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ORIGINAL ARTICLE

Neutrophil gelatinase-associated lipocalin and liver-type fatty acid-binding protein as biomarkers for acute kidney injury after organ transplantation

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Abstract
Purpose Neutrophil gelatinase-associated lipocalin (NGAL) and liver-type fatty acid-binding protein (L-FABP) are promising early biomarkers for acute kidney injury (AKI). In organ transplant recipients, AKI predictability based on NGAL and L-FABP remains to be elucidated. Furthermore, the association between serial NGAL and L-FABP measurements and AKI outcome is unknown. Therefore, we conducted a study to evaluate the ability of NGAL and L-FABP to predict AKI after organ transplantation and investigate the association between NGAL, L-FABP and AKI outcome.
Methods Twenty-five organ transplant recipients admitted to the intensive care unit (ICU) immediately after transplant surgery were studied prospectively. Plasma NGAL (P-NGAL), urinary NGAL (U-NGAL) and L-FABP were measured from ICU admission to ICU discharge. U-NGAL and L-FABP were corrected for dilution/concentration by calculating U-NGAL/urine creatinine ratios (U-NGAL/Cr) and L-FABP/urine creatinine ratios (L-FABP/Cr). AKI was defined according to the Kidney Disease: Improving Global Outcomes criteria.
Results AKI occurred in 11 patients. P-NGAL, U-NGAL/Cr and L-FABP/Cr upon ICU admission were unrelated to AKI development ($p = 0.24$, 0.22, and 0.53, respectively). There were no differences in P-NGAL, U-NGAL/Cr, and L-FABP/Cr levels from day 1 to day 6 between patients who did not recover from AKI and patients who recovered from AKI ($p = 0.82$, 0.26, and 0.61, respectively).
Conclusion Our findings suggest that NGAL and L-FABP upon ICU admission are not predictive of AKI and serial NGAL and L-FABP measurements may be ineffective for monitoring the status and treatment of post-transplantation AKI.

Keywords Neutrophil gelatinase-associated lipocalin · Liver-type fatty acid-binding protein · Organ transplantation · Acute kidney injury

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Introduction

Acute kidney injury (AKI) is a common complication after organ transplantation [1]. AKI during the post-transplantation period is associated with an increase in morbidity and mortality [2]. Once AKI occurs in organ transplant recipients, management of immunosuppressants, antibacterial, antifungal, and antiviral agents becomes difficult. Early recognition of AKI is crucial, because the lack of early detection results in delayed initiation of therapy and reduction in renal toxic drugs. Recently, new biomarkers of AKI with high sensitivity and specificity have been introduced. Both neutrophil gelatinase-associated lipocalin (NGAL) and urinary liver-type fatty acid-binding protein (L-FABP) have been reported as promising early biomarkers for AKI in various settings [3–5]. However, the

ability of NGAL and L-FABP to predict AKI in organ transplant recipients has rarely been described.

Although many studies have shown the usefulness of NGAL and L-FABP results upon intensive care unit (ICU) admission [3–5], the association between serial NGAL and L-FABP measurements and AKI outcome has rarely been validated. The cause of post-transplant AKI is multifactorial. Organ transplant recipients suffer multiple insults to their kidneys and have AKI risk factors such as underlying pre-operative renal dysfunction, intra-operative hypotension, large volume transfusions, and renal toxic drugs required after surgery (e.g., calcineurin inhibitors, and ganciclovir) [6]. Because it has recently been reported that NGAL is useful as a real-time indicator during active kidney damage [7], we hypothesized that serial measurements of NGAL and L-FABP during the post-transplantation period may be useful to monitor the current status of kidney function.

Therefore, we conducted a study to evaluate the ability of NGAL and L-FABP to predict AKI after organ transplantation and investigate the association between NGAL, L-FABP and AKI outcome.

Patients and methods

Patients

This study was conducted in the 10-bed ICU of Osaka University Hospital. We enrolled 25 patients who underwent heart, lung, or liver transplantation (dead or alive related) between December 2010 and January 2012. The study was approved by the Osaka University Hospital ethics committee (No. 10194), and written informed consent was obtained from their family members. Patients with preoperative renal failure requiring renal replacement therapy (RRT) were excluded. The treatment of patients including initiation of RRT was at the intensivist's discretion.

Biomarker measurements

Plasma and urine samples were collected from patients upon admission and once a day thereafter in the morning until they were discharged from the ICU. When samples were not available upon ICU admission, values from the next sample within 12 h of ICU admission were used. Samples were frozen and stored at −80 °C until measured. Plasma NGAL (P-NGAL) was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (BioPorto Diagnostics, Grusbakken, Denmark). Urinary NGAL (U-NGAL) was determined using a chemiluminescence immunoassay (ARCHITECT; Abbott Diagnostics, Abbott Park, IL, USA). Urinary L-FABP was measured using a commercially available ELISA kit (CI-MIC CO., Tokyo, Japan), and urinary creatinine (uCr) was measured using a commercially available kit. U-NGAL and L-FABP results were normalized to uCr concentrations and presented as U-NGAL/Cr and L-FABP/Cr which were multiplied by 100 to compensate for possible urinary dilution or concentration.

Definition of acute kidney injury (AKI)

To monitor kidney function, we routinely measured serum creatinine (sCr) and blood urea nitrogen (BUN) every morning during their stay in ICU. We used the Kidney Disease: Improving Global Outcomes (KDIGO) creatinine criteria to define AKI [8]. KDIGO creatinine criteria are based on the increase in sCr compared with the baseline value. Stage 1 represents a 1.5–1.9 times increase or an absolute increase of 0.3 mg/dL, stage 2 represents a 2–2.9 times increase, and stage 3 represents an increase of >3 times or increase in sCr to ≥4.0 mg/dL or initiation of RRT. Baseline sCr (bsCr) was defined as the steady-state level before transplantation. We diagnosed AKI when the sCr value met the KDIGO creatinine criteria during the ICU stay. We defined AKI outcome according to sCr on the day of ICU discharge (dsCr), sustained AKI as $dsCr \geq bsCr + 0.3$ mg/dL, and recovered AKI as $dsCr < bsCr + 0.3$ mg/dL. In our investigation of the relationship between NGAL and L-FABP levels and AKI outcome, we used patients who did not have missing values in NGAL and L-FABP data from day 1 to day 6. We defined day 1 as the day on which the patient entered the ICU. Missing values for some data were because of the inability to collect urine samples due to anuria, discharge from the ICU, or repeat surgery.

Data collection and statistical analysis

Other variables recorded included age, sex, ICU days and mortality at 28 days. Baseline BUN was defined as the steady-state level before transplantation. We recorded the bsCr and baseline BUN on the same day. SPSS version 16 (IBM, North Castle, NY, USA) was used for statistical analysis. Continuous variables are described using mean and standard deviation and these were compared between 2 groups using the Mann–Whitney *U* tests. P-NGAL, U-NGAL/Cr, and L-FABP/Cr were compared between AKI and non-AKI groups using Mann–Whitney *U* tests. Daily changes in P-NGAL, U-NGAL/Cr, and L-FABP/Cr levels were compared between the sustained AKI and recovered AKI groups using two-way repeated measures ANOVA. All reported *p* values are two-tailed, and *p* values <0.05 were considered statistically significant.

Table 1 Patient characteristics

Characteristics	Total ($n = 25$)	AKI ($n = 11$)	Non-AKI ($n = 14$)	p value (AKI vs non-AKI)
Age (years)	43 ± 16	48 ± 13	39 ± 17	NS
Male/female (n)	13/12	6/5	7/7	
Organ: heart/lung/liver (n)	9/6/10	1/3/7	8/3/3	
Donor: dead/live (n)	15/10	4/7	11/3	
Rejection (n)	0	0	0	–
Baseline sCr (mg/dL)	0.80 ± 0.30	0.77 ± 0.25	0.83 ± 0.35	NS
Baseline BUN (mg/dL)	17.1 ± 8.4	14.5 ± 7.0	19.2 ± 9.1	NS
KDIGO stage 1/2/3 (n)		6/1/4		
Mortality at 28 days (%)	0	0	0	–
ICU stay (days)	10.7 ± 11.9	16.0 ± 16.1	6.4 ± 4.2	0.02

Values are expressed as mean ± standard deviation

AKI acute kidney injury, *sCr* serum creatinine, *BUN* blood urea nitrogen, *ICU* intensive care unit

Results

Patient characteristics

Patient characteristics are shown in Table 1. As for the baseline characteristics, there were no significant differences between patients with or without AKI with regard to age, bsCr, and baseline BUN. No patients experienced rejection during their ICU stay.

AKI after organ transplantation

AKI occurred in 11 patients (44 %)—6 developed KDIGO stage 1, 1 developed stage 2, and 4 developed stage 3 AKI. Of the patients with KDIGO stage 3 AKI, all four received RRT (16 % of the overall patient cohort) during each clinical course. The sCr values first met the KDIGO criteria on day 1 for 3 patients, day 2 for 3 patients, day 3 for 4 patients and day 4 for 1 patient. There was a positive correlation between AKI and length of ICU stay. There were no significant differences with respect to organ type and incidence of AKI ($p = 0.10$).

In addition, P-NGAL concentrations, U-NGAL/Cr and L-FABP/Cr upon ICU admission were not related to AKI development ($p = 0.24$, 0.22, and 0.53, respectively) (Fig. 1a–c).

P-NGAL concentrations, U-NGAL/Cr and L-FABP/Cr upon ICU admission were also not significantly related to RRT requirements ($p = 0.48$, 0.85, and 0.39, respectively).

Out of 11 patients of AKI, 5 patients were assessed as sustained AKI and 6 patients were as recovered AKI at ICU discharge. No differences in P-NGAL, U-NGAL/Cr, and L-FABP/Cr levels from day 1 to day 6 were observed between patients who did not recover from AKI (P-NGAL data were available for 5 patients, U-NGAL/Cr and L-FABP/Cr data were available for 4 patients) and patients who recovered from AKI (P-NGAL, U-NGAL/Cr, and L-FABP/Cr data were available for 4 patients) ($p = 0.82$, 0.26, and 0.61, respectively) (Figs. 2, 3, 4).

Discussion

The present study shows that P-NGAL, U-NGAL/Cr and L-FABP/Cr values upon ICU admission could not predict the development of AKI and the initiation of RRT in organ transplant recipients during their ICU stay. This study also shows that using serial NGAL and L-FABP measurements, P-NGAL, U-NGAL/Cr and L-FABP/Cr values did not reflect the clinical course (sustained or recovered) of AKI.

NGAL is considered as a promising biomarker to predict AKI in various clinical settings [9–11]. Previous studies in critically ill adult patients in the ICU [3, 5] and in the emergency department [4] have reported the predictive accuracy of P-NGAL and U-NGAL for AKI at ICU admission; however, in our study, NGAL could not predict AKI in organ transplant patients. There may be several explanations for our results. First, organ transplant recipients may have already had a kidney injury due to underlining heart or liver failure prior to transplantation. A study reported that U-NGAL in adult cardiac surgery patients with a baseline estimated glomerular filtration rate (GFR) of <60 mL/min was useless in detecting AKI [12]. Urinary L-FABP levels in patients with chronic kidney disease were higher than those in healthy control subjects [13]. Therefore, the usefulness of U-NGAL and L-FABP for the prediction of acute-on-chronic renal failure may be questionable. As bsCr in our study population was not high, we speculated that organ transplant recipients had undetectable renal injury before transplantation. Therefore, after transplantation, organ grafts began to function and the kidney injury may then have gradually recovered.

Second, we started measurements of NGAL and L-FABP after ICU admission. A study observed that NGAL concentrations obtained during surgery were highly associated with postoperative AKI in patients undergoing liver transplantation [14]. However, NGAL measurement

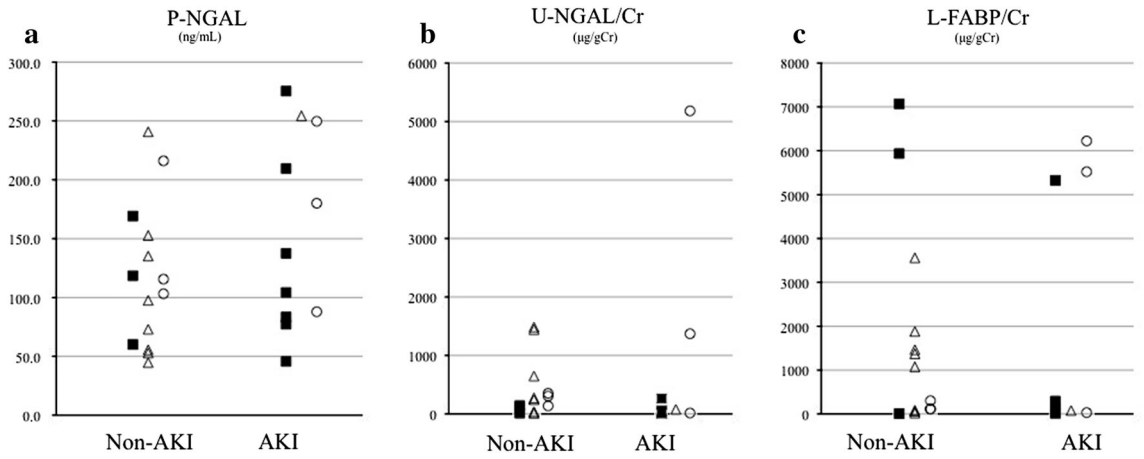

Fig. 1 Scatter plot of neutrophil gelatinase-associated lipocalin (NGAL) and L-type fatty acid-binding protein (L-FABP) concentrations measured on admission day 1 in the intensive care unit in 11 acute kidney injury (AKI) patients versus 14 non-AKI patients (heart *white triangle*, lung *white circle*, liver *black square*)

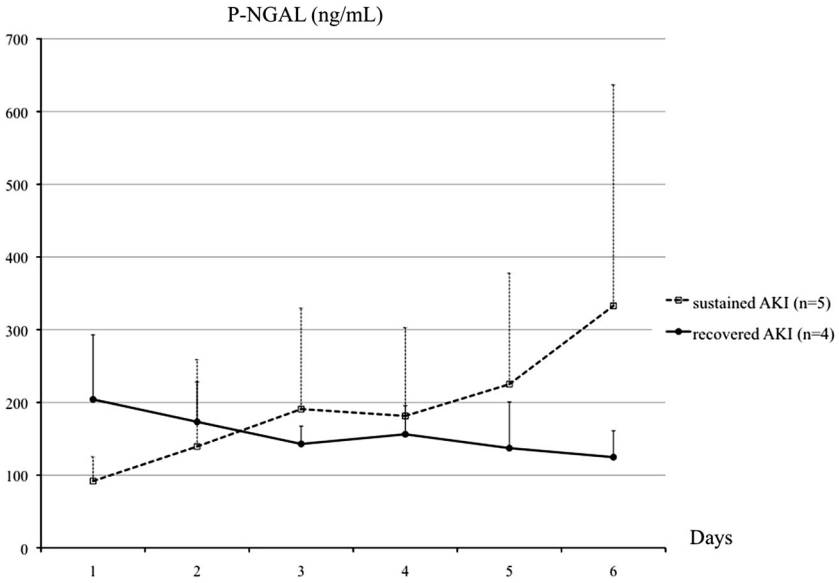

Fig. 2 Plasma neutrophil gelatinase-associated lipocalin (P-NGAL) levels from day 1 to day 6 in sustained and recovered acute kidney injury (AKI) patients

during the pre- or intra-operative period is impractical in the real world, because measurement of NGAL is complicated and takes many hours. The advantage of NGAL to predict AKI should be based on a measurement at a single time point (i.e., within 24 h of ICU or emergency department admission) to predict AKI certainty in heterogeneous populations [3–5]. Therefore, we considered that the timing of NGAL sampling in our study was reasonably realistic.

Third, recent reports have indicated that NGAL is not useful in predicting the development of AKI. P-NGAL is not effective in predicting AKI in patients with contrast-induced nephropathy [15], and U-NGAL is not a reliable predictor of AKI in ICU patients using every different molecular forms of NGAL [16]. In a landmark NGAL report by Mishra et al. [9], an excellent result for AKI prediction was described, which showed an area under the receiver-operating characteristic curve (AUC) value of 0.998. One reason for this result may be that pediatric cardiac surgery patients and AKI insults occurred at one point during the cardiopulmonary bypass. However, NGAL values rise with complications such as infection, ischemia, malignancy, and confounding factors are common. Our study was intended for such patients as described above. Many studies which indicate that NGAL is useful report an AUC of approximately 0.7 [10]. Moreover, a report on NGAL values that examined patients after liver transplant indicated that high NGAL values significantly correlated with postoperative AKI (AUC approximately 0.7) and that AKI could be predicted if the APACHE score was considered along with NGAL values within 24 h after liver

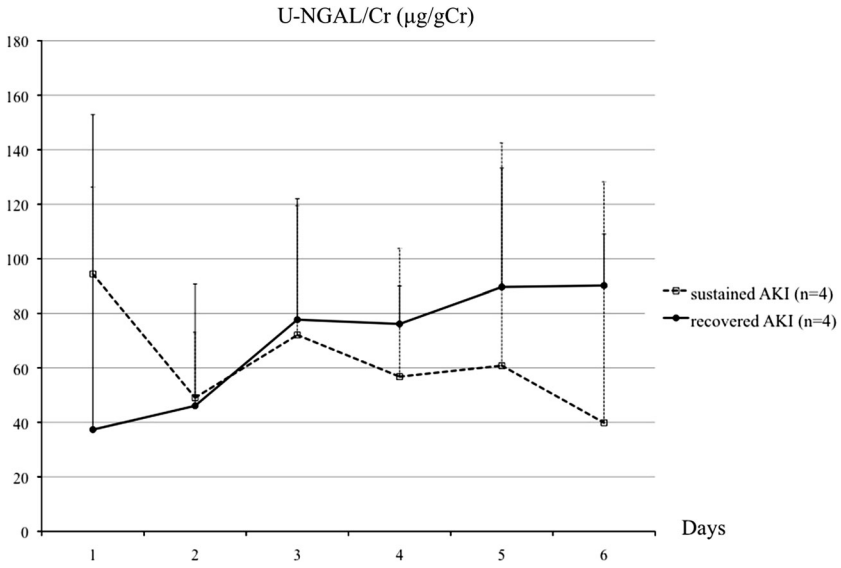

Fig. 3 Urine neutrophil gelatinase-associated lipocalin creatinine ratio (U-NGAL/Cr) from day 1 to day 6 in sustained and recovered acute kidney injury (AKI) patients

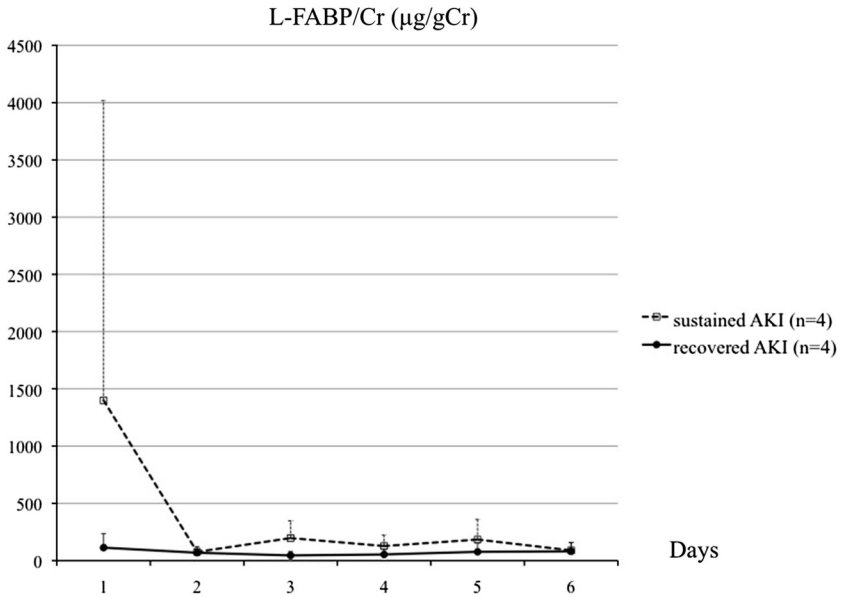

Fig. 4 L-type fatty acid-binding protein creatinine ratio (L-FABP/Cr) from day 1 to day 6 in sustained and recovered acute kidney injury (AKI) patients

transplantation [17]. Rather intricate calculations arrived at AUC = 0.7, which is certainly not considered high, and is very similar to the AUC value of Cr. Our detailed review of the many studies that indicate that NGAL is effective revealed significant variations in actual NGAL values, such that NGAL values often overlapped between the AKI and non-AKI groups [3, 5, 9]. The results of our study also varied similarly. Most recently, the Acute Dialysis Quality Initiative Consensus Conference reported that there are currently insufficient data on damage biomarkers to support their use for AKI staging [18]. Because the accuracy of predicting AKI according to NGAL values differs in each report, we believe that it is reasonable to conclude that there is no consensus, especially the situation regarding multiple insults to the kidney. Therefore, we believe that our results are primarily consistent with those of other studies and that NGAL values are not useful for prediction AKI.

This study provides preliminary evidence that serial NGAL measurements do not reflect the clinical course of AKI. To the best of our knowledge, this is the first report evaluating the association between serial NGAL and L-FABP measurements and AKI outcome. A recent study showed that the U-NGAL level may be useful for monitoring the status and treatment of diverse renal diseases [19]. It has been indicated that NGAL increases with infection and confounding factors [20]. In post-organ transplant period, the timing of renal insults was not

strictly identified and may have occurred more than once. After transplantation, recipients are given nephrotoxic drugs including immunosuppressants, antibacterial, antifungal, and antiviral agent without exception. Although these drugs are given carefully, AKI is not rare after transplantation. Graft rejection might also be injurious to kidneys. During the post-organ transplantation period, recipients are exposed to various nephrotoxic situations. Thus, serial NGAL measurements may not be a reliable tool in monitoring the status and treatment of AKI post-transplantation.

Several limitations may have affected the results obtained in this study. We studied a small number of patients and RRT may have affected P-NGAL levels. Our preliminary study revealed that continuous venovenous hemodiafiltration (CVVHDF) reduced P-NGAL levels to approximately 80 % of that before CVVHDF; however, discontinuation of CVVHDF increased P-NGAL levels and returned them to levels similar to that before CVVHDF [21]. Our study included various types of transplantation (organs: heart, lung, and liver; donor: dead or live-related). Operation time, amount of blood loss and need for transfusion varied and heart transplantation required cardiopulmonary bypass, which could have deleterious effects on renal function.

In conclusion, P-NGAL, U-NGAL/Cr and L-FABP/Cr values upon ICU admission could not predict the development of AKI and the initiation of RRT in organ transplant recipients during their ICU stay. Furthermore, P-NGAL, U-NGAL/Cr and L-FABP/Cr levels do not reflect the clinical course (sustained or recovered) of AKI in organ transplant recipients during their ICU stay. Our findings suggest that serial NGAL and L-FABP measurements may be ineffective for monitoring the status and treatment of post-transplantation AKI.

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