Neutrophil Gelatinase-associated Lipocalin Predicts Post-traumatic Acute Kidney Injury in Severely Injured Patients

KATHARINA LEDITZKE 1, MAXIMILIAN EBERHARD HERMANN WAGNER 2, CLAUDIA NEUNABER 1, JAN-DIERK CLAUSEN 1 and MARCEL WINKELMANN 1

1 Trauma Department, Hannover Medical School, Hannover, Germany; 2 Department of Maxillofacial Surgery, University of Zürich, Zürich, Switzerland

Abstract. Background: Early detection of acute kidney injury (AKI) is crucial in the management of multiple-organ dysfunction syndrome in severely injured patients. Standard laboratory parameters usually increase with temporal delay. Therefore, we evaluated neutrophil gelatinase-associated lipocalin (NGAL) as an early marker for acute kidney injury.

Patients and Methods: We retrospectively evaluated patients admitted to a level 1 trauma center. We collected clinicodemographic data and measured kidney-related factors and plasma cytokines. Results: A total of 39 patients were included. Patients with AKI had significantly higher levels not only of serum creatinine and urea, but also of NGAL (all p<0.001) than patients without AKI. The optimal NGAL cut-off value was determined to be 177 ng/ml, showing significant correlation with imminent or manifest AKI (p<0.001). Other independent markers correlated with AKI included pre-existing chronic kidney disease, use of catecholamines, and severe injury (p<0.001). Conclusion: The serum level of NGAL is a feasible early predictor of AKI.

Multiple-organ dysfunction syndrome (MODS) is a strong indicator of poor outcomes in severely injured patients. At more than 48 hours after trauma, MODS is the leading cause of death, accounting for approximately 50% of all fatalities (1). The incidence and outcome of MODS have only slightly improved over recent years, and its treatment remains challenging (2-4). Early detection of MODS or being able to predict imminent MODS might help lower mortality. However, its diagnosis is difficult. A frequent starting point of MODS is acute kidney injury (AKI) (5, 6), defined as a sudden, rapidly progressing decrease in renal function. AKI is generally reversible when diagnosed immediately (7, 8). Thus, the timing of detection of impending renal failure is crucial for its adequate therapy and achievement of good prognosis. Regardless of its cause, AKI ultimately leads to reduced blood flow to the kidneys. This causes a decrease in the glomerular filtration rate, subsequently resulting in ischemic tubular injury, which in turn leads to acute tubular necrosis, the histologically pathognomonic sign of AKI. As such, the findings of increasing levels of serum creatinine and serum urea are currently used for the detection of AKI. These two standard laboratory parameters correlate with the glomerular filtration rate, enabling the detection of impaired renal function. However, both are retention parameters and are increased with a significant delay. As such, over 70 equations based on the creatinine and or cystatin C levels were suggested over the past decades to overcome this limitation (9).

A promising parameter that is directly correlated with parenchymal injury is neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin 2 (10). NGAL is increasingly formed in renal tubules during kidney damage. NGAL has a neuroprotective effect by reducing the rate of apoptosis and increasing the proliferation of renal tubular cells. After ischemic or nephrotoxic kidney damage, NGAL is one of the first proteins to be increasingly expressed (11). Due to its small molecular weight and high stability in urine, it is detectable in its free form or in complex with matrix metallopeptidase 9. Given that the urinary level of NGAL is correlated with its serum level, it can also be detected in the blood immediately on injury (12). The NGAL level has been found to increase in sepsis patients with AKI. In patients...
with severe burns, pre AKI is correlated with an increase in NGAL (13). However, comparable data are lacking in multiply injured patients. Therefore, this study aimed to analyze the usefulness of NGAL as an early predictor of AKI in patients with multiple injuries, particularly its possible superiority over the currently used predictors, serum creatinine and serum urea levels.

Patients and Methods

Study design and patients. This was a retrospective study of severely injured patients with an Injury Severity Score (ISS) ≥16 who were admitted to a level 1 trauma center within 6 hours after trauma and needed intensive care (14). Patients with incomplete plasma samples prior to death or discharge were excluded. The patients were participants in a superior central plasma databank project that included patients over a period of 4 years. The databank comprises a larger number of patients. However, only a small percentage were eligible for this study. Another study referring to the same global data source was published in 2019 (15). The final analysis was performed between November 2016 and January 2018 as part of this project.

Data collection included multiple blood samples, genotyping, and documentation of clinical data. Clinicodemographic data were gathered parallel to blood collection. Information on age, sex, mortality, initial Glasgow coma scale score, direct kidney injury, length of intensive care and hospital stay, transfusion requirements (packed red blood cells, fresh frozen plasma, and platelet concentrates), and catecholamine (noradrenaline, adrenaline, and dobutamine) use were collected.

This study was approved by the Institutional Review Board (application number 4980). Written informed consent to participate in the study was obtained from the patient or their nearest relatives whenever the patient was unconscious or unable to consent.

Measurements and analysis. Arterial blood samples for plasma cytokine measurements were taken at days 1, 2, 3, 5, 7, 10, and 14. A 10-ml sample was immediately centrifuged at 500×g for 10 minutes, and plasma was extracted and subsequently preserved at −80°C. Cytokine analysis was performed with cytoketric bead arrays. NGAL was measured using a human Lipocalin-2/NGAL Quantikine ELISA kit (R&D Systems Europe Ltd., Abingdon, UK), which has a limit of detection of 0.04 ng/ml and an assay range of 0.2-10 ng/ml. All analyses were performed in a timely manner in a specialized trauma research laboratory. Serum creatinine and serum urea were measured using enzymatic assays (Roche Diagnostics GmbH, Mannheim, Deutschland) at the Institution’s laboratory.

Variable definitions. All injuries were classified according to the Abbreviated Injury Scale (AIS) (2008 update) (16). Total injury severity was calculated using the ISS (14). The diagnosis of acute respiratory distress syndrome was made according to the Berlin definition, a further development of the North American European Consensus Conference criteria (17, 18). Systemic inflammatory response syndrome (SIRS) and sepsis were diagnosed using the 2010 revised criteria of S-2k guidelines of the German Sepsis Society and the German Interdisciplinary Association of Intensive Care and Emergency Medicine (19). MODS was diagnosed according to the MODS score developed by Marshall et al (20).

Specifically, MODS was deemed present when the sum of single-organ dysfunction was >8 in at least one day, representing severe MODS with considerably increased mortality. Kidney-related parameters included fluid intake and output (total and urine output), hemodialysis, and individual susceptibility to AKI according to the stages of susceptibility definition (SSD) (21). Severity of AKI was assessed according to the Acute Kidney Injury Network classification (22). AKI was diagnosed according to the Risk of renal dysfunction, Injury to the kidney, Failure or Loss of kidney function, and End-stage kidney disease (RIFLE) classification (23).

Statistical analysis. Between-group comparisons of plasma concentrations and other continuous variables were conducted using one-way or repeated measures analysis of variance via Greenhouse–Geisser adjustment to correct for violations of sphericity. After testing for Gaussian distribution (Shapiro-Wilk test), statistical analysis included parametric tests (Student’s t-test) for Gaussian-distributed data and Wilcoxon-Mann-Whitney test for non-Gaussian-distributed data. Fisher’s exact test (exact chi-squared-test) was used for contingency table analysis. The diagnostic values of NGAL, serum creatinine and serum urea as predictors of AKI were assessed using the receiver operating characteristic (ROC) analysis and the area under the curve. Odds ratios and 95% confidence intervals were also calculated. The optimal cut-off points were identified according to Youden’s index (J) and the optimal cut-off point that optimized the differentiation capability when equal weight is given to sensitivity and specificity (24). The updated STARD 2015 reporting guideline for diagnostic accuracy studies was used (25). All statistical analyses were performed using IBM SPSS (Version 23; IBM, Armonk, NY, USA). Significance was set at p<0.05.

Results

Patient characteristics. In total, 39 patients (7 women, 32 men) with a mean age of 41±20 years were included. There were no significant differences in clinicodemographic characteristics between the men and the women. The mean ISS was 34±11. The most severely injured body regions were the thorax (AIS score=3.0±1.9) and the head (AIS score=2.9±1.8). The mean duration of intensive care was 24±13 days, and the mean duration of mechanical ventilation was 497±285 h. The mean expected mortality rate was calculated to be 36±19%, but the actual in-hospital mortality rate was 18% (seven patients). Five patients died within the first 14 days, with the deaths occurring within a mean of 9 days. There were 14 patients who developed MODS; 15 patients SIRS; 10 patients sepsis; 15 patients acute respiratory distress syndrome, and 11 patients ventilator-associated pneumonia (see also Table I).

Correlation between AKI and MODS. There were 11 patients who developed imminent and seven patients who developed manifest AKI within a mean of 1.2±1.4 days (range=0-5 days), respectively. Figure 1 illustrates the percentage of patients with imminent or manifest AKI during the first 14 days of intensive care. There were no significant differences in clinicodemographic characteristics between patients with
and without imminent or manifest AKI. However, all patients with manifest AKI developed MODS, indicating that the presence of manifest AKI was positively correlated with the development of MODS [Spearman-rho (ρ)=0.43, p=0.007]. Patients with imminent or manifest AKI had significantly higher average values of serum creatinine (121.5±103.9 vs. 72.9±18.6 μmol/l, p<0.001) and serum urea (9.4±6.3 vs. 6.7±2.4 mmol/l, p<0.001) than did their counterparts without it. Similarly, the average NGAL value was significantly higher in those with imminent or manifest AKI (259.4±90.7 vs. 147.3±89.9 ng/ml, p<0.001) (see also Table II). Figure 2 illustrates the mean values of all three parameters over the 14-day course of admission.

**Diagnostic value of NGAL.** The area under the ROC curve was 0.83 (see also Figure 3). The ROC curve coordinates showed that the optimal cut-off value of NGAL for detecting AKI was 177 ng/ml (Youden’s J=0.61; sensitivity=86.5%, specificity=74.0%, negative predictive value=96.8%, and positive predictive value=37.6%). An NGAL value of 177 ng/ml significantly correlated with imminent or manifest AKI (ρ=0.46, p=0.001). In addition, a pre-existing chronic kidney disease (ρ=0.3, p<0.001), use of catecholamines (ρ=0.3, p<0.001), and severe injury (ρ=0.2, p<0.001) were also correlated with an imminent of manifest AKI. In contrast, age (ρ=−0.02, p=0.7) and sex (ρ=0.04, p=0.5) did not.

In a logistic regression model comprising ISS>33, catecholamine application, pre-existing chronic kidney disease (SSD≥II), and NGAL>177 ng/ml, NGAL was an independent predictor of imminent or manifest AKI (odds ratio=14.4, p<0.001). Table III outlines the results of the logistic regression analysis. Aside from NGAL, severe injury (ISS>16), pre-existing chronic kidney disease, and catecholamine use were also independent predictors of AKI.

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**Table I. Demographic and clinical data for patients with and without acute kidney injury (AKI).**

| Total (n=39) | Without AKI (n=21) | With AKI (n=18) | p-Value |
|-------------|--------------------|-----------------|---------|
| Age, years  | Mean±SD            | Mean±SD         | Mean±SD |
| Gender, n   | Male               | Female          |         |
| GCS at admission | Median (IQR) | Median (IQR) | Median (IQR) | >0.99b |
| Injure severity score, | Median (IQR) | Median (IQR) | Median (IQR) | >0.99b |
| AIS, median (IQR) | Head | Median (IQR) | Median (IQR) | Median (IQR) | >0.99b |
| Face       | 0 (2)              | 0 (2)           | 0.5 (2)  | 0.43a  |
| Thorax     | 4 (3)              | 4 (2)           | 4 (4)    | 0.88a  |
| Abdomen    | 0 (2)              | 0 (2)           | 0 (2)    | 0.88a  |
| Extremities| 3 (1)              | 3 (1)           | 3 (3)    | 0.75a  |
| External   | 0 (0)              | 0 (0)           | 0 (0)    | 0.53a  |
| RTS        | 5.48±2.32          | 5.55±2.49       | 5.39±2.17| 0.61a  |
| TRISS      | 0.60±0.35          | 0.63±0.34       | 0.56±0.38| 0.84a  |
| APACHE II  | 20.2±8.1           | 18.4±6.7        | 22.3±9.1 | >0.99a |
| Intensive care, days | Mean±SD | Mean±SD | Mean±SD | >0.99a |
| In-patient care, days | Mean±SD | Mean±SD | Mean±SD | >0.99a |
| Duration of mechanical ventilation, h | Mean±SD | Mean±SD | Mean±SD | >0.99a |
| Transfusion, mean no. units±SD | | | |
| 48 h        | PRBC               | FFP             | PC |
| Mortality, n| 8                  | 3               | 5   | 0.43b  |
| SSD≥II, n   | 9                  | 4               | 5   | 0.71b  |
| ARDS, n     | 16                 | 8               | 8   | 0.75b  |
| VAP, n      | 17                 | 9               | 8   | >0.99b |
| SIRS, n     | 20                 | 9               | 11  | 0.34b  |
| Sepsis, n   | 11                 | 4               | 7   | 0.29b  |
| MODS, n     | 14                 | 5               | 9   | 0.11b  |

AIS: Abbreviated injury scale; APACHE II: acute physiology and chronic health disease classification system II; ARDS: acute respiratory distress syndrome; FFP: fresh frozen plasma; GCS: Glasgow coma score; MODS: multiple-organ dysfunction syndrome. ISS: injury severity score; PRBC: packed red blood cells; RTS: revised trauma score; SIRS: systemic inflammatory response syndrome; SSD: stages of susceptibility definition; TRISS: trauma and injury severity score; VAP: ventilator-associated pneumonia. aMann-Whitney U-test; bFisher’s exact test; cStudent’s t-test.
Discussion

The delayed increase of serum creatinine and urea as indicators of impaired renal function limits their use in early detection of AKI. This study demonstrated the feasibility of NGAL as an independent predictor of imminent and manifest AKI. We also found that pre-existing chronic kidney injury, use of catecholamines, and severe injury were independent factors of AKI.

The incidence of AKI in multiply injured patients substantially varies in the literature. Podoll et al. reported a 6% incidence in a group of 901 US patients, (26) whereas Brandt et al. reported a rate of 23.8% in a study of 1,003 US patients. Eriksson et al. reported that AKI occurred in 24.9% of 413 Swedish patients (27, 28), while Bagshaw et al. reported that early AKI developed in 18.1% of 9,449 Australian trauma patients (29). In our study, 18% of the patients developed AKI, this rate being consistent with that in the current literature. Furthermore, all patients who developed AKI developed MODS, highlighting the high impact of AKI in severely injured patients. Given that the majority of cases of AKI are due to hypovolemic shock (usually hemorrhagic), early detection is of particular importance. However, current gold standard laboratory parameters only increase after a considerable delay, which hampers the early detection of AKI, especially the imminent type. In addition, creatinine is actively secreted in the tubules above a serum level of more than 2 mg/dl, making it impossible to determine the glomerular filtration rate using creatinine clearance. This can result in an overestimation of the glomerular filtration rate as serum creatinine increases (30). Moreover, the serum creatinine level only increases significantly if the glomerular filtration rate is reduced by more than 50%. Thus, an inconspicuous creatinine value does not preclude the onset of AKI. In addition, severely injured patients have fluid imbalance, and fluid balance is a prerequisite for a close correlation between glomerular filtration rate and creatinine clearance (31). Urea, on the other hand, does not exceed the serum threshold until the glomerular filtration rate has already decreased by more than 75%. Its significance is further restricted by its dependence on protein metabolism. Urea is only suitable for a partial
survey and follow-up evaluation of AKI. The validity of urea for the assessment of renal function is even lower than that of creatinine.

The use of NGAL can overcome these limitations and NGAL has the potential to be an early indicator of AKI. Several studies have shown that NGAL is a useful biomarker of AKI (32-34). NGAL was also found to be associated with the mortality of severely injured patients (35, 36). In our study, all patients with AKI also developed MODS, but not all patients with MODS had AKI, indicating a correlation between AKI and NGAL, although this should not be regarded as absolute. A major advantage of the use of NGAL, however, is that its level increases earlier than other conventional biomarkers. In a study of 72 children undergoing cardiopulmonary bypass, the plasma NGAL concentration indicated possible AKI as early as 2 hours postoperatively. NGAL was the most significant predictor of AKI, with a sensitivity of 100% and a specificity of 98% (37). An increased urine NGAL level was also measured as early as 1 to 3 hours after cardiac surgery in adult patients with AKI. In contrast, an increase in creatinine was detected only after 24-48 hours (38). Our findings are in line with these previous results, further confirming the potential of NGAL as a direct and independent early biomarker of renal failure in multiply injured patients.

This study has some limitations. There was a gap between blood sampling including cytokine measurement and data analysis in our study because the initial cytokine measurement served a different purpose in the superior database project. The idea of analyzing the potential

Table II. Serum levels of neutrophil gelatinase-associated lipocalin (NGAL), creatinine and urea over the first 14 days after trauma in patients with and without acute kidney injury (AKI).

| Day | Total (n=39) | Without AKI (n=21) | With AKI (n=18) | p-Value |
|-----|-------------|-------------------|----------------|----------|
| NGAL, ng/ml | | | | |
| 1 | 192±83 | 181±85 | 204±82 | 0.117\(a\) |
| 2 | 186±96 | 144±79 | 233±92 | 0.001\(a\) |
| 3 | 178±106 | 128±94 | 234±91 | <0.001\(a\) |
| 5 | 194±137 | 160±147 | 233±116 | 0.017\(b\) |
| 7 | 153±79 | 126±58 | 188±91 | 0.030\(b\) |
| 10 | 133±66 | 118±53 | 155±78 | 0.137\(b\) |
| 14 | 114±66 | 102±52 | 133±83 | 0.524\(a\) |
| Serum creatinine, ng/ml | | | | |
| 1 | 107±79 | 91±23 | 126±112 | 0.148\(a\) |
| 2 | 113±55 | 91±18 | 139±71 | 0.001\(a\) |
| 3 | 110±81 | 81±14 | 142±111 | 0.001\(a\) |
| 5 | 111±93 | 74±15 | 156±125 | 0.003\(a\) |
| 7 | 103±102 | 68±14 | 148±144 | 0.008\(a\) |
| 10 | 79±58 | 64±12 | 100±86 | 0.294\(a\) |
| 14 | 63±20 | 58±12 | 70±27 | 0.149\(b\) |
| Serum urea, ng/ml | | | | |
| 1 | 5.9±2.4 | 5.6±1.5 | 6.3±3.2 | 0.813\(a\) |
| 2 | 5.8±2.9 | 5.1±2.0 | 6.5±3.7 | 0.321\(a\) |
| 3 | 5.8±3.9 | 4.8±1.7 | 6.8±5.3 | 0.967\(a\) |
| 5 | 8.0±5.3 | 6.0±1.8 | 10.5±7.0 | 0.107\(a\) |
| 7 | 9.0±5.4 | 7.1±1.7 | 11.4±7.4 | 0.185\(a\) |
| 10 | 9.4±5.6 | 7.9±2.4 | 11.6±7.8 | 0.202\(a\) |
| 14 | 8.1±3.6 | 7.5±2.8 | 8.8±4.4 | 0.691\(a\) |

\(a\)Mann-Whitney \(U\)-test; \(b\)Student’s \(t\)-test. Data are mean±standard deviation.

Figure 3. Receiver operating characteristics curve for prediction of imminent or manifest acute kidney injury by serum neutrophil gelatinase-associated lipocalin.
correlation of NGAL and AKI in multiply injured patients was developed later. However, cytokine measurements and clinical data acquisition were performed in close time proximity. Only data merging and analysis were performed recently. Therefore, data quality is reliable and high. Some limitations refer to NGAL itself. Unlike creatinine and cystatin C, NGAL is not a marker of renal function but is a specific marker of kidney damage (12). After only 30 minutes, the level of NGAL in both blood and urine increase in proportion to the severity and duration of kidney damage (39). NGAL concentration decreases as the renal parenchyma recovers (40). Therefore, it is necessary to demonstrate that impairment of renal function is correlated with tubular damage. Additionally, the measurement of NGAL depends on different internal and external factors, and the performance of different NGAL assays varies (41, 42). Bacterial infections, pre-existing diseases of the respiratory tract, pre-existing chronic heart disease, long operative time, and age influence the NGAL values measured (43, 44). During angiography, the NGAL concentration may increase since increased NGAL values are found in arteriosclerotic plaques (45). Additionally, NGAL is significantly increased in patients with various tumor types as the NGAL gene (LCN2) is induced by tumor-promoting substances (46).

Despite these limitations, our findings are consistent with previous reports that NGAL is a promising marker for the detection of AKI in severely injured patients (13, 47, 48). In this context, new techniques such automated machine-learning platforms have recently been proposed to facilitate its use (49). The optimal cut-off value and the correlation between NGAL and AKI independent from serum creatinine and urea levels need to be analyzed in a larger patient population. The small number of patients examined in this study justifies a first evaluation, and a longer observational period and a multi-center study design are needed for future analyses.

In conclusion, serum NGAL is independently correlated with AKI and increases earlier than does serum creatinine and urea. As such, it can be an early indicator of AKI. Combining NGAL with other predictive factors (pre-existing chronic kidney disease, use of catecholamines, and severe injury) might help in the earlier detection of AKI in severely injured patients and the prevention of its subsequent sequelae.

Conflicts of Interest

No Author has any conflicts of interest to declare.

Authors’ Contributions

Study concept, design, and coordination: WM, NC, CJD. Data acquisition and study concept: LK, WM, WMEH. Article drafting/preparation: LK, WM, WMEH. Article revision: NC and CJD. All Authors reviewed the article and approved the final version.

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Table III. Logistic regression analysis of significant independent predictors of imminent or manifest acute kidney injury.

| Parameter                  | OR (95%-CI)   | p-Value |
|----------------------------|---------------|---------|
| ISS (>33)                  | 3.6 (1.5-8.8) | 0.004   |
| NGAL (>177 ng/ml)          | 14.4 (5.0-41.6) | <0.001 |
| SSD (CKD ≥II)              | 3.2 (1.3-8.0) | 0.01    |
| Catecholamine use (yes)    | 5.7 (1.2-28.0) | 0.03    |

CI: Confidence interval; CKD: chronic kidney disease; ISS: injury severity score; NGAL: neutrophil gelatinase-associated lipocalin; OR: odds ratio; SSD: stage of susceptibility definition.
