Efficacy of urinary Midkine as a biomarker in patients with acute kidney injury

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Abstract

Background: The mortality and morbidity associated with acute kidney injury (AKI) remains high, despite advances in interventions. A multifunctional heparin-binding growth factor, Midkine (MK), is involved in the pathogenesis of ischemic kidney injury. However, the clinical relevance of MK has not yet been elucidated. The present study investigated whether urinary MK can serve as a novel biomarker of AKI.

Methods: We initially compared the predictive value of MK with other urinary biomarkers, including N-acetyl-β-D-glucosaminidase (NAG), interleukin (IL)-18 and neutrophil gelatinase-associated lipocalin (NGAL), for the detection and differential diagnosis of established AKI (549 patients). Subsequently, the reliability of MK for the early detection of AKI was prospectively evaluated in 40 patients undergoing elective abdominal aortic aneurysm surgery. Urine samples were obtained at baseline, the period of aortic cross-clamping and declamping, the end of the surgery, and on post-operative day 1.

Results: The areas under the receiver operating characteristic curves for the diagnosis of AKI in various kidney diseases were 0.88, 0.70, 0.72, and 0.84 for MK, NAG, IL-18, and NGAL, respectively. When the optimal cut-off value of urinary MK was set at 11.5 pg/mL, the sensitivity and specificity were 0.87 and 0.85, respectively. In the second study, urinary MK peaked at the period of aortic declamping, about 1 hour after cross-clamping in patients with AKI. Interestingly, the rise of MK in AKI patients was very precipitous compared with other biomarker candidates.

Conclusion: Urinary MK was prominent in its ability to detect AKI and may allow the start of preemptive medication.

Introduction

Acute kidney injury (AKI) develops due to complex interactions between acute insults, including ischemia and toxic, and ultimate tissue damage, occurring over a cycle of minutes to days [1, 2]. Current outcome for treating AKI remains insufficient, despite aggressive medical challenges [3, 4]. Accurate understanding of the process of the tissue damage and kidney dysfunction inevitably leads to the ideal approach to seek appropriate therapeutics. Earlier identification of at-risk patients would be extremely helpful for improving morbidity and mortality rates. Serum creatinine (sCr), typically used to diagnose AKI, is not sensitive or reliable for improving these rates [5, 6]. Therefore, clinical studies to date have emphasized the necessity for exploring new biomarker candidates. These include neutrophil gelatinase-associated lipocalin (NGAL) [7], kidney injury molecule (KIM)-1 [8], N-acetyl-β-D-glucosaminidase (NAG) [9], cystatin C [10] and interleukin (IL)-18 [11]. Since AKI represents various aspects and is caused by multiple complex pathogeneses, many biomarker candidates have not satisfied the requirements with regards to specificity, sensitivity and clinical relevance of validated cut-off values [12]. In addition to earlier detection for AKI, these molecules would be needed to achieve high diagnostic accuracy (area under the curves: AUC > 0.9).

Midkine (MK) is a heparin-binding multifunctional growth factor with biological roles such as cell growth and survival, migration, and chemotaxis [13]. Particularly, it has been implicated in neuronal survival, cancer development and tissue inflammation [14]. In the kidney diseases, the pathophysiological roles of MK are diverse, ranging from the occurrence of AKI to progression of chronic kidney disease, often accompanied by renal ischemia [15], hypertension [16, 17], diabetes mellitus [18, 19] and drug toxicity [20]. MK is originally distributed in renal tubular epithelial cells, but to a lesser extent in endothelial cells. In damaged kidney, MK expression is strikingly induced both in epithelial and endothelial

cells. In the underlying mechanism, oxidative stress, inflammation with macrophage recruitment and endothelial dysfunction involving the activation of renin-angiotensin system might be involved [14].

Early and accurate elucidation inevitably implies to avoid the irreversible consequences of acute tissue necrosis as well as the prevention for the development of AKI. Based on our evidence, MK may be a prime candidate for the elucidation of AKI as a noninvasive diagnostic tool compared to previous reported molecules. The present study examined whether MK can be one of novel biomarker candidates by conducting a cross-sectional study and a prospective observational study. First, the sensitivity and specificity of urinary MK for the differential diagnosis of AKI, was evaluated in the cross-sectional study. Subsequently, we prospectively investigated the value of urinary MK for the early detection of AKI in patients with elective open abdominal aortic aneurysm (AAA) repair surgery.

Subjects and Methods

Patients and procedures

In a cross-sectional study, spot urine and plasma samples were collected from 582 participants (male, n = 335; female, n = 247), including 33 healthy controls and 549 patients with various renal diseases diagnosed pathologically (n = 491) and clinically (n = 58) at Nagoya University Hospital and affiliated hospitals between November 2003 and June 2007, during renal biopsy for the differential diagnosis of various kidney diseases, or during nephrology consultation because of acute decline in kidney function. Samples from patients with a history of end-stage renal disease or chronic dialysis and post-kidney transplantation were excluded. We defined established AKI as an increase in serum creatinine (≥0.3 mg/dL within 48 hours or ≥50% within 7 days) and/or oliguria (≤400 mL/day). In principle, AKI

was diagnosed at the time of sample collection of plasma, urine and kidney tissues. Urinary and plasma samples were centrifuged to remove cellular components and debris, and then supernatants were stored at -80°C. All samples were tested using a dipstick before centrifugation. The concentrations of sCr were measured using an enzymatic method (creatininase-sarcosine oxidase-POD).

In a prospective observational study, we prospectively registered 40 patients undergoing elective open surgery for the treatment of non-ruptured AAA between August 2005 and January 2009 at Nagoya University Hospital to investigate the ability of swiftness to detect AKI. Exclusion criteria included the daily use of nephrotoxic drugs (e.g., glycopeptide antibiotics, aminoglycoside antibiotics, non-steroidal anti-inflammatory drugs, and selective COX-2 inhibitors) before or during the study period. Urine samples were obtained from all patients at the start of anesthesia, the period of aortic cross-clamping, the period of aortic declamping, the end of the surgery, and on post-operative day (POD) 1. Baseline sCr levels of all patients were obtained at the last preoperative assessment before index admission. Post-operatively, sCr levels were measured and monitored daily until post-operative day 7. The diagnostic criteria for post-operative AKI were defined according to the current Kidney Disease: Improving Global Outcomes (KDIGO) criteria [21] as an increase in the serum creatinine level (≥0.3 mg/dL within 48 hours or ≥50% within 7 days) and/or reductions in urine output (0.5 mL/kg/h for >6 hours).

Both studies were conducted according to the principles of the Declaration of Helsinki and the Japanese National Ethical Guidelines. Ethical approval was obtained from the institutional review boards of Nagoya University Hospital and the affiliated hospitals (approval number: 1135 and 178013). All patients provided written informed consent.

Measurement of urinary MK, NAG, IL-18, and NGAL

Urinary MK levels were measured using an enzyme-linked immunosorbent assay (ELISA) technique, as previously described [22]. The lowest detectable value of MK is 1.0 pg/mL. Urinary NAG levels were measured using a colorimetric assay (Boehringer Mannheim, Mannheim, Germany). Urinary IL-18 and NGAL values were measured using an ELISA kit according to the manufacturer's instructions (Medical and Biological Laboratories, Nagoya, Japan; BioPorto Diagnostics, Grusbakken, Denmark). All values were measured in duplicate and in a blinded fashion to determine the AKI status.

Statistical analysis

Continuous variables were compared using Student's t-test or the non-parametric Mann-Whitney U-test, as appropriate. Correlations between plasma MK and urinary MK levels were assessed using linear regression analysis. Receiver operating characteristic (ROC) curves were constructed to compare the accuracy of diagnosing AKI following ATN, and diagnostic performance was quantified as areas under curves (AUCs) [23]. The optimal cutoff value was determined by the maximal Youden index (sensitivity + specificity − 1). To examine differences in the longitudinal changes in MK, NAG, IL-18, and NGAL over the course of the open repair of AAA surgery, we evaluated the interaction between the presence of AKI and the time course using a repeated-measures analysis of variance with a mixed effects model. Two-tailed *P*-values of <0.05 were considered statistically significant. Data were statistically analyzed using R, version 2.72 (http://www.r-project.org) and StatView-J 5.0 (SAS Institute, Cary, NC).

Results

Characteristics of patients in the cross-sectional study

Table 1 summarized the clinical characteristics of 582 participants enrolled in the cross-sectional study. This study population includes 33 individuals without a history of kidney or other diseases as healthy controls. All participants were Japanese, aged 53 ± 18 (mean ± standard deviation) years, and were 57.6% male. According to the dipstick test, 89.2% of all 582 patients had proteinuria. The patients included those with AKI (n = 67) and non-AKIs (n = 482). The number of AKI patients stratified by disease categories is as follows: AKI was observed in all 32 patients with acute tubular necrosis (ATN) and in 35 patients with other renal diseases (Figure 1, Table 1). Almost half of total patients with AKI were caused by ATN, resulted from renal ischemia (e.g., hypoperfusion due to cardiogenic factors, sepsis, severe nephrotic syndrome, and hepato-renal syndrome) or toxic tubular injury (e.g., from radiocontrast agents and cytotoxic drugs) [1].

Urinary MK levels in the cross-sectional study

In Figure 2A, the frequency distribution of urinary MK levels in the cross-sectional study is shown. Urinary MK was undetectable in 86.7% of all participants. The detected MK values ranged from 0–1,794 pg/mL, with 99% of participants showing values ranging from 0–500 pg/mL. In contrast, plasma MK values were relatively widely distributed, ranging from 0–25,601 pg/mL (Figure 2B). Urinary MK was statistically associated with plasma MK ($r = 0.368$, $p < 0.0001$), serum creatinine ($r = 0.411$, $p < 0.0001$) and dipstick urinary protein ($\varrho = 0.098$, $p = 0.022$) (Supplemental Figures A-C). Urinary MK levels were markedly higher in patients with ATN than in those with other kidney diseases or in the healthy controls, although urinary MK values were slightly elevated in several other diseases (Figure 3).

In addition to the analysis of urinary MK values in various kidney diseases, we next examined the usefulness of urinary MK in the differential diagnosis of AKI. Urinary levels of MK showed a greater increase in patients with AKI (268 ± 342 pg/mL) than in those with

non-AKI (21 ± 73 pg/mL) or in the healthy controls (2 ± 11 pg/mL) (Table 2). Particularly, urinary MK values in AKI from ATN patients (448 ± 410 pg/mL) are prominent in those of AKI patients from the other kidney diseases (104 ± 128 pg/mL). Based on our data and previous reports [15], severe tubular damage such as ATN caused by ischemia may show greater induction of MK from renal tubules in themselves and inflammatory cells. Collectively, urinary MK may be a useful biomarker candidate for detecting the established AKI, especially AKI from ATN (Table 3).

Comparison of the sensitivity and specificity of MK and other biomarkers in AKI patients

To investigate the ability of MK as a hallmark for AKI, we examined the comparison between urinary MK and novel biomarker candidates such as NAG, IL-18 and NGAL. Besides these biomarker candidates, urinary MK levels were significantly elevated in patients with AKI compared to those with other renal diseases and the healthy controls (Figure 4). ROC curves were generated to evaluate the diagnostic performance as determined with AUCs and the sensitivity and specificity of these biomarker candidates involving MK for detecting AKI (Figure 5). The AUCs were 0.88, 0.70, 0.72, and 0.84 for MK, NAG, IL-18, and NGAL, respectively (Table 4). Additionally, that of plasma MK was 0.68 (data not shown). The AUC of urinary MK was statistically greater than those of NAG and IL-18 ($p < 0.001$) (Figure 5). When the optimal cut-off value of urinary MK as derived from the ROC curve analysis was set at 11.5 pg/mL, the sensitivity and specificity were 0.87 and 0.85, respectively. Urinary MK achieved the diagnostic accuracy of the same extent as NGAL. Taken together, urinary MK would be more reliable for diagnosing AKI, similar to the other biomarker candidates.

Notably, urinary MK showed the highest AUC for the differential diagnosis of ATN (Figure 6). When the optimal cut-off value of urinary MK as derived from the ROC curve

analysis was set at 69.5 pg/mL, the sensitivity and specificity were 0.97 and 0.90, respectively (Table 5). These data suggest the close relationship between MK and ATN.

Characteristics of patients in patients with AKI in the prospective study

As summarized in Table 6, early detection of AKI was prospectively examined in 40 patients undergoing AAA surgery. AKI developed in 12 of 40 patients, and eleven patients with AKI showed the adaptable to the diagnostic criteria of the KDIGO within 48 hours after the end of surgery. No significant differences in the age, sex ratio, estimated glomerular filtration rate and sCr levels before surgery, and aortic cross-cramp duration were found in patients with/without AKI.

Urinary MK level as a biomarker for the early detection of AKI

Urinary MK values showed similar ones in patients with and without AKI before AAA operation. Perioperative urinary MK levels in AKI patients were consistently higher than in those without AKI, and peaked at the time of aortic declamping (~1 hour after clamping) during AAA surgery (Figure 7). Interestingly, the rise of urinary MK in AKI patients after the start of operation was very precipitous compared with other biomarker candidates. This peak then showed a subsequent sharp decline at POD 1. Serum Cr, typically used to diagnose AKI, gradually increased after this procedure and peaked at post-operative day 2 in patients with AKI. Results of the repeated-measures analysis of variance showed a significant interaction between the presence of AKI and the time course for longitudinal changes in MK ($p = 0.02$). However, there was no significant interaction for changes in urinary NAG, IL-18, or NGAL ($p = 0.37$, 0.15, and 0.19 vs. non-AKI, respectively). These data suggest that urinary MK may surpass sCr for detecting early AKI among AKI patients, similar to other biomarker candidates.

Discussion

The biological understanding for AKI allows the start-up of preemptive medication, thus increasing the importance of early biomarkers [4, 12]. To the best of our knowledge from basic research, MK is up-regulated in damaged tubular epithelial cells during the extremely early phase in *in vivo* and *in vitro* studies involving ischemia and hypoxia [14, 15]. On the basis of these findings, renal ischemia provokes AKI via MK induction. The present study demonstrated that the application of MK as a practical biomarker could diagnose AKI at the earliest stage with the greater sensitivity and specificity. Malyszko also documented the usefulness of serum MK in contrast-induced acute kidney injury after coronary angiography [24]. In the present study, however, the sensitivity and specificity of urinary MK as a potential biomarker for AKI were superior to those of serum MK. MK rich in basic amino acids and cysteine residues can thus maintain structural stability, which allows accurate measurements of urinary MK levels. In addition, MK can be easily detected in plasma using our established ELISA technique [25], but is rarely found in urine in healthy individuals. Since MK forms high molecular weight complexes (<250 kDa) with MK-binding proteins in sera, MK cannot be filtrated through the glomerular basement membranes. In spite of the filtration of massive protein from circulating blood in various kidney diseases, indeed, urinary MK levels in patients without AKI are extremely lower. Urinary MK may become one of crucial biomarker candidates to detect early AKI as a noninvasive diagnostic tool.

In the evolving area of clinical research and care, crucial biomarkers would be inevitably required for the specificity and sensitivity and appropriate relevant of validated cut-off values in the earlier stage of AKI [12]. Despite numerous efforts and clinical publications, the requirements remain to be achieved insufficiently. Clinically relevant and validated cut-off values help guide biomarker use to build an appropriate therapeutic strategy.

Indeed, new biomarker candidates are needed to achieve high diagnostic accuracy. The optimal cut-off value of urinary MK could be achieved higher sensitivity and specificity in the differential diagnosis of AKI, especially ATN-induced AKI. Since any morphological evidence of tubular necrosis on biopsy is often either patchy or nonexistent, ATN refer to the clinical syndrome of intrarenal AKI secondary to ischemia and/or nephrotoxicity rather than as a morphological description [26]. In addition, AKI patients in the other kidney diseases, showed a significant rise of urinary MK than non-AKI patients. Subsequently, we compared on the reliability and usefulness of urinary MK with other promising biomarker candidates, including NAG, IL-18, and NGAL. All of these biomarker candidates showed the expected results as an invasive diagnostic tool. Notably, the AUC of MK was excellent, and the biomarker showed higher sensitivity and specificity than NAG and IL-18.

AKI has been reported to develop in 15–33.7% of patients undergoing elective AAA surgery [27, 28]. With increased advanced therapeutics, the frequency of AKI is gradually increasing. There is a common consensus that early recognition of renal dysfunction can be reduced in the intensity of the insult to the kidney tissues by conducting preemptive medication [29]. In our prospective study, urinary MK showed an earlier diagnostic ability for AKI after AAA operation, compared with the sCr typically used to diagnose AKI and the novel markers IL-18 and NGAL. A peak of MK values in AKI patients was found at the period of aortic declamping, which provides severe ischemic exposure. In contrast, established AKI continued to be exposed to various pathogenesis untill effective treatment was started. Higher values of urinary MK were therefore found in the cross-sectional study. Presumably, the performance of AAA surgery would also induce oxidative stress in non-AKI patients, leading to the slight increase of MK expression. Indeed, MK induction is due to the intensity of exposure to hydrogen peroxide in tubular epithelial cells [14]. After reaching a peak, the response then showed a sharp decline at POD 1. In AKI

caused by multifactorial and complex pathogenesis, prediction using a single molecule might be quite difficult [30]. To further improve the accuracy and timeliness for the diagnosis of AKI, MK should time-spatially complement by promising other biomarker candidates, including as IL-18 and NGAL, and which may allow the start of preemptive medication.

The mechanisms underlying multiple organ failure involve several complicated pathway and numerous molecules [1, 2, 12]. These elucidations involving effects on distant organ inevitably leads to the improvement of clinical outcome. Among various concurrent illnesses, AKI may give a serious impact to remote organ, including lung, liver and cardiovascular system. Indeed, its incident has been reported to vary from 5% in hospitalized patients to 30-50% in intensive care units [31, 32]. Combination of biomarker candidates would therefore be needed to achieve higher diagnostic accuracy for diagnosing early AKI. In the near future, a prospective longitudinal cohort of patients with AKI will be assembled to confirm standardized platforms for reliable measurement of promising molecules. We propose that urinary MK may be prominent in its ability to detect earlier AKI as a noninvasive diagnostic tool and allow the stat of preemptive medication.

Conflict of interest

None.

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Table 1. Characteristics of patients in the cross-sectional study (n = 582)

	AKI (n = 67)	non-AKI (n = 482)	Control (n = 33)
Male sex, n (%)	46 (68.7)	274 (56.8)	15 (45.5)
Age, years	57 ± 17	52 ± 18	59 ± 19
Serum creatinine, mg/dL	4.6 ± 4.1	1.2 ± 1.1	0.7 ± 0.1
Clinical and/or pathological diagnosis			
Acute tubular necrosis, n (%)	32 (47.8)	0 (0.0)	
Other kidney diseases, n (%)	35 (52.3)	482 (100)	
MCNS[a], n	4	46	
FSGS[b], n	2	27	
MN[c], n	0	74	
MPGN[d], n	0	9	
AGN[e], n	2	3	
IgAN[f], n	0	124	
Paraprotein-related kidney disease, n	3	12	
Lupus nephritis, n	3	39	
HSPN[g], n	1	9	
AAV[h], n	5	36	
Diabetic nephropathy, n	0	35	
TMA[i], n	5	3	
Benign nephrosclerosis, n	0	15	
TIN[j], n	7	8	
ADPKD[k], n	0	8	
Miscellaneous, n	3	34	

Continuous variables are presented as mean ± standard deviation, and categorical variables as numbers and ratios (%).

[a]minimal change nephrotic syndrome

[b]focal segmental glomerulosclerosis

[c]membranous nephropathy

[d]membranoproliferative glomerulonephritis

[e]acute glomerulonephritis

[f]IgA nephropathy

[g]Henoch-Schönlein purpura nephritis

[b] antineutrophil cytoplasmic antibody-associated vasculitis

[c] thrombotic microangiopathy

[d] tubulointerstitial nephritis

[e] autosomal dominant polycystic kidney disease

Table 2. Urinary MK levels of patients stratified by disease categories (n = 582)

	Total	Patients with various kidney diseases (n = 549)		Controls (n = 33)
		ATN[a] (n = 32)	Other diseases (n = 517)	
AKI[b]	268 ± 342	448 ± 410	104 ± 128	n.a.[c]
Non-AKI	21 ± 73	n.a.	21 ± 73	n.a.
Control	2.0 ± 11	n.a.	n.a.	2 ± 11

Data are presented as mean ± standard deviation (pg/mL).

[a]acute tubular necrosis

[b]acute kidney injury

[c]not available

Table 3. Difference of urinary MK levels of patients stratified by disease categories

	Difference (pg/mL)	p value	95% CI[a] (pg/mL)	
ATN[b] vs. Other renal diseases	421	<0.0001	273	569
ATN vs. Healthy controls	446	<0.0001	298	593
Other renal diseases vs. Healthy controls	25	<0.0001	17	33
AKI[c] (ATN) vs. AKI (other renal diseases)	343	<0.0001	190	496
AKI vs. non-AKI in the other renal diseases	83	0.0005	39	127
Other renal diseases (AKI) vs. Healthy controls	102	<0.0001	61	148
Other renal diseases (non-AKI) vs. Healthy controls	19	<0.0001	12	27

[a]confidence interval

[b]acute tubular necrosis

[c]acute kidney injury

Table 4. Accuracy of MK and other biomarkers for detecting established AKI in various kidney diseases (n = 542*)

	AUC[a]	95% CI[b]	Optimal cutoff value	Sensitivity	Specificity	LR (+)[c]	LR (–)[d]
MK[e]	0.88	0.83-0.92	11.5 pg/mL	0.87	0.85	5.63	0.16
NAG[f]	0.70	0.63-0.78	20.3 U/L	0.52	0.83	3.03	0.58
IL-18[g]	0.72	0.66-0.79	18.5 pg/mL	0.75	0.63	2.03	0.40
NGAL[h]	0.84	0.79-0.89	51.4 ng/mL	0.82	0.80	4.19	0.22

*Forty subjects were excluded from total patients (n = 582): 7 due to missing values and 33 due to healthy controls.

[a]area under the curve

[b]confidence interval

[c]LR (+), positive likelihood ratio

[d]LR (–), negative likelihood ratio

[e]midkine

[f]NAG, N-acetyl-β-D-glucosaminidase

[g]IL-18, interleukin-18

[h]NGAL, neutrophil gelatinase-associated lipocalin

Table 5. Accuracy of MK and other biomarkers for detecting ATN in various kidney diseases (n = 542*)

	AUC[a]	95% CI[b]	Optimal cutoff value	Sensitivity	Specificity	LR (+)[c]	LR (–)[d]
MK[e]	0.96	0.95–0.98	69.5 pg/mL	0.97	0.90	9.88	0.03
NAG[f]	0.75	0.66–0.85	22.5 U/L	0.63	0.83	3.66	0.44
IL-18[g]	0.78	0.70–0.86	60.5 pg/mL	0.66	0.78	2.99	0.44
NGAL[h]	0.85	0.78–0.92	64.8 ng/mL	0.84	0.83	4.89	0.19

*Forty subjects were excluded from total patients (n = 582): 7 due to missing values and 33 due to healthy controls.

[a]area under the curve

[b]confidence interval

[c]LR (+), positive likelihood ratio

[d]LR (–), negative likelihood ratio

[e]midkine

[f]NAG, N-acetyl-β-D-glucosaminidase

[g]IL-18, interleukin-18

[h]NGAL, neutrophil gelatinase-associated lipocalin

Table 6. Characteristics of patients in the prospective study (n = 40)

	Total (n = 40)	AKI[a] (n = 12)	Non-AKI (n = 28)	p value
Male sex, n (%)	36 (90)	10 (83)	26 (93)	0.7301
Age, years	72 ± 8	72 ± 7	72 ± 8	0.9655
Body mass index, kg/m^2	23.2 ± 3.1	23.2 ± 3.7	23.2 ± 2.8	0.9831
Co-morbidities, n (%)				
Hypertension	33 (83)	9 (75)	24 (86)	0.7164
Coronary artery disease	18 (45)	9 (75)	9 (32)	0.0316
Stroke	5 (13)	0 (0)	5 (18)	0.2968
Diabetes	10 (25)	3(25)	7 (25)	>0.9999
Dyslipidemia	30 (75)	8 (67)	22 (79)	0.6903
Interval between CE-CT[b] and operation, days	54 ± 32	53 ± 24	55 ± 35	0.8663
Pre-operative medications, n (%)				
Beta-blockers	9 (23)	3 (25)	6 (21)	>0.9999
Calcium channel blockers	24 (60)	6 (50)	18 (64)	0.6220
ACE[c] inhibitor/ARB[d]	20 (50)	6 (50)	14 (50)	>0.9999
Diuretics	4 (10)	1 (8)	3 (11)	>0.9999
Anti-platelets	19 (48)	7 (58)	12 (43)	0.5804
HMG-CoA[e] reductase inhibitor	16 (40)	9 (75)	7 (25)	0.0092
Pre-operative laboratory data				
Alb[f], mg/dL	4.0 ± 0.3	3.9 ± 0.3	4.1 ± 0.3	0.0912
Hgb[g], mg/dL	12.5 ± 1.7	11.4 ± 1.8	13.0 ± 1.4	0.0054
sCr[h], mg/dL	1.09 ± 0.45	1.29 ± 0.60	1.00 ± 0.34	0.0654
eGFR[i], mL/min/1.73 m^2	58.8 ± 21.2	51.7 ± 25.6	61.9 ± 18.6	0.1668
Intra-operative variables				
Mean arterial pressure, mmHg	73 ± 6	74 ± 7	72 ± 6	0.4803
Bilateral suprarenal aortic cross-clamp, n (%)	2 (5)	2 (17)	0 (0)	0.1542
Aortic cross-clamp duration, min	63 ± 23	74 ± 25	59 ± 22	0.0579
Total surgical duration, min	271 ± 83	318 ± 102	251 ± 67	0.0185
Blood loss, mL	1974 ± 1328	2362 ± 2049	1807 ± 860	0.2305
Urine output, mL/kg/h	3.3 ± 1.9	2.4 ± 1.4	3.8 ± 2.0	0.0291

Red cell transfusion, mL	291 ± 677	641 ± 1056	141 ± 361	0.0305

Data are presented as mean ± SD.

[a]acute kidney injury

[b]contrast-enhanced computed tomography

[c]angiotensin-converting enzyme

[d]angiotensin II receptor blocker

[e]3-hydroxy-3-methylglutaryl–coenzyme

[f]albumin

[g]hemoglobin

[h]serum creatinine

[i]estimated glomerular filtration rate

Figure legends

Figure 1. Venn diagram of the various kidney diseases included in the cross-sectional study

All patients with ATN (n=32) and 35 (6.8%) of 517 patients with other renal diseases are observed in AKI category. AKI, acute kidney injury; ATN, acute tubular necrosis; AGN, acute glomerulonephritis; IgAN, IgA nephropathy; HSPN, Henoch-Schönlein purpura nephritis; LN, lupus nephritis; MPGN, membranoproliferative glomerulonephritis; AAV, antineutrophil cytoplasmic antibody-associated vasculitis; DMN, diabetic nephropathy; MCNS, minimal change nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; MN, membranous nephropathy; TMA, thrombotic microangiopathy; NSC, benign nephrosclerosis; TIN, tubulointerstitial nephritis; ADPKD, autosomal dominant polycystic kidney disease; Para., paraprotein-related kidney disease.

Figure 2. Frequency distribution of urinary MK and plasma MK levels

A. Frequency distribution of urinary MK levels (n = 582). Numbers on the x-axis indicate urinary MK values (pg/mL) in increments of 50 pg/mL. **B.** Frequency distribution of plasma MK levels (n = 526). Numbers on the x-axis indicate plasma MK values (pg/mL) in increments of 100 pg/mL.

Figure 3. Urinary MK levels in patients with various kidney diseases and the controls

Data are presented as box plots with median lines, 25- and 75-percentile boxes, and 10- and 90-percentile error bars. n = 582.

Figure 4. Comparison of urinary biomarkers between AKI, non-AKI, and the healthy controls

Data are presented as box plots with median lines, 25- and 75-percentile boxes, and 10- and 90-percentile error bars. The relative levels of these biomarkers show a greater increase in individuals with AKI (n = 67) than in patients without AKI (n = 482) and the healthy controls (n = 33); $^{*}p < 0.001$. NAG, *N*-acetyl-β-D-glucosaminidase; IL-18, interleukin-18; NGAL, neutrophil gelatinase-associated lipocalin. n = 582.

Figure 5. ROC curve analysis for the differentiation between AKI and non-AKI

ROC curve analysis of each biomarker indicates the ability to diagnose AKI (n = 67). $^{*}p < 0.001$ vs. MK. ROC, receiver operating characteristics; IL-18, interleukin-18; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin. n = 543.

Figure 6. ROC curve analysis for the differentiation between ATN and other kidney diseases

ROC curve analysis of each biomarker indicates the ability to diagnose ATN (n = 32). $^{*}p < 0.01$ and $^{**}p < 0.001$ vs. MK. ROC, receiver operating characteristics; IL-18, interleukin-18; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin. n = 543.

Figure 7. Changes in urinary biomarkers in AKI and non-AKI patients undergoing AAA surgery

To examine the difference of the longitudinal changes in MK, NAG, IL-18, and NGAL over the course of the open repair of AAA, the interaction between the presence of AKI and the surgery time course were evaluated by repeated-measures analysis of variance with mixed effect model. Data are mean ± standard error of the mean. POD, post-operative day. n = 40.