The urinary levels of prostanoid metabolites predict acute kidney injury in heterogeneous adult Japanese ICU patients: a prospective observational study

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Abstract

Background

Acute kidney injury (AKI) is frequently observed in critically ill patients in the intensive care unit (ICU) and is associated with increased mortality. Prostanoids regulate numerous biological functions, including hemodynamics and renal tubular transport. We herein investigated the ability of urinary prostanoid metabolites to predict the onset of AKI in critically ill adult patients.

Methods

The current study was conducted as a prospective observational study. Urine of patients admitted to the ICU at Okayama University Hospital was collected and the urinary levels of prostaglandin E_2 (PGE_2), PGI_2 metabolite (2,3-dinor-6-OXO-$PGF_{1\alpha}$), thromboxane A_2 (TXA_2) metabolite (11-dehydro-TXB_2) were determined.

Results

Of the 93 patients, 24 developed AKI (AKIN criteria). Surgical intervention (93%, 75%) was the leading cause of ICU admission. Overall, the ratio of the level of serum Cr on Day 1 after ICU admission to that observed at baseline positively correlated with the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ($r = 0.57$, $p < 0.0001$) and 11-dehydro-TXB_2/Cr ($r = 0.47$, $p < 0.0001$) ratios. In 16 cases of de novo AKI, the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values were significantly elevated compared with that observed in the non-AKI group, whereas the urinary PGE_2/Cr values were not. The urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratio exhibited the best diagnostic and predictive performance among the prostanoid metabolites according to the receiver operating characteristic (ROC) analysis (ROC-area under the curve (AUC): 0.75).

Conclusions

Taken together, these results demonstrate that the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr ratios are associated with the subsequent onset of AKI and poor outcomes in adult heterogeneous ICU patients.

Key words: acute kidney injury, prostaglandin, thromboxane, ICU

Introduction

Acute kidney injury (AKI) is frequently observed in critically ill patients in the ICU and is associated with increased mortality [1]. AKI occurs in 5-7% of hospitalized patients and 20-25% of ICU admissions [2]. Unfortunately, the serum creatinine (sCr) level increases at later time points and cannot be used to detect or diagnose early renal tubular injury prior to a decline in the glomerular filtration rate (GFR). Therefore, the identification of more reliable and early biomarkers for AKI is required. To date, urinary kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), L-type fatty acid-binding protein (L-FABP) and a number of other markers have been examined with respect to their role in the early and accurate detection of AKI [3-5].

Prostaglandins (PGs) are derived from the metabolism of arachidonic acid, with the first step being catalyzed by the cyclooxygenase (COX) system. PGs are produced at various sites in the kidneys and have been implicated to regulate glomerular hemodynamics, renin secretion and tubular transport [6]. Prostacyclin (prostaglandin I_2: PGI_2), a major PG, exerts vasoprotective effects, including vasodilation, inhibition of platelet aggregation and the proliferation of vascular smooth muscle cells [7]. Prostacyclin-deficient mice with a genetic disruption in prostaglandin I_2 synthetase (PGIS) develop renal fibrosis and arterial sclerosis [8], suggesting its involvement in the maintenance of a normal kidney structure. In addition, the renoprotective effects of prostacyclin and its analogues have been reported in various experimental models, including gentamycin-induced tubular injury [9], diabetic nephropathy [10-12] and unilateral ureteral obstruction [13, 14]. In contrast, thromboxane A_2 (TXA_2) exerts effects opposite to those of PGI_2, in association with the development of vascular lesions [7]. PGE_2 is one of the major prostanoids generated by the kidneys [15] and affects both vascular tone and the epithelial function. PGE_2 also modulates renal sodium and water excretion [16].

PGI_2 is rapidly metabolized into a nearly inactive product, 6-keto-$PGF_{1\alpha}$. Thromboxane is similar to prostaglandins in that its rate of production is relatively low in normal subjects and inhibiting its synthesis has little effect on the renal function [17]. Meanwhile, urinary prostaglandin E_2 (PGE_2), PGI_2 metabolite (2,3-dinor-6-OXO-$PGF_{1\alpha}$) and TXA_2 metabolite (11-dehydro-TXB_2) are relatively stable and reflect the production of each prostanoid.

In a previous report of 10 patients undergoing coronary artery bypass grafting, the urinary and plasma levels of TXB_2 and 6-keto $PGF_{1\alpha}$ increased, and a significant correlation (r = 0.87, p less than 0.01) was observed between the urinary NAG and TXB_2 levels [18]. Low-dose prostaglandin E_1 (PGE_1), which has a similar function to that of PGE_2, was also found to prevent deterioration of the renal function under surgical anesthesia in 109 adult patients [19]. Furthermore, patients with acute oliguric renal failure immediately after transplantation show high urinary PGE_2 concentrations, and rejection crises are characterized by a two-fold increase in urinary PGE_2 excretion [20].

To date, the performance of urinary prostanoid metabolites for predicting AKI in critically ill patients has not been examined. Therefore, in the present study, we aimed to evaluate the potential of urinary prostanoid metabolites in diagnosing and predicting AKI in heterogeneous ICU patients.

Materials and methods

Study design and population

The current study was conducted as a prospective observational study. We prospectively collected data for adult patients > 20 years of age admitted to the ICU at Okayama University Hospital, Okayama, Japan, between November 2010 and July 2011. Ninety-three Japanese patients were randomly enrolled in total, and written informed consent was obtained from each subject. However, if prior consent could not be obtained due to critical illness or the use of sedatives or anesthetics, delayed consent was obtained [21]. The study protocol was approved by the Institutional Ethics Review Board of Okayama University Hospital (No. 673). This study was registered with the Clinical Trial Registry of the University Hospital Medical Information Network (registration number: UMIN 000004503). End-stage renal disease (ESRD) was defined as chronic kidney failure treated with either dialysis or transplantation and non-dialyzed patients, whose baseline eGFR was less than 15 mL/min/1.73 m^2. Patients with ESRD and those who were discharged within 24 hours after admission to the ICU were excluded.

The following clinical variables were evaluated: age, sex, body mass index (BMI), reason for admission (medical issue or type of surgery), complications of diabetes mellitus, a diagnosis of sepsis, as defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee [22], the use of contrast exposure within 48 hours preceding ICU admission, prior medication with non-steroidal anti-inflammatory drugs (NSAIDs), the use of diuretics or human atrial natriuretic peptide (hANP) medications during the ICU stay, the initiation of RRT, in-hospital mortality and the length of in-hospital and ICU stay. Disease severity was evaluated according to the Acute Physiology and Chronic Health Evaluation (APACHE) II score within 24 hours of ICU admission [23].

The primary outcome was the development of AKI within seven days after ICU admission, according to the Acute Kidney Injury Network (AKIN) classification [24]. The presence of AKI was assessed daily by calculating the change in sCr from baseline to the maximum sCr level and based on the cumulative daily urine output. The severity of AKI was categorized according to the AKIN classification [24], as follows: stage 1, an increase in sCr of 0.3 mg/dl or 1.5-2 times the baseline level or a reduction in urine output (< 0.5 mL/kg/hour) for six hours; stage 2, an increase in sCr 2-3 times the baseline level or a reduction in urine output (< 0.5 mL/kg/hour) for 12 hours; stage 3, an increase in sCr more than 3 times the baseline level or a sCr level greater than 4.0 mg/dl after a rise of at least 0.5 mg/dl and/or the need for acute RRT or a reduction in urine output (< 0.5 mL/kg/hour) for 24 hours or anuria for 12 hours.

The baseline renal function of all 93 patients was assessed based on a retrospective analysis of the sCr levels recorded one to two months prior to ICU admission. Additionally, the sCr level at the time of ICU discharge was evaluated. The GFR was estimated according to the Modification of Diet in Renal disease equation for Japanese [25].

In the AKI group, established AKI was defined as a diagnosis of AKI by Day 1 (the day after ICU admission), and newly diagnosed AKI was defined as a diagnosis of AKI after Day 1, and with subsequent fulfillment of the AKIN criteria within one week.

Measurement of urinary biomarkers

After ICU admission (Day 0), urinary collection was initiated until the following morning (Day 1). Serum samples were obtained on Days 1 and 3. The samples were stored at -80°C until use soon after centrifugation. All samples were aliquoted and stored to avoid repeated cycles of freezing and thawing. Therefore, the duration of storage in the freezer did not differ between the groups.

EIA for PGE_2, 2,3-dinor-6-OXO-$PGF_{1\alpha}$ and 11-dehydro-TXB_2

Enzyme immunoassays (EIAs) of the urinary levels of human PGE_2, 2,3-dinor-6-OXO-$PGF_{1\alpha}$ and 11-dehydro-TXB_2 were performed using the EIA kit for each prostanoid according to the manufacturer's instructions (Cayman Chemical, USA). All samples were examined in duplicate, and the mean value for an individual's serum was utilized for the statistical analysis. The mean minimum detectable dose of PGE_2 2,3-dinor-6-OXO-$PGF_{1\alpha}$ and 11-dehydro-TXB_2 was 15.6 pg/mL, 78 pg/mL and 15.6 pg/mL, respectively. In each assay, we observed a proper standard curve using serial dilutions of recombinant human prostanoids as described in the manufacturer's instructions. The stability of the urinary samples with respect to each prostanoid was confirmed after freezing and thawing (data not shown).

Other clinical parameters

The concentrations of sCr, blood urea nitrogen (BUN), white blood cells (WBCs) and C-reactive protein (CRP) were measured using routine laboratory methods (SRL, Inc., Okayama, Japan). The urinary levels of albumin, NAG and creatinine were also determined (SRL, Inc.), and the serum and urinary creatinine levels were measured according to the enzymatic colorimetric method.

Statistical analysis

All values are expressed as proportions or the median [interquartile range]. Continuous variables were compared using the Wilcoxon rank-sum test, while categorical variables were expressed as proportions and compared using the chi-squared test. The correlation analyses were performed using Spearman's test. The predictive performance of the urinary biomarkers was determined using a ROC curve analysis, and a p value of < 0.05 was considered to be

statistically significant. All statistical analyses were performed using the JMP version 9.0 software program (SAS Institute, Inc., Cary, NC).

Results

Characteristics of study groups

Of the 93 patients, 24 (25.8%) developed AKI within seven days after ICU admission (**Table 1**). Among all AKI patients, fourteen (58.3%) developed AKIN stage 1 disease, eight (33.3%) developed AKIN stage 2 disease and two (8.3%) developed AKIN stage 3 disease. The median age was 66 years in the non-AKI group and 60 years in the AKI group, although the difference was not statistically significant. The leading cause of ICU admission was surgical intervention in 92.8% of the patients in the non-AKI group and 75% of the patients in the AKI group. In the AKI group, two patients (8.3%) required RRT and one patient died. The length of both ICU and hospital stay was significantly longer in the AKI group than in the non-AKI group. In this study, CKD was defined by the baseline eGFR under 60 mL/min/1.73 m^2. There were 16 patients with CKD (n=7 for AKI group, n=9 for Non-AKI) as shown in Table 1. CKD stage of those patients is shown in Supplementary table 1. As compared with patients in the CKD stage 3a, patients in the CKD stage 3b developed AKI more frequently.

There were no differences with respect to age, BMI or the use of NSAIDs prior to admission to the ICU. In addition, the AKI group exhibited higher APACHE II scores than the non-AKI group, whereas the mean baseline eGFR values were similar between the groups.

Estimated causes of AKI

The two major causes of developing AKI in the hospital are prerenal disease and acute tubular necrosis (ATN), although AKI occurs due to multifactorial etiologies. In the present study population, AKI most commonly occurred in postoperative patients, particularly recipients of liver transplantation (**Table 2**).

Associations between the urinary biomarkers and the fold increase in sCr

The ratio of the serum Cr level on Day 1 (after admission to the ICU) to that observed at baseline was determined as an indicator of a rapid decline in the renal function. The fold-increase in sCr positively correlated with both the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and urinary 11-dehydro-TXB_2/Cr ratios, but not the urinary PGE_2/Cr ratio (**Fig. 1**).

Associations between the urinary prostanoids and other urinary markers

We evaluated the potential correlations between the levels of urinary prostanoid metabolites and the other urinary parameters. Significant positive correlations were observed between the urinary 2, 3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and urinary NAG/Cr ratios (r = 0.60, p < 0.0001, **Fig. 2b**) and between the urinary 11-dehydro-TXB_2/Cr and urinary NAG/Cr ratios (r = 0.75, p < 0.0001, **Fig. 2c**) in all cases. In contrast, there were no significant correlations between the urinary PGE_2/Cr and urinary NAG/Cr ratios (**Fig. 2a**) or between the urinary prostanoid/Cr and urinary albumin/Cr ratios (**Fig. 2d-f**).

Comparison of the efficacy of the urinary biomarkers for detecting and predicting of AKI

Newly diagnosed AKI was defined as a diagnosis of AKI after Day 1 (the day after ICU admission), with subsequent fulfillment of the AKIN criteria within one week. In 16 cases of de novo AKI, the urinary 2,3-dinor-6-OXO- $PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values were significantly elevated compared with that observed in the non-AKI group (18,477 vs. 8,277 ng/gCr, p = 0.002; 18,637 vs. 7,926 ng/gCr, p < 0.002), whereas the urinary PGE_2/Cr values were not (**Table 3**). The urinary concentrations of 2,3-dinor-6-OXO-$PGF_{1\alpha}$ and 11-dehydro-TXB_2 were also significantly elevated in the AKI cases compared with those observed in the non-AKI cases. In addition, each parameter in pooled urine showed similar findings to the

results obtained by normalizing the levels of the urinary metabolites to that of creatinine (**Table 4**).

We also evaluated the performance of the urinary biomarkers in the subgroup without liver transplantation (**Supplementary Table 2**) to exclude the possibility that these biomarkers were affected by liver transplantation in our population. In 16 cases of total AKI and 9 cases of newly diagnosed AKI, the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values were significantly elevated compared with that observed in the non-AKI group. These results were similar to all analysis including the cases with liver transplantation.

ROC-AUC analysis for the detection and prediction of AKI

We next performed a ROC-AUC analysis to evaluate the performance of the urinary concentrations of prostanoids in detecting and predicting AKI, as shown in **Fig. 3**. The best AUC for the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$ levels was 0.75, compared with 0.71 for the urinary 11-dehydro-TXB_2 levels (**Table 5**). Meanwhile, the AUC for the urinary PGE_2/Cr ratio was much lower than that for the other two prostanoids.

Associations between the quartiles of the urinary levels of prostanoids and the outcomes

We next graded the levels of urinary prostanoids into quartiles and evaluated their capacity to predict AKI (**Table 6**). After adjusting for clinical variables, such as age, gender, baseline sCr, the use of NSAIDs, sepsis, contrast agents and type of admission, the highest quartiles (Q4) of the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values were found to be associated with a 54- and 8-fold adjusted odds ratio, respectively, when compared with the lowest quartiles (Q1).

Comparison of the efficacy of the urinary biomarkers in predicting the outcomes

We next evaluated the correlations between the levels of urinary prostanoids and various outcomes (**Fig. 4 and 5**). In the AKI group, the urinary PGE_2/Cr ratio exhibited an inverse correlation with both the length of ICU stay (r = -0.28, p = 0.007, **Fig. 4a**) and the length of hospital stay (r = -0.25, p = 0.015, **Fig. 4d**). In all subjects, there was a positive correlation between the urinary 11-dehydro-TXB_2/Cr ratio and both the length of ICU stay (r = 0.27, p = 0.008, **Fig. 5c**) and the length of hospital stay (r = 0.26, p = 0.01, **Fig. 5f**). In contrast, no significant correlations were observed between the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratios and outcomes (**Fig. 4 and 5**).

Discussion

AKI is common in critically ill patients in the ICU and is associated with significant morbidity and mortality [26]. At present, AKI is diagnosed according to the serum creatinine level and urinary output [27]. Numerous investigational biomarkers have been evaluated to detect AKI preceding a rise in the serum creatinine level. For example, NGAL, KIM-1, IL-18 and L-FABP are representative biomarkers for AKI [4, 28, 29]. In the present study, we investigated the levels of urinary prostanoid metabolites in heterogeneous ICU patients and demonstrated that an increase in the urinary levels of 2,3-dinor-6-OXO-$PGF_{1\alpha}$ and 11-dehydro-TXB_2, could be used to predict and detect the onset of AKI.

In general, prostanoids are synthesized and exert biological effects locally including kidneys. They are rapidly inactivated/metabolized locally or during pulmonary circulation, thus they do not circulate systemically. In the kidneys, glomeruli produce both PGI_2 and PGE_2, while tubules primarily synthesize PGE_2 [15]. In the recent clinical study, prophylactic administration of the prostacyclin analog iloprost protected against contrast-induced nephropathy in high-risk patients undergoing a coronary procedure [30]. TXA_2 exerts the opposite effects to PGI_2 and has been shown to be elevated in a glycerol-induced acute renal failure (ARF) model [17], whereas selective inhibition of its synthesis protects rats against ARF induced by glycerol [31]. PGE_2 also affects vascular tone and the epithelial cell function and modulates renal sodium and water excretion [15, 16]. Furthermore, treatment with PGE_2 improves the course of established nephritis by limiting inflammation, blunting the immune response and alleviating oxidative stress.

Urinary NAG is a well-known parameter of acute tubular injury. In the present cohort composed of adult heterogeneous ICU patients, we confirmed significant positive correlations between the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and urinary NAG/Cr values and between the

urinary 11-dehydro-TXB_2/Cr and urinary NAG/Cr values in all cases. Our data are consistent with those of a previous study demonstrating a significant correlation between the urinary NAG and TXB_2 levels in patients undergoing cardiopulmonary bypass surgery [32]. In addition, the urinary albumin level serves as a biomarker for the early diagnosis of AKI, being specific to intrinsic (renal) causes of AKI and not altered by AKI due to prerenal or postrenal etiologies [33]. In the setting of AKI, tubular injury results in dysfunction of the proximal tubules in reabsorbing albumin, thus leading to albuminuria. In addition, previous reports have demonstrated that albuminuria can be attributed to direct increases in albumin gene transcription in the renal cortex in animal models of intrinsic AKI [34, 35]. Interestingly, the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values were not found to significantly correlate with the urinary excretion of albumin in the present study. On the other hand, a significant positive correlation was observed between the urinary PGE_2/Cr and urinary Albumin/Cr ratios.

In general, urinary biomarkers are considered to reflect the state of the kidney including glomerular and tubular portions. Histological alterations including acute tubular necrosis is observed in AKI as extensively studied in experimental models of AKI. In this study, the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$ and 11-dehydro-TXB_2 levels were elevated in the AKI patients. Accordingly, we speculate that urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$ and 11-dehydro-TXB_2 were derived from damaged tubular epithelial cells, at least in part, and their levels may thus reflect the presence of tubular injury accompanied by AKI.

Among the prostanoid metabolites evaluated in the present analysis, the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr ratios were significantly elevated in the newly diagnosed AKI and total AKI groups compared with that observed in the non-AKI group, whereas the urinary PGE_2/Cr ratios were not. A recent report by Doi et al. evaluated the urinary levels of L-FABP, N-gal, IL-18, NAG and albumin in a mixed ICU population [36]. The

authors observed marked elevation of the urinary L-FABP levels in the established AKI patients, rather than in those with newly diagnosed AKI. In contrast, in the present study, the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values were significantly elevated in the newly diagnosed AKI patients, rather than the established AKI patients. Therefore, elevated urinary values of 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr may reflect distinct mechanisms predisposing patients to developing AKI. Spot urine measurements were obtained in the study described above [36], whereas we utilized collected urine samples, which may account for the discrepancy in findings. A previous study by Waikar S.S. et al. suggested [37] that the most accurate method for quantifying biomarkers requires the collection of timed urine specimens to estimate the actual excretion rate, provided that the biomarkers are stable over the period of collection [37]. Urinary metabolites of prostanoids are considered to be stable, and we observed equivalent results by normalizing the levels of the urinary metabolites to that of creatinine and assessing the concentrations in pooled urine.

The urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratio exhibited the best diagnostic and predictive performance among the three prostanoid metabolites, as confirmed in the ROC analysis (ROC-AUC: 0.75). In comparison with the results obtained by Doi et al. [36], the ROC-AUC for the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratios was equivalent to that of urinary L-FABP in detecting AKI. Importantly, the type of admission differed between the present and previous study. For example, the majority of patients were admitted for elective surgery rather than medical reasons in the current study. In spite of this difference in patient background characteristics, the performance of the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratio is considered to be excellent, similar to that of urinary L-FABP or NGAL, suggesting the potential usefulness of this biomarker in predicting and managing AKI.

After adjusting for clinical variables, the highest quartiles of the 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values were found to be associated with a 54- and 8-fold

adjusted odds ratio, respectively, when compared with the lowest quartiles. Collectively, these results demonstrate the potential usefulness of evaluating the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and 11-dehydro-TXB_2/Cr values in patients newly admitted to the ICU in order to predict the onset of AKI. Considering the renoprotective role of PGI_2 in the setting of AKI and other renal disorders, we speculate that the synthesis of PGI_2 is increased in patients at an elevated risk for AKI in a compensatory manner. We recently reported renoprotective effects of ONO-1301, a novel sustained-release prostacyclin analogue in experimental models of diabetic nephropathy and obstructive uropathy [12, 14]. ONO-1301 might have therapeutic effects on AKI, and the measurement of urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr might be a suitable biomarker in considering the indication of ONO-1301 and assessing the therapeutic effects in patients with AKI. The majority of PGI_2 is produced by the glomeruli in human kidneys, whereas the synthesis of PGI_2 by the inner medulla is dominant in rodents [15]. Immunohistochemical analysis demonstrated that prostacyclin synthetase was localized to the peritubular capillaries, renal interstitial cells and glomerular mesangial cells [38]. The mechanism of the excretion of PGI_2 from tubular epithelial cells is still unclear, however COXs and PGIS mRNA were present in the whole kidney *in vivo* and endogenous PGI_2 protects renal tubular cells from doxorubicin-induced apoptosis through interacting with peroxisome proliferator-activated receptor-α (PPAR-α) [39]. It is also possible that the circulating levels of PGI_2 are elevated in the high-risk populations, resulting in elevated urinary values of 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr. Since we did not determine the circulating levels of prostanoids in the present study, this possibility remains to be investigated. A recent report assessed the influence of surgical tissue injury on the levels of urinary prostaglandin metabolites. In patients receiving surgery, urinary levels of PGE_2/Cr was elevated as compared to those levels obtained before surgery, but urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr levels were not elevated [40]. Therefore,

elevated urinary levels of 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr in the current study may not reflect systemic injuries due to surgical operations.

Oxidative stress and hypoxia/ischemia in the renal tubulointerstitial microenvironment are known potential mechanisms of AKI. Ischemia/hypoxia in the peritubular capillaries may induce the enhanced synthesis of PGI_2, thereby resulting in elevated urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratios in high-risk populations.

As previously reported in the experimental model of AKI in rodents, the TXA_2 levels are elevated in the setting of AKI [17], consistent with our present observation in ICU patients. In this regard, the elevated urinary 11-dehydro-TXB_2/Cr values observed in high-risk populations may reflect early renal injury and susceptibility to *de novo* AKI.

In this study, we also evaluated the length of ICU and hospital stay in association with the prognosis. In all patients, the urinary 11-dehydro-TXB_2/Cr ratios were found to be positively correlated with both the length of ICU and hospital stay. In addition, in the AKI group, the urinary PGE_2/Cr ratio exhibited an inverse correlation with the length of ICU and hospital stay. Further studies should evaluate whether urinary prostanoid metabolites can serve as prognostic markers of renal outcomes, such as end-stage renal disease.

There are several limitations associated with the current study. First, the degree of AKI was relatively mild, without the need for RRT, and the mortality rate was low. In addition, the background characteristics of the patients, i.e. the reason for ICU admission, may differ from those observed in other studies. Second, pooled urine samples were used instead of spot urine samples in this study. However, the urinary metabolites of the evaluated prostanoids are stable, and the results obtained based on the concentrations in pooled urine were similar to those obtained by normalizing the levels of the urinary metabolites to that of creatinine.

Conclusion

The urinary levels of PGI_2 and TXA_2 metabolites on admission to the ICU may serve as prognostic and diagnostic markers of AKI. Further studies are warranted to evaluate the usefulness of these parameters in ICU patients with distinct etiological backgrounds.

Conflict of interests

Financial competing interests

Prof. Yohei Maeshima belonged to endowed department by Chugai pharmaceutical, MSD, Boehringer ingelheim and Kawanishi Holdings. Prof. Hitoshi Sugiyama belongs to endowed department by Baxter. Prof. Hirofumi Makino is a consultant for AbbVie, Astellas and Teijin, receives speaker honoraria from Astellas, Boehringer-ingelheim, Chugai, Daiichi Sankyo, Dainippon Sumitomo, Kyowa Hakko Kirin, MSD, Novartis, Pfizer, Takeda, and Tanabe Mitsubishi, and receives grant support from Astellas, Boehringer-ingelheim, Daiichi Sankyo, Dainippon Sumitomo, Kyowa Hakko Kirin, Mochida, MSD, Novartis, Novo Nordisk, Pfizer, Takeda, and Tanabe Mitsubishi.

Non-financial competing interest

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Table 1. Patient characteristics and clinical outcomes

	Non-AKI (n=69, 74.2%)	AKI (n=24, 25.8%)	*P*-value (vs. Non-AKI)
Age, yrs	66.0 [59.0-71.5]	60[52.5-68.7]	0.075
Male, *n* (%)	52 (75.4%)	17 (24.6%)	0.0077
BMI, kg/m^2	21.6 [19.0-24.3]	24.0 [20.1-27.6]	0.0814
Patient type, *n* (%)			0.018
Medical	5 (7.3%)	6 (25.0%)	
Elective surgery	59 (85.5%)	14 (58.3%)	
Emergency surgery	5(7.3%)	4 (16.7%)	
APACHE Ⅱ	12 [8.5-15]	14.5 [10-19.8]	0.0438
Diabetes, *n* (%)	13 (19.1%)	7 (29.1)	0.304
Sepsis, *n* (%)	5 (7.2%)	4 (16.7%)	0.179
Contrast exposure in preceding 48 hrs. *n* (%)	13 (18.8%)	4 (16.7%)	0.812
NSAIDs, *n* (%)	7 (10.1%)	1 (4.17%)	0.368
Medication used during ICU stay (initial 7 days), *n* (%)			
Furosemide	18 (26.1%)	20 (83.3%)	＜0.0001
hANP	4 (5.8%)	12(50.0%)	＜0.0001
Baseline Cr, mg/dl	0.79 [0.68-0.93]	0.78 [0.62-0.97]	0.990
Baseline eGFR, ml/min per 1.73m2	71.5 [61.7-86.2]	72.5 [49.2-92.3]	0.819
Baseline eGFR＜60, n.(%)	9 (13.0%)	7 (29.1%)	0.0714
Day 1 following ICU admission			
WBC, $10^4/\mu l$	8495 [6185-11340]	8195 [5301-11928]	0.8589
CRP, mg/dL	6.32 [3.21-8.90]	5.99 [1.96-11.52]	0.9044
BUN, mg/dL	12.9 [9.7-16.7]	23.6 [14.1-29.9]	0.0003
sCr, mg/dL	0.77 [0.63-0.93]	0.94 [0.64-1.29]	0.0735
sAlbumin, mg/dL	2.5 [2.2-2.9]	3 [2.6-3.3]	0.0057
sCr (ICU discharge), mg/dl	0.66 [0.56-0.79]	0.62 [0.47-0.98]	0.853
Outcome			
ICU stay, day	6.0 [5.0-8.5]	11.5 [8.5-21]	＜0.0001
ICU stay, hrs	114.2 [86.4-171.3]	244.7 [188.5-471.0]	＜0.0001
RRT during ICU stay, *n* (%)	0	2 (8.3%)	0.0153
Hospital mortality, *n* (%)	2 (2.9%)	1 (4.2%)	0.762

Abbreviations: BMI, body mass index; NSAIDs, non-steroidal anti-inflammatory drugs; ICU, intensive care unit; eGFR, estimated glomerular filtration rate; WBC, white blood cell; CRP, C-reactive protein; BUN, blood urea nitrogen
The baseline Cr level was measured approximately 1-2 months before ICU admission.
The values are expressed as the median [interquartile range].

Table 2. Estimated causes of the ICU admission

Estimated causes for the ICU admission	Non-AKI (n=69)	AKI (n=24)
Surgery for solid tumor	47	6
Liver transplantation	3	8
Surgery for benign disease	4	0
Cardiac surgery	5	0
Pneumonia, respiratory failure	4	3
peritonitis	0	2
Abscess	2	0
Infective endocarditis, Myocarditis	2	1
Severe pancreatitis (MOF)	0	1
Ileus	0	1
Rupture of an abdominal aortic aneurysm	0	1
Hemorrhagic shock	0	1
Subarachnoid hemorrhage	2	0

Abbreviations: MOF, multiple organ failure

Table 3. Comparison of the efficacy of the urinary biomarkers (normalized to the urinary creatinine concentration) in detecting and predicting AKI

	Non-AKI (n=69)	Total AKI (n=24) Established AKI (n=8) Newly Diagnosed AKI (n=16)	*P*-value (vs. Non-AKI)
PGE_2/Cr, ng/gCr	706.8 [417.5-988.2]	926.3 [442.6-1693.4]	0.145
		983.2 [432.4-1903.1]	0.249
		926.3 [442.6-1630.0]	0.276
2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr, ng/gCr	8276.9 [5556.9-14739.8]	17197.6 [9627.2-40883.1]	[a]0.0002
		14737.4 [9627.2-60058.5]	0.058
		18477.4 [9994.9-40883]	[a]0.0006
11-dehydro-TXB_2/Cr, ng/gCr	7925.9 [4910.0-15621.1]	15176 [10350.8-41583.3]	[a]0.0022
		11264.3 [6456.3-111983.6]	0.229
		18636.7 [11639.8-41583.3]	[a]0.0018

AKI, acute kidney injury
The values are presented as the median [interquartile range].
[a]$P < 0.01$

Table 4. Comparison of the efficacy of the urinary biomarkers (absolute concentrations) in detecting and predicting AKI

	Non-AKI (n=69)	Total AKI (n=24) / Established AKI (n=8) / Newly Diagnosed AKI (n=16)	*P*-value (vs. Non-AKI)
PGE_2, pg/mL	363.6 [204.4-674.2]	426.1 [207.9-917.4]	0.375
		669.0 [415.2-1216.9]	0.146
		401.0 [179.4-917.4]	0.875
2,3-dinor-6-OXO-$PGF_{1\alpha}$, pg/mL	6131.6 [2941.2-9408]	10705 [5086.5.2-22473.1]	[b]0.0062
		11085.6 [7579.4-22924.5]	[a]0.0222
		9892.2 [4612.7-22473.1]	[a]0.0491
11-dehydro-TXB_2, pg/mL	4920.4 [2534.3-8890.5]	12290 [4390.7.8-31624]	[b]0.0055
		13184 [4468.6-57830]	0.0592
		11017.9 [3722.9-25184]	[a]0.0225

AKI, acute kidney injury
The values are presented as the median [interquartile range].
[a]$P < 0.05$, [b]$P < 0.01$

Table 5. ROC-AUC evaluation of the performance of the biomarkers for detecting and predicting AKI

	AUC	Cut-off point (ng/gCr)	Sensitivity	Specificity	PPV	NPV
PGE_2/Cr, ng/gCr	0.60	908.3	0.54	0.71	0.38	0.81
2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr, ng/gCr	0.75	8512.0	0.92	0.53	0.4	0.95
11-dehydro-TXB_2/Cr, ng/gCr	0.71	10005.7	0.79	0.59	0.36	0.87

N=93. Non-AKI: 69, AKI: 24, including established (n=8) and newly diagnosed AKI (n=16) patients

Abbreviations: AUC, area under the receiver operator characteristic (ROC) curve; NPV, negative predictive value; PPV, positive predictive value

Table 6. Association between the biomarker quartile and outcome

	AKI			
Quartile (Cut point)	Unadjusted OR (95% Cl)	*p*-value	Adjusted OR (95% Cl)	*p*-value
PGE_2/Cr, ng/gCr				
Q1 (<425.9)	1 (referent)		1 (referent)	
Q2 (425.9 to 750)	0.4 (0.1, 1.9)	0.26	0.48 (0.08, 3)	0.48
Q3 (750 to 1107.4)	0.94 (0.2, 3.6)	0.93	0.85 (0.2, 4.4)	0.85
Q4 (≧1107.4)	1.82 (0.5, 6.6)	0.34	0.45 (0.4, 8.9)	0.38
unadjusted *p* for trend	0.2392			
adjusted *p* for trend			<0.0095	
2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr, ng/gCr				
Q1 (<6239.1)	1 (referent)		1 (referent)	
Q2 (6239.1 to 10418.1)	2.8 (0.5, 20.9)	0.24	7.6 (0.85, 184)	0.07
Q3 (10418.1 to 16541.1)	2.2 (0.4, 17.3)	0.38	0.6 (0.03, 15)	0.72
Q4 (≧16541.1)	13.7 (3.0, 98.8)	0.0003	54 (5.2, 1909)	0.0002
unadjusted *p* for trend	0.0016			
adjusted *p* for trend			<0.0001	
11-dehydro-TXB_2/Cr, ng/gCr				
Q1 (<5178.6)	1 (referent)		1 (referent)	
Q2 (5178.6 to 10005.7)	0.95 (0.2, 5.7)	0.96	1.2 (0.15, 9.6)	0.87
Q3 (10005.7 to 19142.9)	2.9 (0.7, 15)	0.15	4.3 (0.77, 32)	010
Q4 (≧19142.9)	6.1 (1.5, 31)	0.0087	8.0 (1.4, 62)	0.017
unadjusted *p* for trend	0.0169			
adjusted *p* for trend			<0.0015	

Multiple logistic regression analysis
Adjusted for age, sex, baseline Cr, NSAIDs, sepsis, contrast agent and admission type

Figure Legends

Figure 1. Ratios of the serum Cr level on Day 1 to that observed at baseline and the ratios of prostanoids/Cr in all patients

The serum Cr level on Day 1 was measured the day after admission to the ICU. The baseline Cr level was measured approximately 1-2 months prior to ICU admission. (a) The urinary PGE_2/Cr ratio was not found to be associated with the ratio of the serum Cr level on Day 1 (after admission to the ICU) compared to that observed at baseline (r = -0.005, p = 0.96). (b, c) The fold-increase in s-Cr was positively correlated with the urinary 2, 3-dinor-6-OXO-$PGF_{1\alpha}$/Cr ratio and urinary 11-dehydro-TXB_2/Cr ratio (r = 0.57, $p < 0.0001$ and r = 0.47, $p < 0.0001$, respectively).

Figure 2. Associations between the levels of urinary prostanoids and other urinary markers

Associations between the levels of urinary prostanoids and other urinary markers in all cases. (a-c) Associations between the levels of urinary prostanoids and urinary albumin. (d-f) Associations between the levels of urinary prostanoids and urinary NAG. (a) There were no significant correlations between the urinary PGE_2/Cr ratio and the urinary NAG/Cr ratio (r = 0.08, p = 0.44). (b, c) Significant positive correlations were observed between the urinary 2, 3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and urinary NAG/Cr ratios (r = 0.60, $p < 0.0001$) and between the urinary 11-dehydro-TXB_2/Cr and urinary NAG/Cr ratios (r = 0.75, $p < 0.0001$). (d) A tendency toward a positive correlation was observed between the urinary PGE_2/Cr ratio and urinary albumin/Cr ratio only (r = 0.26, p = 0.01). (e, f) No significant correlations were observed between the urinary 2, 3-dinor-6-OXO-$PGF_{1\alpha}$/Cr and urinary albumin/Cr ratios (r = 0.03, p = 0.78) or between the urinary 11-dehydro-TXB_2/Cr and urinary albumin/Cr ratios (r = 0.02, p = 0.87). Abbreviations: Alb, albumin; NAG, N-acetyl- β -D-glucosaminidase.

Figure 3. ROC-AUC analysis for the detection and prediction of AKI

Diagnostic performance of the urinary PGE_2 (ng/g creatinine), 2,3-dinor-6-OXO-$PGF_{1\alpha}$ (ng/g creatinine) and 11-dehydro-TXB_2 (ng/g creatinine) levels in detecting and predicting AKI. Among the 93 critically ill patients, 24 (25.8%) were diagnosed with AKI within one week. The AUC-ROC values are presented in Table 5. Abbreviations: AUC, area under the curve; ROC, receiver operating characteristic.

Figure 4. Comparison of the efficacy of the urinary biomarkers in predicting the outcomes in the AKI group

Correlations between the levels of urinary prostanoids and various outcomes in the AKI group (n=24). (a, d) The urinary PGE_2/Cr ratio exhibited an inverse correlation with both the length of ICU stay (r = -0.28, p = 0.007) and the length of hospital stay(r = -0.25, p = 0.015). (b, c) No significant correlations were observed between the length of ICU stay (hours) and the urinary 2, 3-dinor-6-OXO-$PGF_{1\alpha}$/Cr or urinary 11-dehydro-TXB_2/Cr values (r = 0.02, p = 0.87 or r = 0.15, p = 0.16, respectively). The reciprocal relationships between the urinary 2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr values and the length of hospital stay (days: e) and between the urinary 11-dehydro-TXB_2/Cr values and the length of hospital stay (days: f) are shown.

Figure 5. Comparison of the efficacy of the urinary biomarkers in predicting the outcomes in all patients

Correlations between the levels of urinary prostanoids and various outcomes in all patients (n=93). Non-AKI samples (open circles) and AKI samples (plus symbols) are shown. (c, f) There was a positive correlation between the urinary 11-dehydro-TXB_2/Cr and both the length of ICU stay (r = 0.27, p= 0.008) and the length of hospital stay (r = 0.26, p = 0.01). (a, b,

d, e) No significant correlations were observed between the urinary PGE_2/Cr or 2, 3-dinor-6-OXO-$PGF_{1\alpha}$/Cr values and outcomes.

Supplemental Table 1. CKD Patient in this study

Baseline eGFR＜60, *n*(%)	Non-AKI (n=9)	AKI (n=7)
Stage 3a, 45≦Baseline eGFR＜60	7	3
Stage 3b, 30≦Baseline eGFR＜45	1	3
Stage 4, 15≦Baseline eGFR＜30	1	1

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; AKI, acute kidney injury
The baseline eGFR was calculated from serum Cr measured approximately 1-2 months before ICU admission.

Supplemental Table 2. Comparison of the efficacy of the urinary biomarkers (normalized to the urinary creatinine concentration) in detecting and predicting AKI (without liver transplantations)

	Non-AKI (n=66)	Total AKI (n=16) Established AKI (n=7) Newly Diagnosed AKI (n=9)	*P*-value (vs. Non-AKI)
PGE_2/Cr, ng/gCr	689.1 [402.0-982.1]	926.3 [464.2-1693.4]	0.157
		828.4.2 [323.4-1696.8]	0.477
		944.3 [505.3-1698.5]	0.176
2,3-dinor-6-OXO-$PGF_{1\alpha}$/Cr, ng/gCr	8221 [5478.2-14475.8]	16442 [9191.7-33493.1]	[b]0.0071
		11622.6 [9533.6-19108.1]	0.134
		16543.2 [8794.9-43361.1]	[a]0.0145
11-dehydro-TXB_2/Cr, ng/gCr	7593.6 [4865.1-15534.4]	14754.4 [7370.9-39916.8]	[a]0.0199
		10005.7 [6444.2-24084.9]	0.454
		20287.5 [11486.1-54499.2]	[a]0.0095

AKI, acute kidney injury
The values are presented as the median [interquartile range].
[a]$P < 0.05$, [b]$P < 0.01$