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Performance of plasma measurement of neutrophil gelatinase-associated lipocalin as a biomarker of bacterial infections in the intensive care unit

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ABSTRACT

Purpose: To assess the value of dimeric neutrophil-gelatinase associated lipocalin (NGAL) as an early marker of bacterial infection and its response to antibiotic therapy in intensive care unit (ICU) patients.
Materials & methods: We measured daily plasma dNGAL in 198 patients admitted to a mixed ICU. Likelihood of infection was determined with International Sepsis Forum criteria. We measured dNGAL in 145 healthy controls to establish normal values.
Results: ICU patients had higher dNGAL than healthy controls. A suspected or confirmed infection was independently associated with 90% (95% CI 15–215%) higher dNGAL than absence of infection. We observed no association between acute kidney injury and dNGAL. Diagnostic accuracy at antibiotic treatment initiation, assessed with area under the receiver-operating characteristics curve (AUC-ROC), for dNGAL was 0.70 (95% CI 0.60–0.79). AUC-ROC for dNGAL 24 h before antibiotic treatment initiation was 0.54 (95% CI 0.41–0.66). The mean (95% CI) change of dNGAL in the first 2 days after appropriate antibiotic therapy initiation was −31 (−49,−13)%.
Conclusions: In our cohort of ICU patients, plasma dNGAL was associated with presence of bacterial infections independent of AKI but it performed poor as a predictor of infections. Following antibiotic therapy, dNGAL markedly decreased–supporting further exploration of dNGAL-guided antibiotic de-escalation.

1. Introduction

Early and accurate detection of infections in intensive care unit (ICU) patients is important but clinically challenging. In such patients, delayed initiation of appropriate empirical antibiotic therapy is associated with worse outcomes [1,2]. In addition, both unnecessary treatment of non-infected patients and excessively prolonged therapy in patients with established infection contributes to antimicrobial resistance [3], increased consumption of health care resources and greater mortality [4].

The use of biomarkers to guide antibiotic therapy initiation and de-escalation has been suggested but data on their clinical benefits are conflicting. Routine biomarkers such as procalcitonin (PCT), C-reactive protein (CRP) and white blood cell count (WBC) demonstrate limited ability to distinguish bacterial infections from other causes of systemic inflammation in the ICU setting [5,6]. Based on data from randomized trials, recent guidelines suggest that PCT can be safely used to guide antibiotic de-escalation [7]. This recommendation is, however, weak and some studies have failed to demonstrate any benefit [8,9].

Neutrophil-gelatinase associated lipocalin (NGAL) also called Human neutrophil lipocalin (HNL) or Lipocalin 2, a protein with bacteriostatic properties, is released from activated neutrophils in response to bacterial infections. Previous data suggest that NGAL outperforms CRP and PCT as a marker of bacterial infections in ICU patients [10]. However, NGAL is also upregulated and released by the kidney and other non-hematopoietic epithelial cells during systemic stress and plasma levels are markedly increased in patients with acute kidney injury (AKI) [11]. In contrast to epithelial cells, which only release the monomeric and heteromeric molecular forms of NGAL (25 and > 90 kDa), dimeric NGAL (45 kDa) appears unique to neutrophils [12].

We recently developed an assay that specifically measures dimeric NGAL [12]. However, the value of plasma dimeric NGAL as a marker of bacterial infections in the ICU has never been assessed. Furthermore, whether the presence of AKI modifies the potential relationship between bacterial infection and dimeric NGAL levels remains unknown.

Accordingly, we aimed to study the association of bacterial infections and AKI with plasma dimeric NGAL levels in ICU patients. In

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addition, we aimed to assess the value of dimeric NGAL as an early marker of bacterial infections and its response to antibiotic therapy in such patients. We hypothesized that the association between bacterial infections and dimeric NGAL would not be affected by the presence of AKI. Furthermore, we hypothesized that dimeric NGAL would be superior to total NGAL, PCT, CRP and WBC as an early marker of bacterial infection, similar to what was shown by the measurement of NGAL in serum [13-15] or after activation of whole blood [16,17]. Finally, we hypothesized that the concentration of dimeric NGAL in plasma would significantly decrease in response to successful antibiotic therapy.

2. Material and methods

The regional ethical review boards in Stockholm and Uppsala approved this study. Written informed consent was obtained from patients or next of kin.

2.1. Selection of patients and control subjects

We assessed patients admitted to the mixed medical/surgical ICU at Karolinska University Hospital Solna, Sweden for eligibility between August 2007 and November 2014. We included 198 patients without acute or chronic kidney disease (estimated glomerular filtration rate [eGFR] >60 mL/min/1.73 m^2) on ICU admission and an expected length of stay >24 h. During the same period, approximately 7000 patients were treated in the ICU. The relatively low number of patients included in this study is partly explained by the restrictive inclusion criteria. In addition, we were unable to include patients on weekends, nights/on-call hours and during holidays. Furthermore, conflict with other active studies prevented consecutive recruitment. The original purpose of collecting data from these patients was to assess the predictive values of AKI biomarkers. The present study was a secondary analysis of the prospectively collected database.

We also included a control population consisting of 145 healthy, non-infected volunteers in order to establish assay specific normal reference values for NGAL in plasma.

2.2. Operational definitions

An infectious disease specialist blinded to the NGAL results classified patients as having no infection (no infection-group) or a probable infection, possible infection or confirmed infection (infection-group) according to the international sepsis forum (ISF) criteria (Table 2) [18]. In infection group-patients, onset of infection was the time when the clinician prescribed antibiotic therapy.

We further classified the infection group-patients as having sepsis, severe sepsis or septic shock according to the modified "Sepsis−2" criteria using three or more systemic inflammatory response syndrome (SIRS) criteria (instead of two or more) as suggested by the PROWESS study group [19-21].

Empiric antibiotic therapy was defined as appropriate if isolated pathogens were susceptible, according to the susceptibility testing report, to the antibiotic therapy administered.

We defined AKI according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria [22]. The lowest creatinine level found within three months prior to ICU admission was used as baseline. When no preadmission creatinine value was available, baseline creatinine was imputed based on the modification of diet in renal disease (MDRD) equation and a GFR of 75 mL/min/1.73 m^2.

2.3. Plasma sampling and biochemical analyses

We collected EDTA-blood as soon as possible after arrival in the ICU and daily thereafter until ICU discharge or initiation of renal replacement therapy (RRT). After centrifugation at 2000 rpm at 4 °C for 10 min, the supernatant plasma was stored at −80 °C. Study samples were analyzed at the Department of Clinical Chemistry, Uppsala University Hospital, Uppsala, Sweden. Laboratory personnel were blinded to clinical patient data.

We quantified dimeric and total NGAL concentrations using two different enzyme-linked immunosorbent assays (ELISAs). When two daily blood tests were available we used the mean value. The antibody configurations of the ELISAs were as follows: in both assays the microtiter plates were coated with the monoclonal antibody clone 763 (Diagnostics Development, Uppsala, Sweden). In the total NGAL assay the detecting antibody was the monoclonal antibody clone 764 (Diagnostics Development, Uppsala, Sweden) and in the dimeric NGAL assay the detecting antibody was the monoclonal antibody clone 765 (Diagnostics Development, Uppsala, Sweden). The expected normal dimeric and total NGAL levels were defined as less than the upper 97.5th percentile in the healthy population.

PCT, CRP and WBC were quantified daily as part of routine care. PCT was analyzed using an ELISA (CV 6% at 0.25 ng/mL and 3% at 10.4 ng/mL) on the Cobas EE (Roche Diagnostics, Mannheim, Germany). The expected normal PCT level was <0.05 ng/mL. CRP was analyzed by turbidimetry (CV 4%) on the Architect Ci8200 (Abbott Laboratories, Abbott Park, IL). The expected normal CRP level was <5 mg/L.

2.4. Statistical analysis

We analyzed data using STATA® version 13.0 (Stata Corp., College Station, TX, USA). Continuous variables were summarized by median and interquartile range (IQR) and categorical variables as n (%). The Kruskal-Wallis test (for >2 groups) or Mann–Whitney *U* test (for two groups) were used for comparison between continuous variables. The Fisher's exact test or χ^2 test was used for comparison between categorical variables.

As biomarker levels were found to be well approximated by a log-normal distribution, they were log-transformed before analysis. We used generalized linear mixed modeling with each patient treated as a random effect to assess changes in biomarker concentrations over time. The interaction between group and time was introduced in the mixed model to compare the change over time between groups. Results were graphically presented as geometric means with 95% confidence interval (CI).

We used the following approach to compare biomarker levels obtained from the infection-group patients at onset of infection (day 0): firstly, we calculated the median ICU day for antibiotic therapy initiation; secondly, we identified all biomarker levels obtained on the corresponding ICU day in the no infection-group patients; finally, we compared biomarker levels obtained on day 0 (infection-group) with biomarker levels from the corresponding ICU day in no infection-group patients. We adopted the same approach to compare biomarker levels on the day before onset of infection (day −1). The relationship between infection status, AKI and log-transformed biomarkers levels on day 0 was assessed by multivariable linear regression analysis adjusting for male sex and acute physiology and chronic health evaluation (APACHE) II score. The regression coefficients were expressed as $100 \times (e^{coeff} - 1)$ and represent the geometric mean percent change in biomarker concentration associated with a one-unit change in the variable.

We assessed predictive and diagnostic accuracy by calculating the area under the receiver-operating characteristics curve (AUC-ROC). A two-sided *p*-value below 0.05 was considered statistically significant.

3. Results

3.1. ICU patients

We included 198 ICU patients (69.2% males). Of these, 144 (72.7%) had or developed a possible, probable or confirmed infection

(infection-group) and 54 (27.3%) had no infection (no infection-group). Compared to no infection-group patients, infection-group patients were older, had higher APACHE II score and were more likely to have AKI on admission. The first study sample was obtained after a median (IQR) of 11 (5.5, 22) h since admission to ICU in the infection group and 9 (4.3, 19) h in the no infection-group ($p = .19$). Asthma, chronic obstructive pulmonary disease and malignancy were more common in the infection-group.

Approximately one-third of the infection-group patients and two-thirds of the no infection-group patients were admitted following multi-trauma (Table 1 and Supplementary table S1). Infection-group patients stayed longer in the ICU, had more severe AKI, and were more likely to receive RRT. By 30 days, 20 (14%) patients in the infection group and 4 (7%) patients in the no infection-group had died ($P = .21$) (Table 1).

3.2. Infection characteristics

Of the 144 infection group-patients, 29 (20.1%) were commenced on antibiotics before ICU admission and 115 (79.9%) had antibiotic therapy initiated after a median of 2 (0–3) days after admission to the ICU. According to the ISF criteria, 93 (64.6%) patients had a confirmed infection, 30 (20.8%) had a probable infection and 21 (14.6%) had a possible infection. Pneumonia was the primary source in 78 (54.2%) patients. *Staphylococcus aureus* was the most common pathogen with 24 (16.7%) confirmed cases (Supplementary table S2). Overall, 41 (28.5%) and 82 (56.9%) patients developed severe sepsis and septic shock, respectively (Table 2).

Table 1
Patient characteristics and clinical outcomes of intensive care unit patients.

Characteristic	Infection-group $n = 144$	No infection-group $n = 54$	*P* Value
Age, y	55 (36, 66)	38 (26, 64)	0.01
Male sex, n (%)	101 (70)	36 (67)	0.64
APACHE II score	17 (14, 23)	13 (9, 19)	0.001
Baseline creatinine, μmol/L	79 (59, 90)	77 (67, 88)	0.43
AKI baseline, n (%)	34 (24)	6 (11)	0.05
Time from ICU admission to enrolment, h	11 (5.5, 22)	9 (4.3, 19)	0.19
Comorbidity, n (%)			
Cardiovascular disease	41 (28)	14 (26)	0.72
Diabetes	14 (10)	4 (7)	0.61
GI/Liver disease	16 (11)	2 (4)	0.11
Any malignancy	31 (22)	3 (6)	0.008
COPD/Asthma	24 (17)	2 (4)	0.016
Other disease	26 (18)	7 (13)	0.39
Admission diagnosis, n (%)			0.40
Neurologic	6 (4)	3 (6)	
Respiratory	40 (28)	4 (7)	
Cardiovascular	5 (3)	5 (9)	
Trauma	48 (33)	39 (72)	
Gastrointestinal	15 (10)	3 (6)	
Sepsis	30 (21)	0 (0)	
Clinical outcomes			
ICU length of stay, days	7 (4.5, 12)	3 (2.8, 5)	<0.0001
Mortality ICU n (%)	6 (4)	3 (6)	0.68
Mortality 30 days n (%)	20 (14)	4 (7)	0.21
Renal replacement therapy	8 (6)	0	0.08
Worst AKI score (KDIGO)			0.004
No acute kidney injury	77 (53)	43 (80)	
Stage 1	34 (24)	1 (2)	
Stage 2	21 (15)	3 (6)	
Stage 3	12 (8)	1 (2)	

Median and interquartile range (IQR) or natural number (n) and %. APACHE II: Acute Physiology And Chronic Health Evaluation II. ICU: Intensive Care Unit. COPD: Chronic Pulmonary Obstructive Disease. AKI: Acute Kidney Injury. KDIGO: Kidney Disease Improving Global Outcomes.

Table 2
Likelihood, primary site, severity of infection, microbiological findings and sepsis severity, n (%).

Variable	Infection-group (n = 144)
Antibiotic therapy commenced in the ICU, n (%)	115 (79.9)
Time from ICU admission to antibiotic therapy, days	2
Likelihood of infection according to ISF, n (%)	
Confirmed infection	93 (64.6)
Probable infection	30 (20.8)
Possible infection	21 (14.6)
Primary site of infection, n (%)	
Pneumonia	78 (54.2)
Catheter-related infection	2 (1.4)
Peritonitis	11 (7.6)
Skin and soft-tissue infection	26 (18.1)
Intraabdominal abscess	2 (1.4)
Endocarditis	2 (1.4)
Primary bloodstream infection	1 (0.7)
Meningitis	2 (1.4)
Urosepsis	3 (2.1)
Epiglottitis	1 (0.7)
Unknown (ISF not applicable)	16 (11.0)
Microbiological findings, n (%)	
Positive blood culture	25 (17.4)
Positive PBS or BAL	50 (34.7)
Positive urine	17 (11.8)
Positive CSF/tissue/abscess/endotracheal	82 (56.9)
Any positive culture	107 (74.3)
Primary pathogen gram stain positive	59 (41)
Primary pathogen gram stain negative	46 (32)
Worst SIRS/sepsis severity, n (%)	
No SIRS	6 (4.2)
SIRS	7 (4.9)
Sepsis	8 (5.6)
Severe sepsis	41 (28.5)
Septic shock	82 (56.9)

ICU: intensive care unit, ISF: international sepsis forum, PBS: Protected brush specimen, BAL: Bronchoalveolar lavage, CSF: Cerebrospinal fluid.

3.3. Control subjects

The 145 healthy controls consisted of 88 females and 57 males with a median (IQR) age of 43 (32, 55) years. The median dimeric NGAL value in plasma was 3.62 μg/L and the median total NGAL was 34.5 μg/L. The upper normal values (97.5th percentile) were 8.3 μg/L and 56 μg/L, respectively.

3.4. Biomarker levels

Compared to healthy controls, ICU patients had significantly higher peak dimeric and total NGAL plasma levels (Fig. 1). Additionally, infection-group patients had significantly higher peak dimeric NGAL, total NGAL, CRP and PCT levels than no infection-group patients whereas WBC levels were similar (Supplementary table S3). Higher likelihood (no infection/possible infection/probable infection/confirmed infection) of infection and worse sepsis severity were associated with higher dimeric and total NGAL, CRP and PCT levels (Supplementary figs. S2, S3). WBC showed no such association with likelihood of infection.

Biomarker kinetics relative to the day when antibiotic treatment was initiated (day 0) is shown in Fig. 2. For no infection group-patients, biomarker levels obtained on ICU day 3 were chosen to represent day 0.

On day −1, we observed similar dimeric NGAL, PCT and WBC levels in the two groups. Conversely, total NGAL and CRP levels were greater in infection group-patients and remained higher during the subsequent five days in ICU.

In no infection-group patients, we observed an early decline in dimeric NGAL and PCT. In infection-group patients, dimeric NGAL and PCT increased until or beyond antibiotic therapy initiation and markedly decreased thereafter.

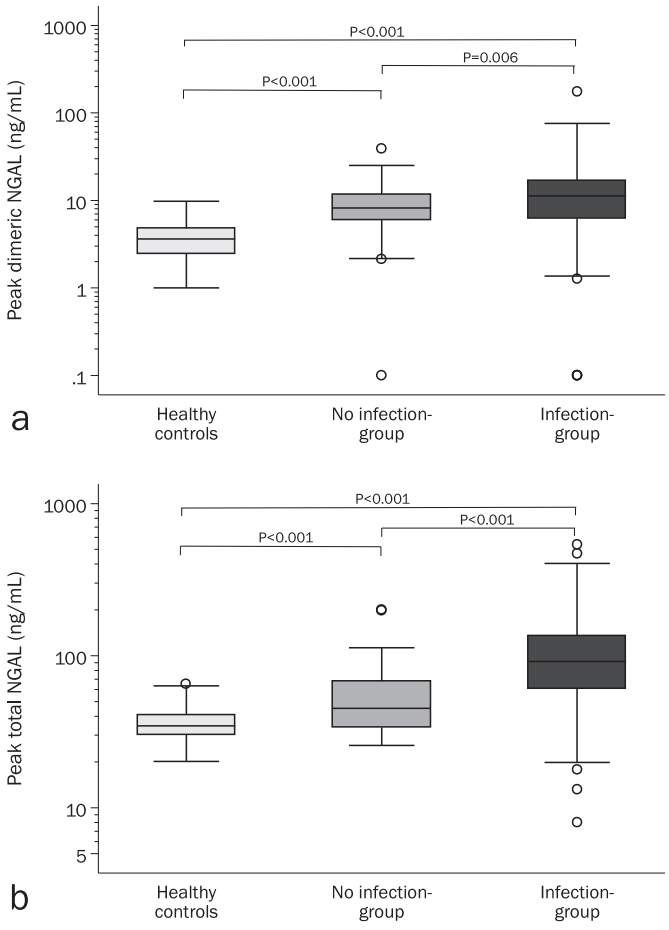

Fig. 1. Peak levels of dimeric NGAL (a) and total NGAL (b) in infection group-patients and no infection-group patients and in healthy controls.

3.5. Association of infection and acute kidney injury with biomarker levels

On separate multivariable linear regression analyses, the presence of a suspected or confirmed infection was independently associated with 90% (95% CI 15–215%) higher dimeric NGAL; 51% (95% CI 21–90%) higher total NGAL, and a 106% (95% CI 61–164%) higher CRP level, respectively. However, we found no independent association between infection status and PCT or WBC levels on day 0. The presence of AKI was independently associated with a 35% (95% CI 0.6–81%) higher total NGAL level but not with levels of dimeric NGAL, PCT, CRP or WBC (Table 3).

3.6. Diagnostic and predictive accuracy as markers of bacterial infection

Diagnostic accuracy on day 0 was fair with plasma levels of dimeric NGAL (AUC-ROC 0.70, 95% CI 0.60–0.79), total NGAL (AUC-ROC 0.77, 95% CI 0.69–0.86) or CRP (AUC-ROC 0.74, 95% CI 0.64–0.83) and poor with PCT and WBC. Overall, diagnostic accuracies, using the percentage change in biomarker level on day 0 relative day −1, were poor.

We observed a pattern towards greater predictive accuracy with CRP compared with the other biomarkers (Table 4).

3.7. Change in biomarker levels after antibiotic therapy initiation

We identified 31 patients with a confirmed infection who also had biomarkers available from the day of onset and the following two days. Only two of those received inadequate antibiotic therapy and were excluded from the comparative analysis. The mean (95% CI) percent change in the first 2 days after initiation of antibiotic therapy in the ICU was −31 (−49 to −13) % for dimeric NGAL in plasma, −6 (+ 2 to −14) % for total NGAL, −4 (+ 14 to −21) % for PCT, and 26 (+ 9 to +42) % for CRP (Fig. 3). A subgroup of 19 patients with possible or probable infection were also analyzed in a similar fashion, showing a significant decrease in dimeric NGAL and PCT levels in the first 2 days following empiric antibiotic treatment (Supplementary fig. S4). Furthermore, we identified 14 patients in the no infection-group who also had biomarkers available on ICU day 3 and the following two days. Analysis of biomarker levels in this subgroup of non-infected patients shows that dimeric NGAL did not decrease significantly from ICU day 3 and the following two days ($P = .07$). Howver, PCT decreased significantly also in non-infected patients from ICU day 3 and the following two days (Supplementary fig. S5).

4. Discussion

4.1. Key findings

We conducted a prospective, observational study to assess the association between dimeric NGAL, a protein released from activated neutrophils, and infection disease development and resolution in ICU patients. We found that our patients had greater plasma dimeric NGAL levels than healthy controls. Furthermore, we found that the presence of a possible, probable or confirmed infection, but not the presence of AKI, was independently associated with greater dimeric NGAL levels. However, dimeric NGAL did not outperform total NGAL, PCT, CRP or WBC as an early marker of infection. In contrast, following appropriate antibiotic therapy initiation, dimeric NGAL plasma levels decreased more rapidly than any of the other biomarkers.

4.2. Relationship with previous studies

This is the first study to explore the predictive and diagnostic value of dimeric NGAL in plasma for bacterial infection and its day-to-day trajectory in ICU patients with and without antibiotic therapy. It is also the first study to compare such values with total NGAL in plasma, CRP, PCT and WBC. However, a recent study assessed the performance of dimeric NGAL in serum to differentiate between bacterial and viral infections in non-critically ill adults and found dimeric NGAL superior to CRP and blood neutrophil count [15]. Another study of non-critically ill adults assessed the performance of total NGAL in activated whole blood to differentiate between bacterial and viral infections and found total NGAL superior to CRP and PCT [17].

In the present study, we found peak dimeric NGAL and peak total NGAL levels on the day of antibiotic therapy initiation. Similar kinetics, with peak NGAL values on the day of antibiotic treatment initiation, was found in a previous study of total NGAL in children (median age 26 months, range one month to 13 years) with suspected bacterial infections [14].

We found that both sepsis and AKI were associated with greater total plasma NGAL levels. This is in accordance with a large body of evidence showing that ongoing inflammation or sepsis affects the performance of total plasma NGAL as a marker of AKI [23-25]. In contrast to total NGAL, we showed that the association between dimeric NGAL and infection was not modified by the presence of AKI. This supports that our dimer-specific assay mainly detects NGAL released from neutrophils and to a lesser extent monomeric NGAL released from kidney epithelial cells.

In the present study, PCT and CRP levels peaked 24 and 48 h, respectively, after adequate antibiotic therapy initiation. These findings are consistent with a number of studies of PCT and CRP kinetics after empiric antibiotic therapy [26]. However, differences in timing of infection onset (before vs. during ICU admission) in relation to antibiotic

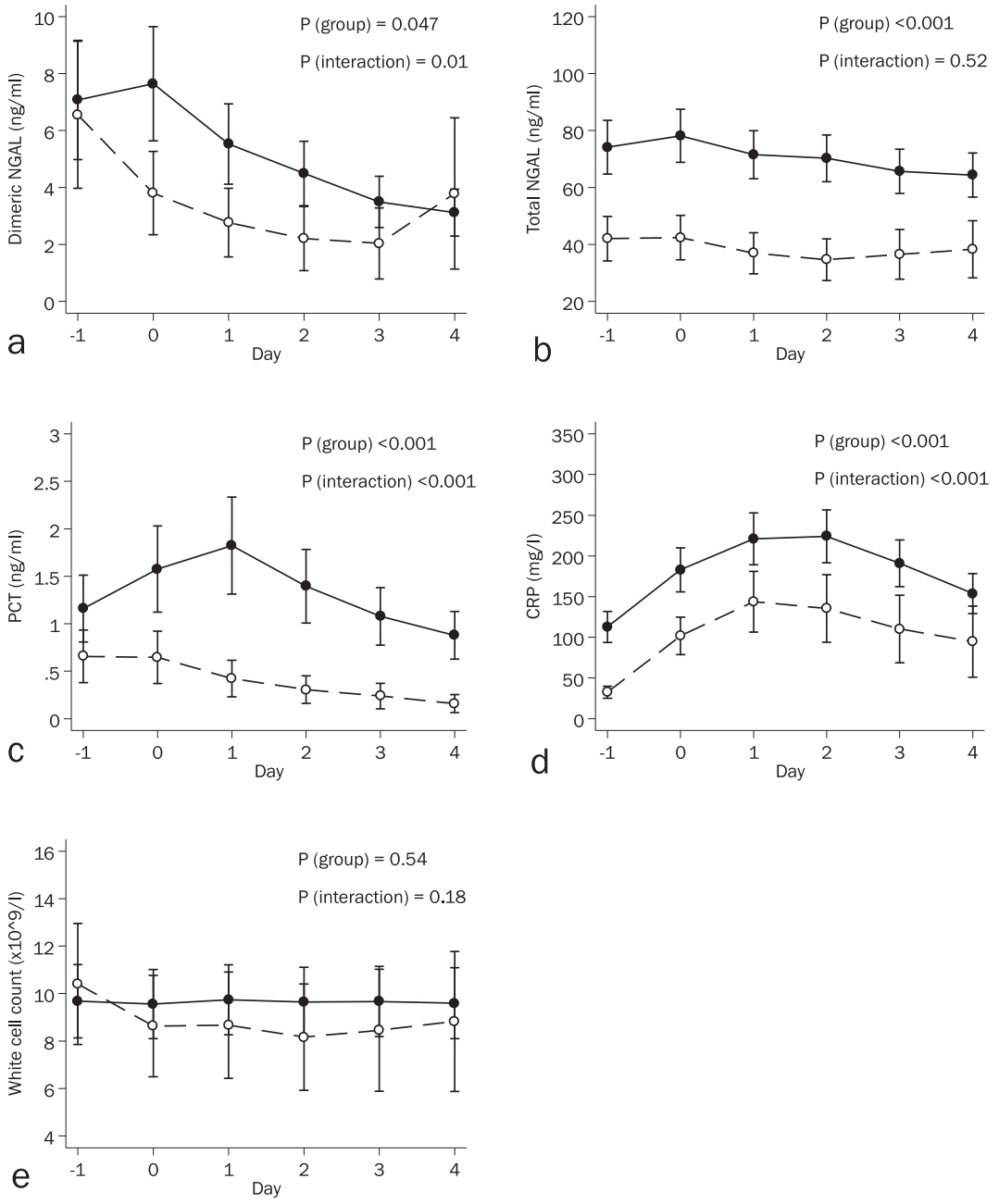

Fig. 2. Mean (95% CI) biomarker levels at different timepoints in infection group-patients (solid circles) and no infection group-patients (hollow circles). Day 0 represents the time when antibiotic therapy was initiated. For no infection group-patients, day 0 represents the third ICU day. The *P* values are derived from the repeated-measures generalized linear mixed model.

initiation and different definitions of appropriate antibiotic therapy restrict the ability to directly compare studies.

PCT guidance has been found to reduce duration of antibiotic therapy in the ICU in several randomized controlled trials. However, results are conflicting regarding clinical impact [8,27-29]. The evidence from the present study suggests that dimeric NGAL may be an alternative to PCT as a tool to assess the response to antibiotic therapy, due to its shorter time-to-peak values and faster descent after antibiotic therapy initiation. This idea is supported by our observation that PCT but not dimeric NGAL rapidly decreased even in non-infected patients. However, our analysis is exploratory and only included a subset of patients with available biomarker data and should there be viewed with caution. The possible utility of dimeric NGAL to guide antibiotic therapy de-escalation should therefore be assessed in future studies.

4.3. Study implications

Our findings imply that neutrophil activation and release of dimeric NGAL is present in critically ill patients and that such activation and release is further accelerated when a bacterial infection is evolving. Moreover, they imply that dimeric plasma NGAL is associated with bacterial infection independent of AKI. Finally, our finding that dimeric plasma NGAL rapidly declines after appropriate antibiotic therapy initiation justifies further prospective assessment of dimeric NGAL-guided antibiotic stewardship in critically ill patients.

4.4. Strengths and limitations

The present study had several strengths. Data was collected prospectively, reducing the risk of selection bias. Biomarkers were collected

Table 3
Separate univariate and multivariable linear regression analyses showing the association of infection and acute kidney injury with each biomarker's levels.

Variable	Not adjusted		Adjusted[a]	
	Coefficient[b] (95% CI)	P	Coefficient[b] (95% CI)	P
Association with dimeric NGAL level				
Infection	100 (26 to 218)	0.004	90 (15 to 215)	0.01
Acute kidney injury	26 (−34 to 140)	0.48	3 (−46 to 99)	0.92
Association with total NGAL level				
Infection	65 (33 to 104)	<0.001	51 (21 to 90)	<0.001
Acute kidney injury	55 (15 to 111)	0.005	35 (0.6 to 81)	0.045
Association with PCT level				
Infection	91 (7 to 241)	0.03	74 (−7 to 227)	0.08
Acute kidney injury	50 (−34 to 241)	0.33	16 (−50 to 170)	0.73
Association with CRP level				
Infection	89 (49 to 142)	<0.001	106 (61 to 164)	<0.001
Acute kidney injury	22 (−11 to 67)	0.21	18 (−12 to 58)	0.28
Association with WBC level				
Infection	7 (−2o to 43)	0.66	8 (−20 to 47)	0.61
Acute kidney injury	0.9 (−28 to 41)	0.96	−2 (−31 to 39)	0.91

NGAL, Neutrophil gelatinase-associated lipocalin; PCT, procalcitonin; CRP, C-reactive protein; WBC, white blood cell count.

[a] Covariates included in the adjusted models were: presence of a suspected or confirmed infection, acute kidney injury, male sex and acute physiology and chronic health evaluation II score.

[b] The coefficient is expressed as 100 x ($e^{coeff} - 1$), which can be interpreted as a geometric mean percent change in biomarker concentration for a one-unit change in the variable.

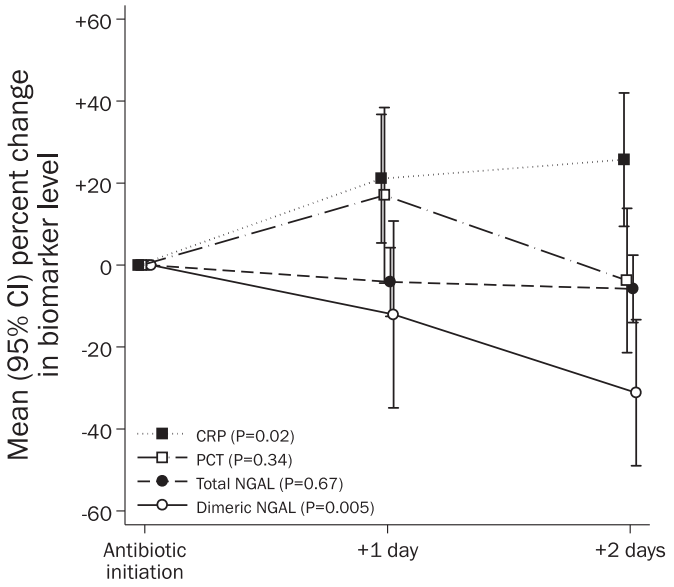

Fig. 3. Percent change in biomarker levels after antibiotic therapy initiation in 31 patients with a confirmed bacterial infection. *P*-values were derived from the Wilcoxon matched-pairs signed-ranks test of equality between biomarker values on day of antibiotic initiation and + 2 days.

daily or twice daily for the duration of the ICU stay and other patient data had high resolution. Treating clinicians, laboratory staff and infectious disease specialists were blinded to total NGAL and dimeric NGAL results.

The present study also presented some limitations. Patients were recruited from a single center. However, our results are comparable with previous studies, which lends robustness to our findings. A majority of admissions were after multiple trauma and patients were younger than most mixed medical/surgical ICU populations, thus reducing the generalizability to older populations. The study was not designed to study guidance of antibiotic treatment duration. It was therefore not possible to study if the use of dimeric NGAL affected outcome. We do not have a gold standard for diagnosis of sepsis in ICU patients. Culture screening methods are limited by a long time-to-positivity and low sensitivities due to administered empirical antibiotic therapy, contaminations and low viable bacterial content in the blood (<100 CFU/mL). The consensus classification we used to define infection entailed inclusion of patients with possible or probable infection. This introduces a risk of misclassification.

The number of patients receiving inadequate antibiotic therapy was too low to compare with those receiving adequate therapy. Additionally, due to missing data, the biomarker response to antibiotic therapy could only be analyzed in a limited number of patients which reduces the robustness of these findings.

We measured NGAL in plasma, which may have attenuated biomarker performance. Indeed, previous studies indicate that NGAL quantified in serum or in activated whole blood outperforms plasma NGAL as a marker of bacterial infections [14,30].

5. Conclusions

In our cohort dimeric NGAL as measured in plasma was a poor predictor of possible, probable or confirmed infection occurring within 24 h. However, it may be used to differentiate between sterile inflammation and bacterial infection in ICU patients, even in patients with

Table 4
Diagnostic and predictive accuracy for a probable, possible or confirmed infection on the day of antibiotic therapy initiation and one day before antibiotic therapy initiation, respectively.

Accuracy	Infection- group (n)	No infection-group (n)	ROC area (95% CI)	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)
Diagnostic accuracy (value at antibiotic therapy initiation)						
Dimeric NGAL	70	40	0.70 (0.60–0.79)	6.6 ng/mL	0.66 (0.53–0.77)	0.60 (0.43–0.75)
Total NGAL	70	40	0.77 (0.69–0.86)	47 ng/mL	0.76 (0.64–0.85)	0.68 (0.51–0.81)
PCT	70	40	0.63 (0.53–0.74)	0.85 ng/mL	0.63 (0.51–0.74)	0.65 (0.48–0.79)
CRP	70	40	0.74 (0.64–0.83)	170 mg/L	0.61 (0.49–0.73)	0.78 (0.62–0.89)
WBC	70	40	0.62 (0.51–0.73)	8.5×10^9/L	0.67 (0.54–0.78)	0.58 (0.41–0.75)
Diagnostic accuracy (% increase from previous value)						
Dimeric NGAL	43	24	0.59 (0.45–0.74)	4%	0.42 (0.27–0.58)	0.75 (0.53–0.90)
Total NGAL	43	24	0.52 (0.38–0.66)	13%	0.47 (0.31–0.62)	0.67 (0.45–0.84)
PCT	43	24	0.58 (0.42–0.74)	6%	0.49 (0.33–0.65)	0.63 (0.41–0.81)
CRP	43	24	0.33 (0.18–0.47)	50%	0.37 (0.23–0.53)	0.29(0.13–0.51)
WBC	43	24	0.45 (0.30–0.61)	2%	0.44 (0.29–0.60)	0.55 (0.32–0.76)
Predictive accuracy (one day before antibiotic therapy initiation)						
Dimeric NGAL	46	35	0.54 (0.41–0.66)	5.3 ng/mL	0.78 (0.64–0.89)	0.31 (0.17–0.49)
Total NGAL	46	35	0.64 (0.52–0.76)	43 ng/mL	0.78 (0.64–0.89)	0.51 (0.34–0.69)
PCT	46	35	0.52 (0.39–0.65)	0.75 ng/mL	0.54 (0.39–0.69)	0.60 (0.42–0.76)
CRP	46	35	0.79 (0.69–0.89)	105 mg/L	0.63 (0.48–0.77)	0.89 (0.73–0.97)
WBC	46	35	0.52 (0.38–0.65)	8.5×10^9/L	0.68 (0.52–0.81)	0.50 (0.32–0.68)

NGAL, Neutrophil gelatinase-associated lipocalin; PCT, procalcitonin; CRP, C-reactive protein; WBC, white blood cell count.

concomitant AKI. In addition, our findings support further assessment of dimeric plasma NGAL-guided antibiotic therapy de-escalation.

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Declaration of Competing Interest

Drs Per Venge and Shengyuan Xu are the owners of world wide granted patents of measuring NGAL in human disease. Dr. Per Venge owns shares in Diagnostics Development (Uppsala, Sweden).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcrc.2019.07.001.

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