 124.5)	174 (128.25 — 233)	p < 0.001*
Minimum GFR (ml/min/1.73 m ²)	62.35 (43.33 — 82.29)	31.68 (23.02 — 46.78)	p < 0.001*
Time from ICU Admission to Peak POC Creatinine (D)	0.93 (0.35 — 1.87)	1.19 (0.53 — 1.61)	p = 0.663
Time from Study Admission to Peak POC Creatinine (D)	0.24 (-0.48 — 1.28)	0.18 (-0.15 — 0.83)	p = 0.758

	AKI Status on Study Admission		Univariate Comparison
	Yes	No	
Biomarkers			
pNGAL	64 (60 — 124)	143.5 (65.5 — 408.5)	p < 0.001 [*]
uNGAL (ng/ml)	51.55 (17.33 — 183.58)	88.95 (28.98 — 320.5)	p = 0.135
uNGAL _{E1} (ng/ml)	31.5 (11.35 — 122)	56.9 (19.85 — 210.73)	p = 0.149
uNGAL _{E2} (ng/ml)	1.82 (0.76 — 4.5)	2.24 (0.73 — 4.96)	p = 0.646
uNGAL _{E1} :uNGAL _{E2}	15.53 (5.77 — 94.16)	22.4 (7.01 — 140.64)	p = 0.433
Outcomes			
Died in Hospital	6 (12.5%)	9 (16.67%)	p = 0.589
Died in ICU	4 (8.34%)	8 (14.82%)	p = 0.369
Length of ICU Stay (days)	2.22 (1.52 — 4.84)	3.12 (2.03 — 6.71)	p = 0.086
Length of Hospital Stay (days)	14.47 (8.17 — 31.19)	16.14 (8.41 — 28.55)	p = 0.995
RIFLE-R or more	19 (39.59%)	53 (98.15%)	p < 0.001 [*]
RIFLE-I or more	3 (6.25%)	36 (66.67%)	p < 0.001 [*]
RIFLE-F alone	1 (2.09%)	7 (12.97%)	p = 0.063
CRRT	1 (2.09%)	6 (11.12%)	p = 0.117

*All values presented as medians with interquartile range (IQR), or as absolute number with percentage in brackets. GFR: glomerular filtration rate; pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2; SIRS: systemic inflammatory response syndrome; AKI: acute kidney injury; CRRT: chronic renal replacement therapy. * indicates significance retained following Bonferroni correction for multiple univariate analyses.*

TABLE E4: Biochemical, demographic and clinical features of patients stratified by early or late study inclusion.

	Inclusion Time Group		Univariate Comparison
	Early	Late	
N	51	51	
Age (years)	64.85 (54.8 — 73.43)	67.31 (48.12 — 75.29)	p = 0.939
Sex (Male)	29 (56.87%)	27 (52.95%)	p = 0.842
Apache 3 Score	64 (48 — 77)	63 (46 — 80.5)	p = 0.92
Diagnoses			
Sepsis	10 (19.61%)	11 (21.57%)	p = 1
Stroke	5 (9.81%)	3 (5.89%)	p = 0.715
Cardiopulmonary bypass	9 (17.65%)	8 (15.69%)	p = 1
Malignancy	5 (9.81%)	3 (5.89%)	p = 0.715
Acute Liver Failure	4 (7.85%)	5 (9.81%)	p = 1
Mechanical ventilation	35 (68.63%)	40 (78.44%)	p = 0.37
Admitted following			
Respiratory Arrest	4 (7.85%)	1 (1.97%)	p = 0.362
Cardiac Arrest	5 (9.81%)	3 (5.89%)	p = 0.715
Emergency/MET call	13 (25.5%)	14 (27.46%)	p = 1
Comorbidities			
Immunosuppressed	5 (9.81%)	4 (7.85%)	p = 1
Chronic Liver Disease	3 (5.89%)	8 (15.69%)	p = 0.2
Malignancy	5 (9.81%)	1 (1.97%)	p = 0.205
Respiratory	1 (1.97%)	2 (3.93%)	p = 1
Cardiovascular	2 (3.93%)	2 (3.93%)	p = 1
Renal Function			
Baseline Creatinine (micromol/l)	85 (66.5 — 112)	78 (64 — 109.5)	p = 0.392
Baseline GFR (ml/min/1.73 m2)	75 (57.98 — 91.65)	75 (64.41 — 101.02)	p = 0.351
Maximum Creatinine (micromol/l)	126 (97 — 191.5)	126 (75.5 — 189.5)	p = 0.278
Minimum GFR (ml/min/1.73 m2)	44.51 (28 — 62.2)	43.86 (26.92 — 74.13)	p = 0.357
Time: ICU Admission to Study Inclusion	0.49 (0.4 — 0.58)	1.28 (0.85 — 1.61)	p < 0.001*
Time: ICU Admission to Peak POC Creatinine (D)	0.72 (0.39 — 1.34)	1.33 (0.79 — 2.69)	p = 0.003
Time: Study Admission to Peak POC Creatinine (D)	0.34 (-0.04 — 0.81)	0 (-0.48 — 1.62)	p = 0.312
AKI on Study Admission	27 (52.95%)	27 (52.95%)	p = 1

	Inclusion Time Group		Univariate Comparison
	Early	Late	
Biomarkers			
pNGAL	108 (63 — 302)	79 (60 — 215.5)	p = 0.115
uNGAL (ng/ml)	76.2 (26.9 — 593.3)	75.3 (18.9 — 158.8)	p = 0.293
uNGAL _{E1} (ng/ml)	49.2 (17.7 — 396)	33.9 (11.75 — 155.5)	p = 0.234
uNGAL _{E2} (ng/ml)	2.29 (0.9 — 4.9)	1.46 (0.53 — 4.06)	p = 0.157
uNGAL _{E1} :uNGAL _{E2}	18.38 (5.55 — 125.84)	19.21 (6.86 — 148.44)	p = 0.751
Outcomes			
Hospital Mortality	8 (15.69%)	7 (13.73%)	p = 1
ICU Mortality	7 (13.73%)	5 (9.81%)	p = 0.76
Length of ICU Stay (days)	2.23 (1.41 — 4.18)	3.21 (2.03 — 7.36)	p = 0.006
Length of Hospital Stay (days)	11.46 (7.27 — 22.65)	22.06 (11.79 — 36.97)	p = 0.016
RIFLE-R or more	36 (70.59%)	36 (70.59%)	p = 1
RIFLE-I or more	18 (35.3%)	21 (41.18%)	p = 0.684
RIFLE-F alone	4 (7.85%)	4 (7.85%)	p = 1
CRRT	3 (5.89%)	4 (7.85%)	p = 1

*All values presented as medians with interquartile range (IQR), or as absolute number with percentage in brackets. GFR: glomerular filtration rate; pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2; SIRS: systemic inflammatory response syndrome; AKI: acute kidney injury; CRRT: chronic renal replacement therapy. The subjects were split into early, if time from ICU admission to study inclusion was less than the median value (0.69 days), and late, greater. * indicates significance retained following Bonferroni correction for multiple univariate analyses.*

TABLE E5: VALUE OF NGAL MEASUREMENT IN PREDICTING AKI, NEED FOR CRRT AND MORTALITY IN PATIENTS WITHOUT SEPSIS

	Area Under the Receiver Operator Curve (95% CI)				
	Any AKI	RIFLE - F	CRRT	Death in ICU	Death in Hospital
pNGAL	0.658 (0.532 — 0.785)	0.843 (0.66 — 1)	0.75 (0.526 — 0.974)	0.468 (0.275 — 0.661)	0.451 (0.276 — 0.626)
uNGAL*	0.552 (0.401 — 0.704)	0.795 (0.586 — 1)	0.716 (0.467 — 0.964)	0.374 (0.224 — 0.524)	0.364 (0.226 — 0.502)
uNGAL_{E1}*	0.551 (0.399 — 0.703)	0.789 (0.582 — 0.997)	0.702 (0.455 — 0.949)	0.384 (0.234 — 0.535)	0.369 (0.231 — 0.507)
uNGAL_{E2}*	0.488 (0.342 — 0.635)	0.577 (0.352 — 0.803)	0.547 (0.314 — 0.779)	0.464 (0.301 — 0.626)	0.43 (0.282 — 0.578)
pNGAL:uNGAL*	0.53 (0.38 — 0.681)	0.298 (0.088 — 0.508)	0.35 (0.121 — 0.579)	0.674 (0.509 — 0.839)	0.67 (0.517 — 0.823)
pNGAL:uNGAL_{E1}*	0.527 (0.378 — 0.676)	0.297 (0.092 — 0.503)	0.38 (0.132 — 0.628)	0.675 (0.506 — 0.843)	0.678 (0.522 — 0.834)
pNGAL:uNGAL_{E2}*	0.571 (0.425 — 0.716)	0.538 (0.305 — 0.77)	0.6 (0.362 — 0.838)	0.548 (0.377 — 0.719)	0.558 (0.404 — 0.712)
uNGAL:uNGAL_{E1}	0.472 (0.316 — 0.629)	0.411 (0.223 — 0.599)	0.583 (0.307 — 0.86)	0.481 (0.331 — 0.632)	0.535 (0.389 — 0.68)
uNGAL:uNGAL_{E2}	0.542 (0.401 — 0.683)	0.613 (0.375 — 0.851)	0.598 (0.396 — 0.799)	0.483 (0.311 — 0.655)	0.497 (0.343 — 0.65)
uNGAL_{E1}:uNGAL_{E2}	0.536 (0.397 — 0.675)	0.63 (0.402 — 0.858)	0.607 (0.422 — 0.792)	0.477 (0.301 — 0.654)	0.493 (0.336 — 0.65)

*pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E2}: NGAL as detected by ELISA-2; AKI: Acute Kidney Injury; CRRT: Continuous Renal Replacement Therapy. *Urinary measures are corrected by urinary creatinine excretion.*

TABLE E6: VALUE OF NGAL MEASUREMENT IN PREDICTING AKI, AND MORTALITY IN PATIENTS WITHOUT AKI ON STUDY RECRUITMENT

	Area Under the Receiver Operator Curve (95% CI)		
	Any AKI	Death in ICU	Death in Hospital
pNGAL	0.431 (0.266 — 0.596)	0.369 (0.074 — 0.665)	0.488 (0.243 — 0.733)
uNGAL*	0.414 (0.25 — 0.578)	0.369 (0.095 — 0.643)	0.433 (0.2 — 0.665)
uNGALE1*	0.412 (0.249 — 0.575)	0.381 (0.11 — 0.652)	0.429 (0.195 — 0.662)
uNGALE2*	0.406 (0.242 — 0.57)	0.557 (0.313 — 0.801)	0.508 (0.293 — 0.723)
pNGAL:uNGAL*	0.592 (0.43 — 0.754)	0.597 (0.351 — 0.842)	0.571 (0.338 — 0.805)
pNGAL:uNGALE1 *	0.58 (0.416 — 0.744)	0.58 (0.337 — 0.822)	0.575 (0.335 — 0.816)
pNGAL:uNGALE2*	0.563 (0.396 — 0.73)	0.42 (0.159 — 0.682)	0.492 (0.275 — 0.709)
uNGAL:uNGALE1	0.406 (0.242 — 0.569)	0.344 (0.181 — 0.506)	0.423 (0.238 — 0.607)
uNGAL:uNGALE2	0.477 (0.308 — 0.647)	0.392 (0.132 — 0.652)	0.476 (0.263 — 0.69)
uNGALE1:uNGALE2	0.483 (0.312 — 0.653)	0.403 (0.131 — 0.675)	0.488 (0.265 — 0.711)

*pNGAL: commercial plasma/serum NGAL; uNGAL: commercial urinary NGAL; uNGAL_{E1}: NGAL as detected by ELISA-1; uNGAL_{E1}: NGAL as detected by ELISA-2; AKI: Acute Kidney Injury; CRRT: Continuous Renal Replacement Therapy.
Urinary measures are corrected by urinary creatinine excretion.