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Urinary kidney injury molecule-1 and monocyte chemotactic protein-1 are noninvasive biomarkers of cisplatin-induced nephrotoxicity in lung cancer patients

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DISCLOSURES: NONE

Abbreviations

AKI, acute kidney injury; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemotactic protein-1; NGAL, neutrophil gelatinase-associated lipocalin; AUC-ROC, area under the receiver operating characteristic curve; Scr, serum creatinine; BUN, blood urea nitrogen.

ABSTRACT

Purpose Acute kidney injury (AKI) is a common and serious adverse effect of cisplatin-based chemotherapy. However, traditional markers of kidney function, such as serum creatinine, are suboptimal, because they are not sensitive measures of proximal tubular injury. We aimed to determine whether the new urinary biomarkers such as kidney injury molecule-1 (KIM-1), monocyte chemotactic protein-1 (MCP-1), and neutrophil gelatinase-associated lipocalin (NGAL) could detect cisplatin-induced AKI in lung cancer patients in comparison with the conventional urinary proteins such as *N*-acetyl-*β*-D-glucosaminidase (NAG) and β2-microglobulin.

Methods We measured KIM-1, MCP-1, NGAL, NAG and β2-microglobulin concentrations in urine samples from 11 lung cancer patients, which were collected the day before cisplatin administration and on days 3, 7, and 14. Subsequently, we evaluated these biomarkers by comparing their concentrations in 30 AKI positive (+) and 12 AKI negative (−) samples and performing receiver operating characteristic (ROC) curve analyses.

Results The urinary levels normalized with urine creatinine of KIM-1 and MCP-1, but not NGAL, NAG and β2-microglobulin in AKI (+) samples were significantly higher than those in AKI (−) samples. In addition, ROC curve analyses revealed that KIM-1 and MCP-1, but not NGAL, could detect AKI with high accuracy (area under the curve [AUC] = 0.858, 0.850, and 0.608, respectively). The combination of KIM-1 and MCP-1 outperformed either biomarker alone (AUC = 0.871).

Conclusions Urinary KIM-1 and MCP-1, either alone or in combination, may represent biomarkers of cisplatin-induced AKI in lung cancer patients.

Key words:

cisplatin, acute kidney injury, biomarker, lung cancer

INTRODUCTION

Cisplatin is a widely used anticancer drug for several types of solid tumors, such as bladder, cervical, head and neck, esophageal, and lung cancers [1]. However, nephrotoxicity, which is a dose-limiting adverse effect, is a serious problem. About 20% of patients treated with high doses of cisplatin have peak serum creatinine (Scr) levels greater than 2.0 mg/dL, which are associated with mortality rates of 30% or more [2, 3]. Although this toxicity is transient in most patients and can be mitigated by other treatments, such as prehydration or concomitant osmotic diuresis [2], long-term treatment with cisplatin requires careful monitoring of kidney function.

In November 2010, the standard dosage of cisplatin for lung cancer treatment in Kyoto University Hospital was increased from 60 to 80 mg/m^2. This is of concern not only due to the dose-dependent nephrotoxicity of cisplatin but also because our previous research on a rat model of acute kidney injury (AKI) showed that proximal tubular injuries are not always associated with significant changes in the Scr levels [4]. Although renal biopsy is the gold standard for diagnosing AKI, not all cancer patients treated with cisplatin can undergo this procedure. Therefore, there is a need for noninvasive biomarkers of cisplatin-induced AKI.

Traditional serum markers of kidney function, such as Scr and blood urea nitrogen (BUN), are suboptimal because they only reflect changes in the glomerular filtration rate [3], which is a nonspecific measure of proximal tubular injury that is usually apparent only after significant kidney damage [5]. As a result, serum biomarkers of kidney function may not be adequate to accurately detect AKI. Other noninvasive urinary biomarkers, such as kidney injury molecule-1 (KIM-1) [6, 7] and neutrophil gelatinase-associated lipocalin (NGAL) [8, 9], may be more useful indicators

of proximal tubular injury or AKI. KIM-1 is a type-1 cell membrane glycoprotein up-regulated in dedifferentiated proximal tubule epithelial cells [10]. Its ectodomain was shed and could be quantitated in the urine following kidney injury in a rodent model of cisplatin-induced AKI [6]. On the other hand, NGAL expression is induced in epithelial cells upon inflammation or malignancy. The expression of NGAL has been shown to be up-regulated in the kidney proximal tubule cells and urine in a murine model following ischemic or cisplatin-induced AKI [11]. In addition, recently we showed that the monocyte chemotactic protein-1 (MCP-1) levels are significantly increased in proximal tubular epithelial cells and urine following cisplatin-induced AKI in rats [12]. MCP-1 is a proinflammatory chemokine that plays a role in the recruitment of monocytes to the sites of injury and infection. Similar to NGAL, its expression levels are also up-regulated in the kidney proximal tubule cells following ischemic injury [13]. Therefore, we here investigated whether KIM-1, NGAL, and MCP-1, either individually or in combination, can detect cisplatin-induced nephrotoxicity in lung cancer patients in comparison with two conventional urinary proteins *N*-acetyl-*β*-D-glucosaminidase (NAG) and β2-microglobulin.

MATERIALS AND METHODS

Patients and study design

We enrolled 11 primary lung cancer patients treated with cisplatin-based chemotherapy at Kyoto University Hospital between June 2011 and June 2012. The administration schedules of chemotherapy and supportive therapy are shown in Figure 1. None of the patients received magnesium supplementation.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. All patients provided written informed consent.

Data collection and diagnostic criteria for acute kidney injury

Clinical information, treatments, and laboratory data were obtained from the patients' electronic medical records. Considering that extensive continuous hydration (3000 mL/24 h) is provided with the administration of cisplatin, cisplatin-induced renal impairment was diagnosed in patients with an increase in the BUN level of more than 20 mg/dL and/or the Scr level of 50% in comparison with the baseline. Patients whose BUN level at the administration of cisplatin was higher than 20 mg/dL were excluded. Urine samples were classified as either AKI positive (+) or negative (−).

Urine collection and biomarker analysis

We collected urine samples on the day before cisplatin administration (day 0) and subsequently, on days 3, 7, and 14. Urine samples were collected into tubes with protease inhibitor cocktail tablets (cOmplete, Mini; Roche Diagnostics, Mannheim, Germany).

We measured the KIM-1 concentrations using Luminex xMAP microspheres with polyclonal antibodies raised against the ectodomain of human KIM-1, as described previously [14]. To measure the MCP-1 and NGAL concentrations, we used the Human CCL2/MCP-1 DuoSet and Human Lipocalin-2/NGAL DuoSet enzyme-linked immunosorbent assay kits (R&D Systems Inc., Minneapolis, MN), respectively, according to the manufacturer's instructions. Briefly, a 96-well microplate was coated with capture antibodies, and then blocked with 1% bovine serum albumin in phosphate-buffered saline. Subsequently, 100-μL samples were incubated in the blocked wells for two hours, followed by incubation with biotinylated detection antibodies for two hours and streptavidin-conjugated horseradish peroxidase (HRP) for 20 minutes at room temperature. Finally, MCP-1 and NGAL were detected by adding HRP substrate and measuring the optical density at 450 nm.

The concentrations of NAG and β2-microglobulin, which are tubular injury markers, were measured by using commercial kits: the NAG test Shionogi (Shionogi Co., Osaka, Japan) and beta-2 Microglobulin Human SimpleStep ELISA™ Kit (Abcam, Cambridge, UK), according to the manufacturers' instructions.

The levels of all biomarkers were normalized to the urinary creatinine concentration, which was measured by using an assay kit (LabAssay™ Creatinine; Wako Pure Chemical Industries, Osaka, Japan).

Statistical analyses

To evaluate the diagnostic accuracy of KIM-1, MCP-1, and NGAL, we calculated the area under the receiver operating characteristic (AUC-ROC) curve using SPSS version 18.0 (SPSS Inc., Chicago, IL). Differences were compared using the

Mann-Whitney U test, and *p*-values less than 0.05 were considered statistically significant. These analyses were performed using Prism Version 5.0 (GraphPad, San Diego, CA).

RESULTS

Patient characteristics

The patient characteristics are shown in Table 1. The mean (standard deviation [SD]) age of the patients in this study was 65.9 (10.1) years (range: 49–77 years). All patients had stage III lung cancer. The baseline mean (SD) levels of Scr and BUN were 0.75 (0.26) mg/dL and 14.3 (3.9) mg/dL, respectively. Seven patients were treated with 80 mg/m^2 cisplatin, while the remaining patients were administered lower doses due to reduced kidney function. The mean total cisplatin dosage was 108.1 (20.1) mg. Ten out of the 11 patients received cisplatin combined with vinorelbine (VNR).

Urinary and serum biomarkers of kidney function exhibit time-dependent changes during cisplatin-induced acute kidney injury

Surprisingly, all but one patient met the diagnostic criteria for AKI throughout the study. We observed time-dependent changes in the levels of serum and urinary biomarkers during cisplatin treatment. Figure 2 shows an example of these changes in a 49-year-old male patient with stage IIIA non-small cell lung adenocarcinoma treated with a combination of vinorelbine (20 mg/m^2, 31 mg/body), cisplatin (80 mg/m^2, 125 mg/body), and radiation. On day 3 after cisplatin treatment, AKI was diagnosed, because the BUN level exceeded 20 mg/dL. Between the cisplatin treatment initiation (day 0) and day 7, the Scr and BUN (Figure 2a) and urinary KIM-1 and MCP-1 levels increased relative to the baseline, whereas the urinary NGAL levels decreased (Figure 2b).

Urinary levels of kidney injury molecule-1 or monocyte chemotactic protein-1 can detect cisplatin-induced acute kidney injury

To determine whether the urinary levels of KIM-1, MCP-1, and NGAL can be used to detect cisplatin-induced AKI, we compared the levels of these biomarkers in 30 AKI (+) and 12 AKI (－) urine samples. First, we compared the absolute concentrations of urinary KIM-1, MCP-1 and NGAL, as shown in SF1. The concentrations of KIM-1 were significantly higher in AKI (+) samples than in AKI (−) samples ($p < 0.01$), while the MCP-1 and NGAL concentrations in the urine did not differ between the two groups. Next, to consider the inter-individual differences of urine samples, the concentrations of these markers normalized to urinary creatinine concentration were compared between AKI (−) samples and AKI (+) samples (Figure 3). The urinary KIM-1 and MCP-1 levels in AKI (+) samples were significantly higher than in AKI (−) samples ($p < 0.01$; Figures 3a, b). However, the NGAL concentrations did not significantly differ between AKI (+) and AKI (−) samples (Figure 3c). These results suggested that the urinary levels of KIM-1 or MCP-1 could detect cisplatin-induced AKI. In addition, we measured the urinary concentrations of NAG and β2-microglobulin, as tubular toxicity markers (SF1d, e and Figures 3d, e). There were no significant differences observed between the two groups, with or without normalization to the urinary creatinine concentrations.

Further, we compared the Scr levels to the concentrations of KIM-1, MCP-1 and NGAL (SF2). However, there was no correlation between the Scr level and any of the urinary biomarkers.

Receiver operating characteristic (ROC) curve analyses of urinary biomarkers

To confirm the above findings, we performed ROC curve analyses (Figure 4). The AUC-ROCs of KIM-1, MCP-1, and NGAL were 0.858 ($p < 0.01$), 0.850 ($p < 0.01$), and 0.608 ($p > 0.05$), respectively (Table 2), supporting the conclusion that urinary KIM-1 or MCP-1 can accurately detect cisplatin-induced AKI in lung cancer patients. In addition, the cut-off values of KIM-1, MCP-1, and NGAL were 2.45, 0.26, and 17.2 ng/mg creatinine, respectively (Table 2).

Combination of kidney injury molecule-1 and monocyte chemotactic protein-1 enhances detection of cisplatin-induced acute kidney injury

Since a combination of two biomarkers may be better than a single biomarker, we tested whether a combination of KIM-1 and MCP-1 can detect cisplatin-induced AKI better than either biomarker alone. We defined the combination biomarker as follows:

$$\frac{(KIM-1)_I}{(KIM-1)_{cut-off}} + \frac{(MCP-1)_i}{(MCP-1)_{cut-off}}$$

In this equation, the KIM-1 and MCP-1 concentrations (denoted by *i*) are normalized to their ROC cut-off values. The AUC-ROC for this combination, 0.871, was higher than that of either KIM-1 or MCP-1 alone ($p < 0.001$; Figure 4d).

DISCUSSION

In this study, we examined whether KIM-1, NGAL, and MCP-1 can detect cisplatin-induced AKI in lung cancer patients. Our results suggested that KIM-1 and MCP-1, but not NGAL as well as NAG and β2-microglobulin, can discriminate between AKI (+) and AKI (−) urine samples. The potential usefulness of KIM-1 is consistent with a previous report that urinary KIM-1 is a sensitive and accurate biomarker of cisplatin-induced AKI in both preclinical and clinical settings [15]. Recently, Tekce et al. [16] also reported that urinary KIM-1 concentrations on the first day after treatment may predict cisplatin-induced AKI with high sensitivity and specificity in the clinical setting. Although there have not been any studies using MCP-1 as a biomarker of cisplatin-induced AKI, our previous findings that cisplatin-induced nephrotoxicity increases urinary MCP-1 in rats [12] also support this conclusion. Further, although the concentrations of these markers did not correlate with the Scr levels, our results indicate the possibility that these new urinary biomarkers are more sensitive and specific than Scr for cisplatin-induced AKI.

However, our NGAL findings contradict recent reports that it may be an early biomarker of AKI in cancer patients treated with cisplatin-based chemotherapy [17-19]. There are two possible reasons for this discrepancy. First, the NGAL levels may have differed among studies because urine samples are collected and analyzed at different time points. For example, Lin et al. [18] analyzed urine samples between four hours and four days after cisplatin infusion and found that urinary NGAL levels significantly increased between 12 hours and three days later in AKI (+) samples compared with the baseline levels. In contrast, we measured NGAL concentrations at later time points when they would be lower in AKI (+) samples and, thus, more similar to those in AKI

(−) samples. In our study, analyzing urine samples at earlier time points was not possible because the patients were prehydrated, so their urine would have been too diluted during the first two or three days after cisplatin administration. Second, the NGAL levels may differ among the studies due to differences in the diagnostic criteria for AKI. Many investigators diagnose AKI using the Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease or Acute Kidney Injury Network classifications, which are based on changes in the Scr levels and urine output. In our study, we used expedient criteria slightly modified from the KDIGO (Kidney Disease: Improving Guideline Outcomes) criteria; if the level of Scr was increased more than 1.5-fold compared to the baseline and/or that of BUN was over than 20 mg/dL after the administration of cisplatin, the sample was classified as AKI (+). There are two reasons for why we used the modified KDIGO criteria in this study. First, because the urine outputs of our patients were too high due to the prehydration, we could not accurately diagnose AKI based on the urine output. Second, because the Scr levels are influenced by the muscle mass, it is inadequate to monitor the kidney function based on only the Scr levels, particularly in elderly patients. Hence, we defined the criteria based on the levels of both Scr and BUN. However, further research is needed to determine more specific diagnostic criteria for cisplatin-induced AKI.

Finally, our finding that the AUC-ROC of the combination of KIM-1 and MCP-1 is higher than that of either biomarker alone (Figure 4d) is consistent with previous reports that a combination of two biomarkers may have better diagnostic performance than a single biomarker [20, 21]. However, unlike these previous studies, which used a logistic regression model to combine two biomarkers, we simply summed the concentrations of two biomarkers normalized to their ROC cut-off values, because

this would be easier to calculate in the clinical setting. Although a dipstick assay for KIM-1 has recently become available [15], there is no similar assay for measuring MCP-1 levels quickly and accurately; such an assays could enable early detection and improved treatment of cisplatin-induced AKI.

In conclusion, we here showed that urinary KIM-1 and MCP-1, either alone or in combination, may represent accurate biomarkers of cisplatin-induced AKI in lung cancer patients. These findings may also facilitate the development of new methods to monitor kidney function. However, because of the small number of patients in the present study, larger studies are required in the future to confirm our findings.

Acknowledgments

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Conflict of interest:

The authors have no conflicts of interest to declare.

TABLES

Table 1. Patient characteristics

Characteristic	*n*
Number of patients	11
Age, mean (SD) (years)	65.9 (10.1) (range: 49–77)
Sex (male/female)	7/4
BSA, mean (SD) (m^2)	1.48 (0.19) (range: 1.17-1.72)
Baseline Scr, mean (SD) (mg/dL)	0.75 (0.26) (range: 0.3–1.3)
Baseline BUN, mean (SD) (mg/dL)	14.3 (3.9) (range: 8–19)
Type of lung cancer	
Small-cell	1
Adenocarcinoma	5
Squamous cell carcinoma	5
Tumor stage	
IIIA	8
IIIB	3
Cisplatin dosage	
80 mg/m^2	7
64 mg/m^2	1
60 mg/m^2	3
Total cisplatin dosage, mean (SD) (mg)	108.1 (20.1) (range: 77-131)
Co-administrated drugs	
VNR	10
ETP	1
Number of chemotherapy courses	
First	9
Second	2
Radiation	
Yes	6
No	5

Abbreviations: SD, standard deviation; BSA, body surface area; Scr, serum creatinine; BUN, blood urea nitrogen; VNR, vinorelbine; ETP, etoposide.

Table 2. Receiver operating characteristic curve analyses of kidney function biomarkers in lung cancer patients

Biomarker	AUC-ROC (95% CI)	*p*-value	Cut-off value (ng/mg creatinine)
KIM-1	0.858 (0.714–1.000)	0.002**	2.45
MCP-1	0.850 (0.651~~9~~6–1.00~~0~~4)	0.002**	0.26
NGAL	0.608 (0.422–0.79~~1~~4)	0.310	17.2

Abbreviations: AUC-ROC, area under the receiver operating characteristic curve; CI, confidence interval; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemotactic protein-1; NGAL, neutrophil gelatinase-associated lipocalin.

Statistical analyses were performed using the Mann-Whitney U test. **p<0.01.

FIGURE LEGENDS

Figure 1. Detail schedules of the chemotherapy regimens used.

Detailed administration schedules of each regimen, VNR/CDDP or ETP/CDDP, for our lung cancer patients are shown. As the supportive therapy, maintenance fluid (Soldem 3A®) and saline were injected along with antiemetics (palonosetron and dexamethasone sodium phosphate). At the first day of chemotherapy, the patients received extensive continuous infusion of approximately 3,000 mL. The standard dosages of VNR and ETP were 25 mg/m^2 and 100 mg/m^2, respectively. CDDP, cisplatin; VNR, vinorelbine; ETP, etoposide.

Figure 2. Changes in biomarker levels in a representative lung cancer patient with cisplatin-induced acute kidney injury.

Time-dependent changes in the levels of serum and urinary biomarkers during cisplatin-based chemotherapy in a 49-year-old male patient with stage IIIA non-small cell lung adenocarcinoma. (a) Changes in serum creatinine (Scr; white circle) and blood urea nitrogen (BUN; black circle). Since BUN was greater than 20 mg/dL on day 3, this patient was diagnosed with acute kidney injury (AKI). (b) Changes in urinary levels of kidney injury molecule-1 (KIM-1; white circle), neutrophil gelatinase-associated lipocalin (NGAL; white triangle), and monocyte chemotactic protein-1 (MCP-1; black circle).

Figure 3. Differences in urinary levels of kidney function biomarkers in lung cancer patients with or without acute kidney injury.

Differences in the urinary levels of kidney injury molecule-1 (KIM-1) (a), monocyte chemotactic protein-1 (MCP-1) (b), neutrophil gelatinase-associated lipocalin (NGAL) (c), *N*-acetyl-*β*-D-glucosaminidase (NAG) (d), and β2-microglobulin (e) in acute kidney injury (AKI) positive (+) and AKI negative (−) samples from lung cancer patients treated with cisplatin. The biomarker concentrations were normalized to the urinary creatinine concentration. Statistical analyses were performed using the Mann-Whitney U test. $^{**}p < 0.01$ vs. AKI (−). Horizontal bar indicates the median value.

Figure 4. Receiver operating characteristic curve analyses of urinary biomarkers of acute kidney injury

Receiver operating characteristic (ROC) curves demonstrating the sensitivity and specificity of kidney injury molecule-1 (KIM-1) (a), monocyte chemotactic protein-1 (MCP-1) (b), ~~and~~ neutrophil gelatinase-associated lipocalin (NGAL) (c), and the combination of KIM-1 and MCP-1 (d) with respect to the definition of acute kidney injury (AKI) by serum creatinine or blood urea nitrogen. AUC, area under the curve.

REFERENCES

1. Lebwohl, D. and R. Canetta, *Clinical development of platinum complexes in cancer therapy: an historical perspective and an update.* Eur J Cancer, 1998. **34**(10): p. 1522-34.
2. Hayes, D.M., et al., *High dose cis-platinum diammine dichloride: amelioration of renal toxicity by mannitol diuresis.* Cancer, 1977. **39**(4): p. 1372-81.
3. Bonventre, J.V., et al., *Next-generation biomarkers for detecting kidney toxicity.* Nat Biotechnol, 2010. **28**(5): p. 436-40.
4. Yonezawa, A., et al., *Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat.* Biochem Pharmacol, 2005. **70**(12): p. 1823-31.
5. Sieber, M., et al., *Comparative analysis of novel noninvasive renal biomarkers and metabonomic changes in a rat model of gentamicin nephrotoxicity.* Toxicol Sci, 2009. **109**(2): p. 336-49.
6. Ichimura, T., et al., *Kidney injury molecule-1: a tissue and urinary biomarker for nephrotoxicant-induced renal injury.* Am J Physiol Renal Physiol, 2004. **286**(3): p. F552-63.
7. Ichimura, T., et al., *Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury.* J Biol Chem, 1998. **273**(7): p. 4135-42.
8. Mishra, J., et al., *Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury.* J Am Soc Nephrol, 2003. **14**(10): p. 2534-43.
9. Mishra, J., et al., *Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery.* Lancet, 2005. **365**(9466): p. 1231-8.
10. Bonventre, J.V., *Kidney injury molecule-1 (KIM-1): a urinary biomarker and much more.* Nephrol Dial Transplant, 2009. **24**(11): p. 3265-8.
11. Mishra, J., et al., *Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity.* Am J Nephrol, 2004. **24**(3): p. 307-15.
12. Nishihara, K., et al., *Urinary chemokine (C-C motif) ligand 2 (monocyte chemotactic protein-1) as a tubular injury marker for early detection of cisplatin-induced nephrotoxicity.* Biochem Pharmacol, 2013. **85**(4): p. 570-82.
13. Rice, J.C., et al., *Monocyte chemoattractant protein-1 expression correlates with monocyte infiltration in the post-ischemic kidney.* Ren Fail, 2002. **24**(6): p.

703-23.

14. Vaidya, V.S., et al., *Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies.* Nat Biotechnol, 2010. **28**(5): p. 478-85.
15. Vaidya, V.S., et al., *A rapid urine test for early detection of kidney injury.* Kidney Int, 2009. **76**(1): p. 108-14.
16. Tekce, B.K., et al., *Does the kidney injury molecule-1 predict cisplatin-induced kidney injury in early stage?* Ann Clin Biochem, 2015. **52**(Pt 1): p. 88-94.
17. Gaspari, F., et al., *Predicting cisplatin-induced acute kidney injury by urinary neutrophil gelatinase-associated lipocalin excretion: a pilot prospective case-control study.* Nephron Clin Pract, 2010. **115**(2): p. c154-60.
18. Lin, H.Y., et al., *Urinary neutrophil gelatinase-associated lipocalin levels predict cisplatin-induced acute kidney injury better than albuminuria or urinary cystatin C levels.* Kaohsiung J Med Sci, 2013. **29**(6): p. 304-11.
19. Peres, L.A., et al., *Evaluation of the cisplatin nephrotoxicity using the urinary neutrophil gelatinase-associated lipocalin (NGAL) in patients with head and neck cancer.* J Bras Nefrol, 2014. **36**(3): p. 280-8.
20. Katagiri, D., et al., *Combination of two urinary biomarkers predicts acute kidney injury after adult cardiac surgery.* Ann Thorac Surg, 2012. **93**(2): p. 577-83.
21. Liang, X.L., et al., *Combination of urinary kidney injury molecule-1 and interleukin-18 as early biomarker for the diagnosis and progressive assessment of acute kidney injury following cardiopulmonary bypass surgery: a prospective nested case-control study.* Biomarkers, 2010. **15**(4): p. 332-9.

1

2 Figure 1.

❖ ETP/CDDP

Day 1

Supportive therapy	Chemotherapy	Supportive therapy
Antiemetic + infusion solution total volume 1,000 mL	ETP + CDDP total volume 1,000 mL	Infusion solution total volume 1,000 mL

Days 2, 3

Supportive therapy	Chemotherapy	Supportive therapy
Antiemetic total volume 500 mL	ETP + CDDP total volume 500 mL	Infusion solution total volume 500 mL

❖ VNR/CDDP

Day 1

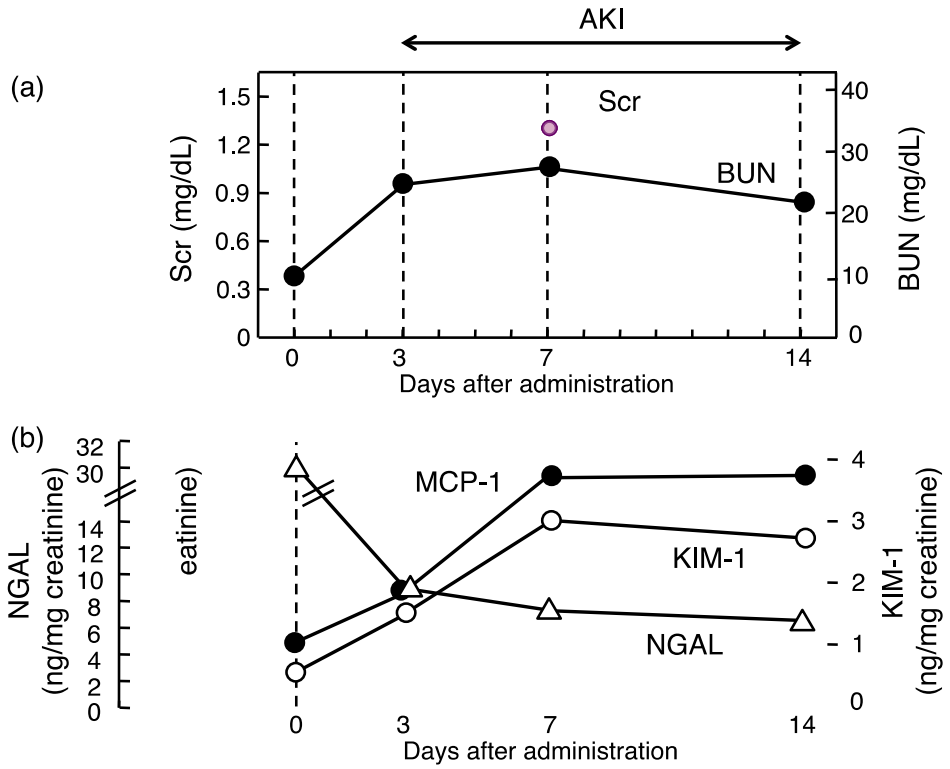
Supportive therapy	Chemotherapy	Supportive therapy
VNR, antiemetic, + infusion solution total volume 1,250 mL	CDDP total volume 500 mL	Infusion solution total volume 1,000 mL

Days 2, 3

Supportive therapy
Antiemetic +infusion solution total volume 1,000 mL

1 Figure 2.

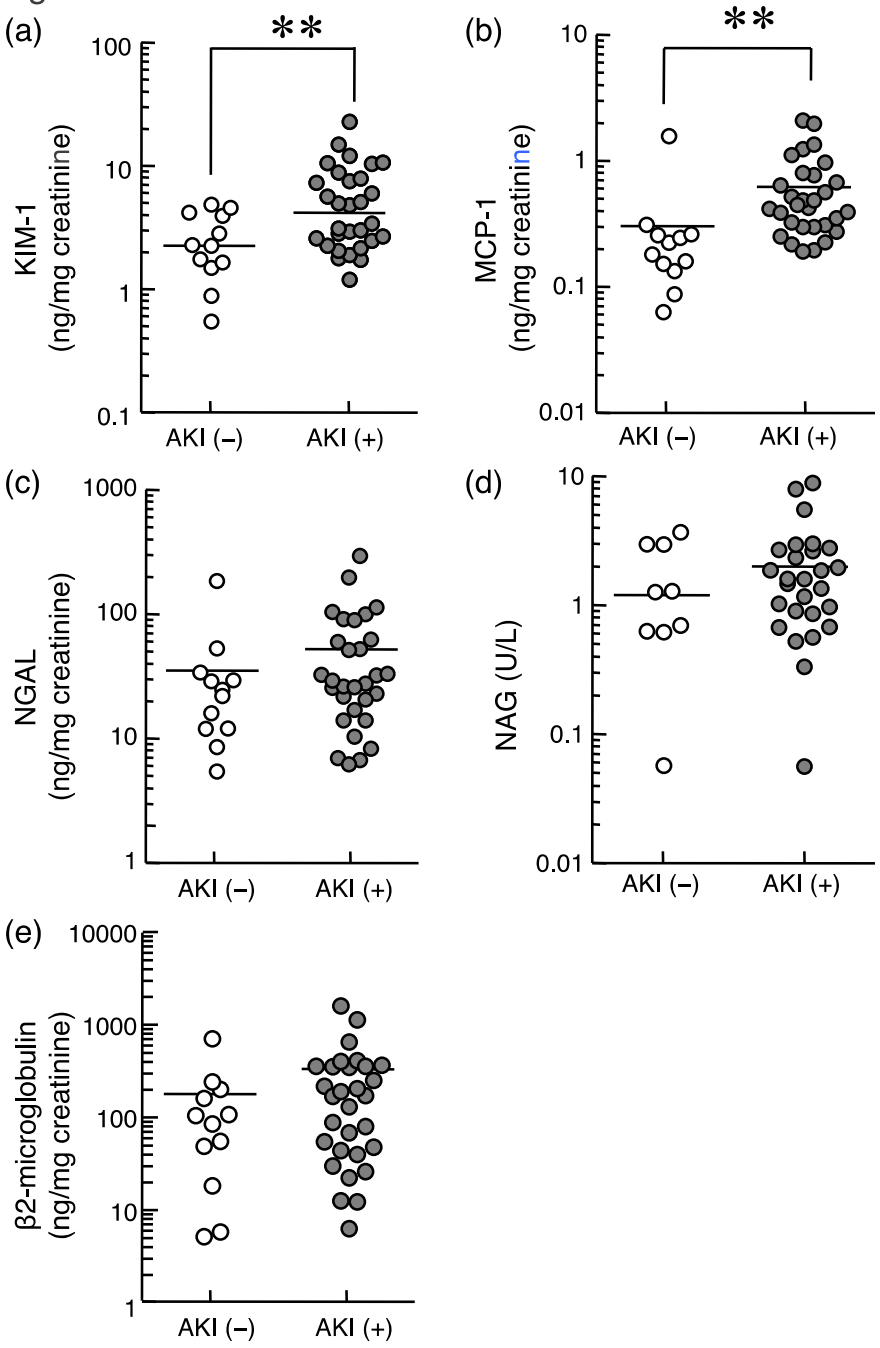
2


AKI
(a)
Scr
BUN
Scr (mg/dL)
BUN (mg/dL)
1.5
1.2
0.9
0.6
0.3
0
40
30
20
10
0
0
3
7
14
Days after administration
(b)
MCP-1
KIM-1
NGAL
NGAL (ng/mg creatinine)
eatinine)
KIM-1 (ng/mg creatinine)
32
30
14
12
10
8
6
4
2
0
4
3
2
1
0
0
3
7
14
Days after administration

1

2

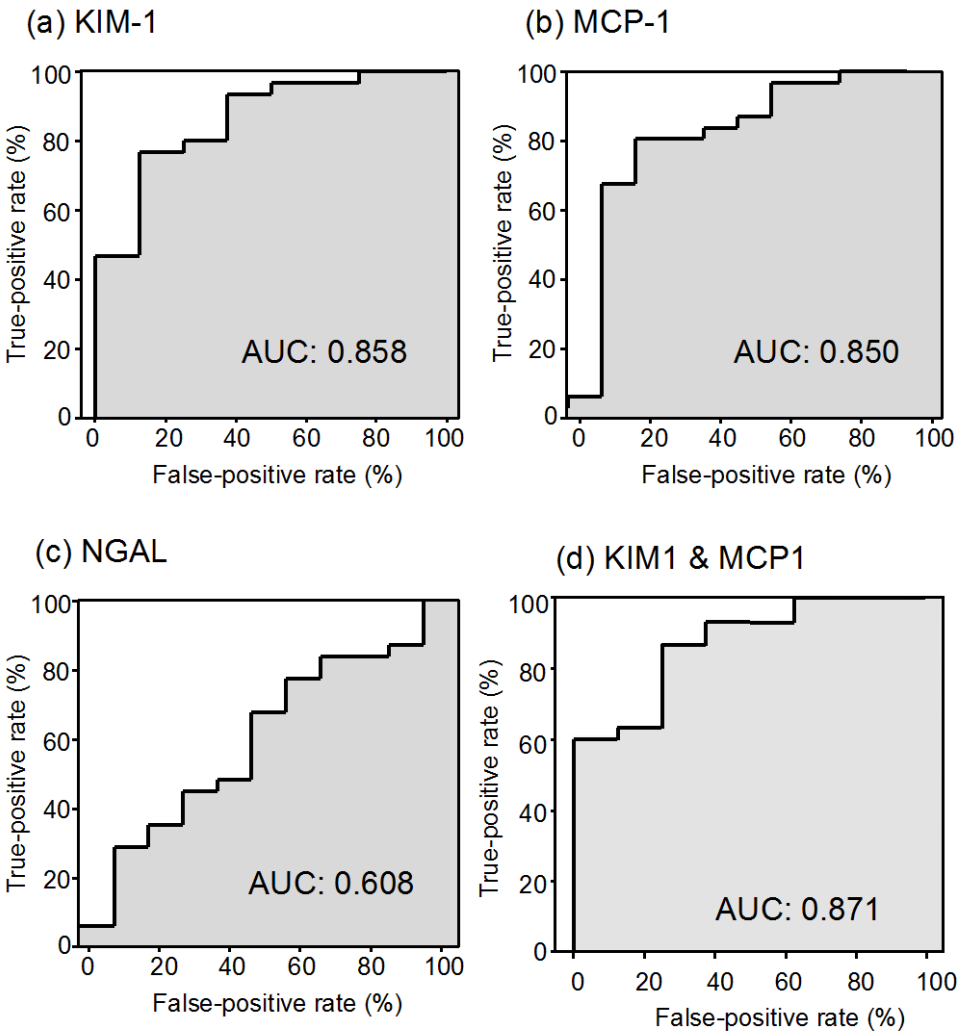
Figure 3.


(a)
**
KIM-1 (ng/mg creatinine)
100
10
1
0.1
AKI (–)
AKI (+)
(b)
**
MCP-1 (ng/mg creatinine)
10
1
0.1
0.01
AKI (–)
AKI (+)
(c)
NGAL (ng/mg creatinine)
1000
100
10
1
AKI (–)
AKI (+)
(d)
NAG (U/L)
10
1
0.1
0.01
AKI (–)
AKI (+)
(e)
β2-microglobulin (ng/mg creatinine)
10000
1000
100
10
1
AKI (–)
AKI (+)

1

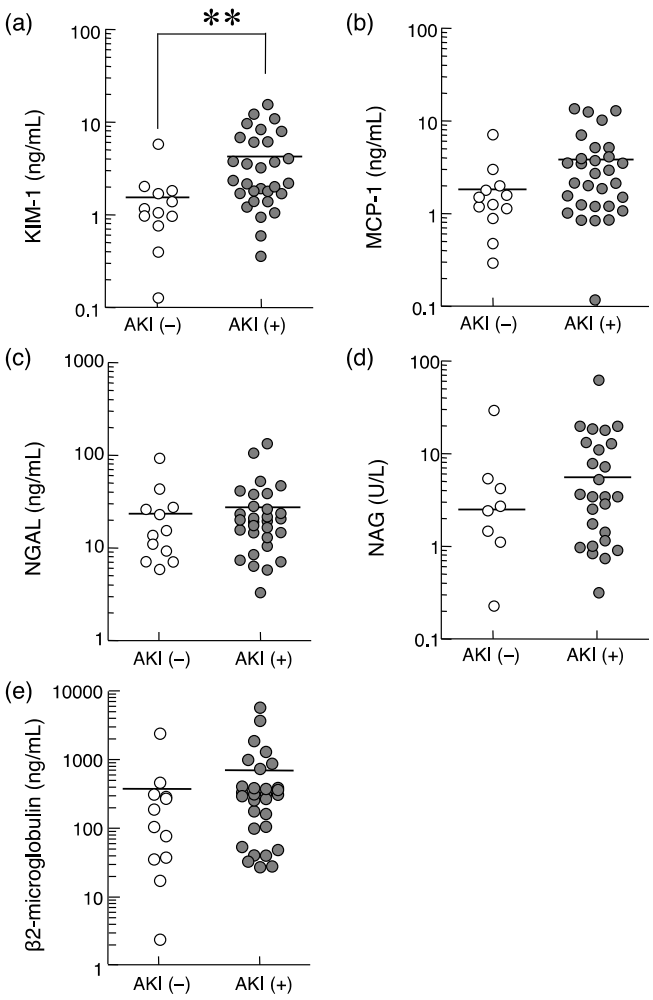
2

Figure 4.


(a) KIM-1
True-positive rate (%)
False-positive rate (%)
AUC: 0.858
(b) MCP-1
True-positive rate (%)
False-positive rate (%)
AUC: 0.850
(c) NGAL
True-positive rate (%)
False-positive rate (%)
AUC: 0.608
(d) KIM1 & MCP1
True-positive rate (%)
False-positive rate (%)
AUC: 0.871

1

2

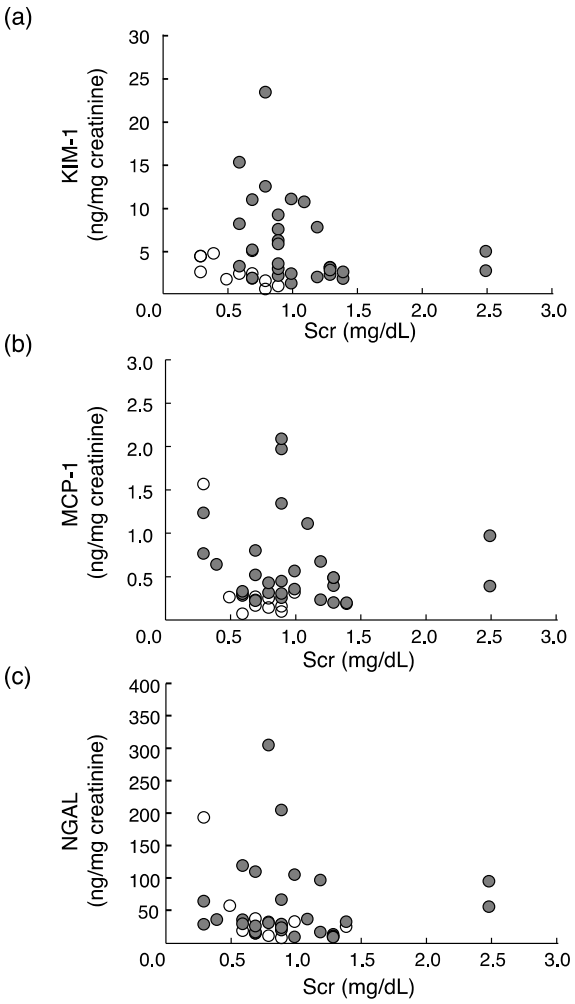


s

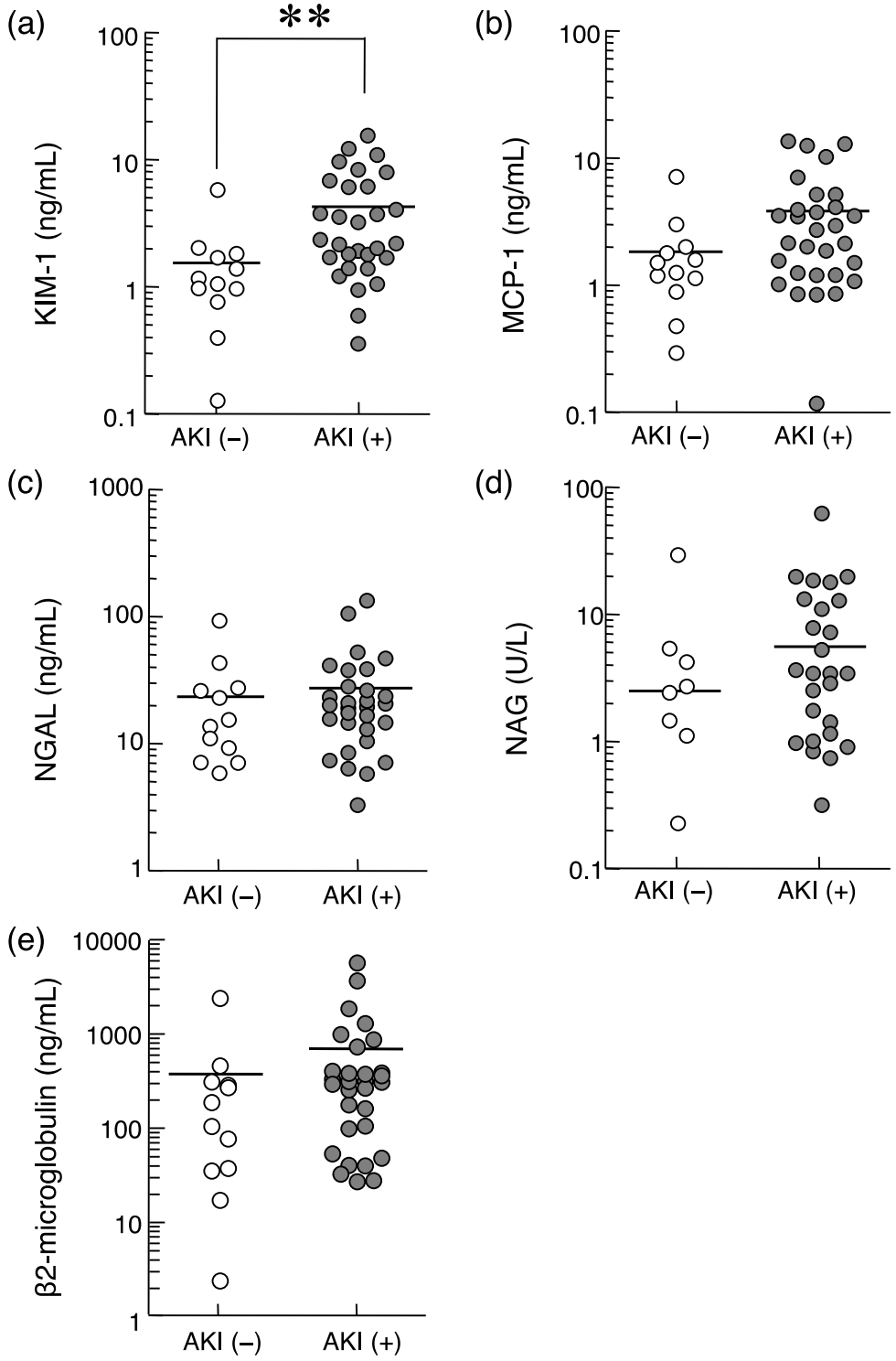
the median value.

1

2

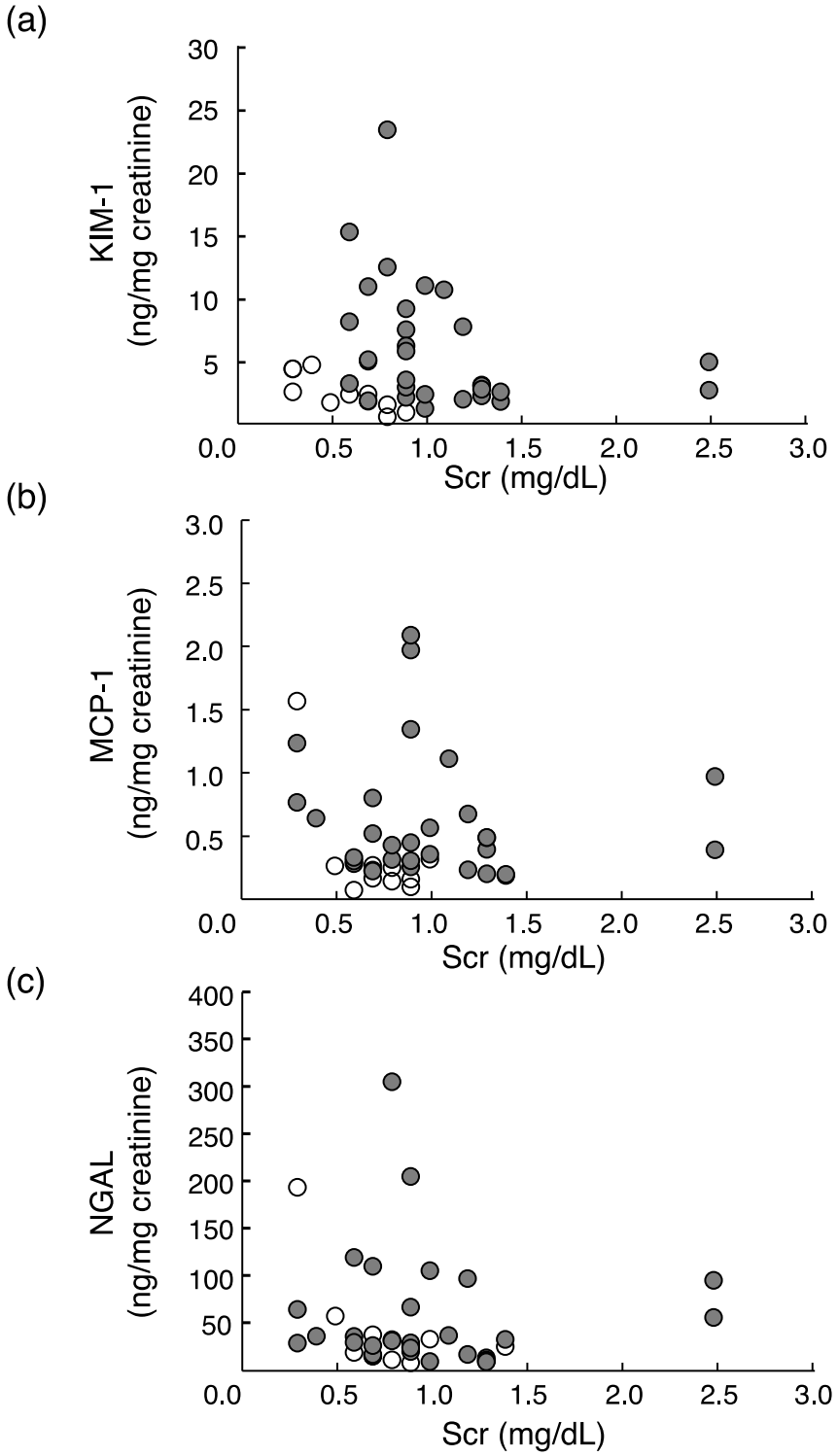


rcles, acute kidney injury (AKI) (−) samples; gray circles, AKI (+) samples.



SF1, Supplementary Figure 1. Differences in the absolute concentrations of urinary biomarkers in lung cancer patients with or without acute kidney injury.

Differences in the absolute urinary levels of kidney injury molecule-1 (KIM-1) (a), monocyte chemotactic protein-1 (MCP-1) (b), neutrophil gelatinase-associated lipocalin (NGAL) (c), *N*-acetyl-*β*-D-glucosaminidase (NAG) (d), and β2-microglobulin (e) in acute kidney injury (AKI) positive (+) and AKI negative (−) samples from lung cancer patients treated with cisplatin. Statistical analyses were performed using the Mann-Whitney U test. **$p < 0.01$ vs. AKI (−). Horizontal bar indicates the median value.



SF2, Supplementary Figure 2. Correlations between the serum creatinine levels and urinary biomarkers.

No correlation between the serum creatinine (Scr) levels and urinary kidney injury molecule-1 (KIM-1) (a), monocyte chemotactic protein-1 (MCP-1) (b), or neutrophil gelatinase-associated lipocalin (NGAL) (c) was observed. White circles, acute kidney injury (AKI) (−) samples; gray circles, AKI (+) samples.