



COVID-19-induced acute renal tubular injury associated with elevation of serum inflammatory cytokine

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Abstract

Background Severe acute respiratory syndrome Coronavirus 2 has rapidly spread worldwide, with acute kidney injury (AKI) as one of the manifestations with unknown causal mechanisms. We aimed to investigate tubular injury by assessing tubular markers and their association with the severity of Coronavirus disease 2019 (COVID-19).

Methods We examined the associations between laboratory markers and urinary levels of N-acetyl- β -D-glucosaminidase (uNAG), β 2-microglobulin (u β 2MG), α 1-microglobulin (u α 1MG), and liver-type fatty acid binding protein (L-FABP). We studied 18 COVID-19 patients without previous chronic kidney disease and analyzed the relationship between the urinary biomarkers and inflammatory markers in patients with severe ($n = 7$) or non-severe ($n = 11$) COVID-19, defined by requirements of supplemental oxygen.

Results Fourteen patients (78%) showed abnormal urinalysis findings and two (11%) developed AKI. Patients with severe COVID-19 had significantly higher levels of proteinuria, uNAG, u β 2MG, u α 1MG, and L-FABP than those with non-severe disease. Serum levels of interleukin-6 (IL-6) were significantly higher on admission in all severe COVID-19 cases and correlated with the levels of L-FABP, u β 2MG, u α 1MG, uNAG, and proteinuria. Moreover, the changes in serum IL-6 (Δ IL-6) levels from baseline to 7 days after admission significantly correlated with Δ L-FABP and Δ u β 2MG.

Conclusions Levels of tubular injury markers, especially L-FABP and u β 2MG, were significantly associated with IL-6 levels even in patients with no evident AKI. This suggests that L-FABP and u β 2MG could be useful as early detective biomarkers for COVID-19 associated renal injury.

Keywords Acute tubular injury · Interleukin-6 · COVID-19 · L-FABP · β 2MG

Introduction

Coronavirus disease 2019 (COVID-19) has rapidly spread worldwide since the novel Coronavirus, severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), was first

detected in Wuhan, China, in December 2019 [1]. COVID-19-associated acute kidney injury (AKI) has been noted in several reports, and the incidence varies depending on criteria for hospitalization in different locales. In a meta-analysis of 25 Chinese papers published between the beginning of the epidemic and July 30, 2020, Zheng et al. found a 32.5% incidence of AKI in ICU patients and a 6.5% incidence of AKI in overall hospital patients [2]. Other recent meta-analyses showed AKI to be closely related to disease severity and mortality for COVID-19 patients [3, 4].

The detailed mechanisms for AKI caused by SARS-CoV-2 have not been elucidated; however, recent studies point to the possibility of direct viral infection to kidney cells via angiotensin-converting enzyme-2 (ACE2) [5–7]. Single-cell RNA-seq data analysis demonstrates that the ACE2 protein is enriched in the bronchus and lung parenchyma, ileum, heart, bladder, and kidney [8]. In the kidney,

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ACE2 is expressed on glomerular endothelial cells, podocytes, and proximal tubule epithelial cells [9].

In autopsies of 26 patients with COVID-19, electron microscopy found Coronavirus particles in both the proximal tubular epithelium and podocytes. The study reported that immunostaining of ACE2 in kidney tissue from the COVID-19 patients was prominent in proximal tubular cells, especially in areas with severe acute tubular injury with loss of brush border or acute tubular necrosis. Occasionally, weaker podocyte staining was also observed [10]. These data suggested that proximal tubules may be the main target of SARS-CoV-2 infection in the kidney.

Interleukin-6 (IL-6), an inflammatory cytokine that can induce cytokine storms, is found in high levels in critically ill COVID-19 patients [11–13]. A previous report found that renal damage in patients with severe acute respiratory syndrome (SARS) occurred because of hemodynamic changes caused by cytokine storms rather than by viral-induced tropism in the kidneys [14]. Thus, it is not known whether COVID-19 causes progressive kidney damage, in the proximal tubules in SARS-CoV-2, by direct viral infection or pro-inflammatory cytokine-mediated mechanisms.

In this study, we aim to investigate the relationships of tubular injury, severity of COVID-19, and inflammation markers, such as IL-6, to address the possible involvements of pro-inflammatory cytokine-mediated mechanisms on developing AKI.

Materials and methods

Patients

Our retrospective study used data from all inpatients with COVID-19 seen in April and May 2020 at Juntendo University Hospital, a 1051-bed university-affiliated hospital, Tokyo, Japan. The study included 21 Japanese adult (≥ 18) COVID-19 patients diagnosed by detecting SARS-CoV-2 RNA from nasopharyngeal swab specimens via reverse transcription-polymerase chain reaction (Ct value ≤ 40.0 , LightCycler[®] 480 System II, Japan).

We excluded patients who lacked urinary data ($n = 2$) and a patient with chronic kidney disease ($n = 1$), resulting in 18 study patients. This study was approved by the ethics committee of Juntendo University Hospital, and informed written consent was obtained from all patients (Process No. 20–036). The authors have complied with the Declaration of Helsinki during this study.

Data collection

Clinical and laboratory information were collected at admission and on the seventh day. Laboratory parameters included

serum creatinine (Cr), serum IL-6, urinary protein excretion, urinary liver-type fatty acid binding protein (L-FABP), urinary *N*-acetyl- β -D-glucosaminidase (uNAG), urinary alpha1-microglobulin (u α 1MG), and urinary β 2-microglobulin (u β 2MG). The urinary data contained a relatively small number of examples with missing values.

We analyzed the relationship between laboratory markers and respiratory status in patients with severe or non-severe COVID-19 as defined by requirements of supplemental oxygen referring to National Institutes of Health and Japanese guidelines [15, 16]. We also investigated the correlation analysis between the patients' levels of urinary tubule markers (uNAG, u β 2MG, u α 1MG, and L-FABP), laboratory markers, proteinuria, and the presence of urinary cast.

Definition of acute kidney injury

AKI was defined as follows: increase in serum Cr by ≥ 0.3 mg/dl within 48 h; or increase in serum Cr to ≥ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or urine volume of < 0.5 mL/kg/h for 6 h [17].

Statistical analyses

Data were expressed as median (interquartile range [IQR]) and n (%), respectively. Continuous and categorical variables were compared between groups using the Mann–Whitney *U* test or Fisher's exact tests. The correlations between IL-6 and other variables were evaluated by Spearman correlation analysis. Statistical significance was defined as $p < 0.05$. These statistical analyses were conducted using the Prism software ver.8.4.2 (GraphPad Software, San Diego, CA, USA).

Results

Baseline demographic, clinical characteristics, and laboratory findings

We retrospectively evaluated 18 patients with COVID-19 whose age ranged from 26 to 78 years. Table 1 presents the clinical and laboratory characteristics and details about the COVID-19 cases (total, non-severe [$n = 11$] and severe [$n = 7$]). There were no significant differences in gender, hypertension, coronary heart disease, and respiratory disease; patients with severe COVID-19 were significantly older (median IQR: 76.0 years [66.5–76.0] vs. 56.0 years [37.5–65.5]) and more likely to have diabetes compared with non-severe cases.

When comparing the two groups, no significant differences were seen in the laboratory data, including white

Table 1 Demographic, clinical, laboratory, and radiographic findings of study patients at admission and patient outcome

	Total (n = 18)	Non-severe (n = 11)	Severe (n = 7)	p value
Demographics and clinical characteristics				
Age, years	64.0 (44.0–74.5)	56.0 (37.5–65.5)	76.0 (66.5–76.0)	0.0059
Gender (% male)	66.7	54.6	85.7	0.32
Comorbidity				
Hypertension	5 (28%)	2 (18%)	3 (43%)	0.33
Diabetes	6 (33%)	2 (18%)	4 (57%)	0.14
Coronary heart disease	2 (11%)	1 (9%)	1 (14%)	1
Chronic kidney disease	0	0	0	
Respiratory disease	0	0	0	
Laboratory findings				
White blood cell count, × 10 ⁹ per L	5.70 (4.63–7.18)	4.90 (4.35–6.55)	6.40 (5.80–7.25)	0.16
Lymphocyte count, × 10 ⁹ per L	1.28 (0.74–1.49)	1.48 (1.00–1.60)	0.93 (0.65–1.28)	0.13
Hemoglobin, g/L	144 (129–153)	143 (128–152)	145 (138–150)	0.88
Platelet count, × 10 ⁹ per L	221 (168–295)	227 (212–287)	175 (160–255)	0.22
Albumin, g/dL	3.30 (3.10–3.80)	3.50 (3.30–4.35)	3.10 (2.85–3.20)	0.01
Ferritin, µg/L	764 (433–973)	469 (238–607)	976 (932–1310)	0.0008
Creatinine, mg/dL	0.68 (0.54–0.77)	0.66 (0.49–0.81)	0.71 (0.67–0.74)	0.64
eGFR, mL/min/1.73 m ²	85.9 (76.1–97.3)	88.5 (77.4–103.3)	85.4 (79.5–87.3)	0.60
D-dimer, µg/mL	2.05 (1.55–2.65)	1.90 (1.40–2.05)	2.70 (2.25–3.95)	0.01
Procalcitonin, ng/mL	0.05 (0.01–0.11)	0.03 (0.00–0.04)	0.12 (0.08–0.15)	0.0019
IL-6, pg/mL	14.3 (3.8–35.0)	5.2 (2.6–12.8)	40.5 (31.1–52.0)	0.0004
CRP, mg/L	4.15 (0.28–8.39)	0.44 (0.19–1.99)	8.40 (8.00–11.88)	0.0012
Radiographic features				
Presence of pneumonia	16 (89%)	9 (82%)	7 (100%)	0.50
Outcome				
Acute kidney injury	2 (11%)	0	2 (29%)	0.14
ICU admission	4 (22%)	0	4 (57%)	0.011
Invasive mechanical ventilation	3 (17%)	0	3 (43%)	0.04
Death	1 (6%)	0	1 (14%)	0.39

Data are expressed as median (IQR); p values were assessed using a Mann–Whitney U test or Fisher's exact test to compare differences between patients with severe and non-severe COVID-19

IL-6 interleukin-6, L-FABP liver-type fatty acid binding protein, uNAG N-acetyl-β-D-glucosaminidase; uβ2MG β2-microglobulin, ua1MG α1-microglobulin

blood cell counts, lymphocyte counts, hemoglobin, platelet counts, creatinine, and estimated glomerular filtration rates; however, serum albumin was decreased, and the serum levels of ferritin, D-dimer, procalcitonin, IL-6, and C-reactive protein (CRP) were increased in the severe group compared with that in the non-severe group.

Urinary levels of tubular markers varied between non-severe and severe cases

Fourteen of the 18 patients (78%) had abnormal urinalysis findings. Patients with severe COVID-19 had significantly higher levels of proteinuria (0.42 g/gCr [0.36–0.78] vs. 0.07 g/gCr [0.05–0.29], $p=0.020$); uNAG (32.5 U/L [26.8–42.0] vs. 5.3 U/L [3.0–18.4], $p=0.001$); uβ2MG (10,516 µg/L [1649–29,237] vs. 160 µg/L [42–297], $p=0.003$); ua1MG (65.8 mg/L [62.3–87.1] vs. 4.4 mg/L

[3.1–15.0], $p=0.005$); L-FABP (47.9 µg/gCr [29.4–75.8] vs. 3.3 µg/gCr [2.1–5.8], $p=0.001$) (Fig. 1); and urinary cast (all; 86% vs. 18%, $p=0.013$: epithelial cast; 86% vs. 9%, $p=0.003$: granular cast; 43% vs. 9%, $p=0.24$) when compared with non-severe cases. Regarding red blood cell and white blood cell cast, there was only 1 case among the 18 patients with COVID-19. We found only two cases (29%) of evident AKI, both in the severe group (Table 1).

Correlation between urinary tubular markers and laboratory markers

The baseline serum levels of IL-6 increased in all severe cases and in six non-severe cases. They were also significantly higher in severe cases than in non-severe cases (40.5 pg/mL [31.1–52.0] vs. 5.2 pg/mL [2.6–12.8], $p=0.0004$) (Table 1). The IL-6 levels on admission were

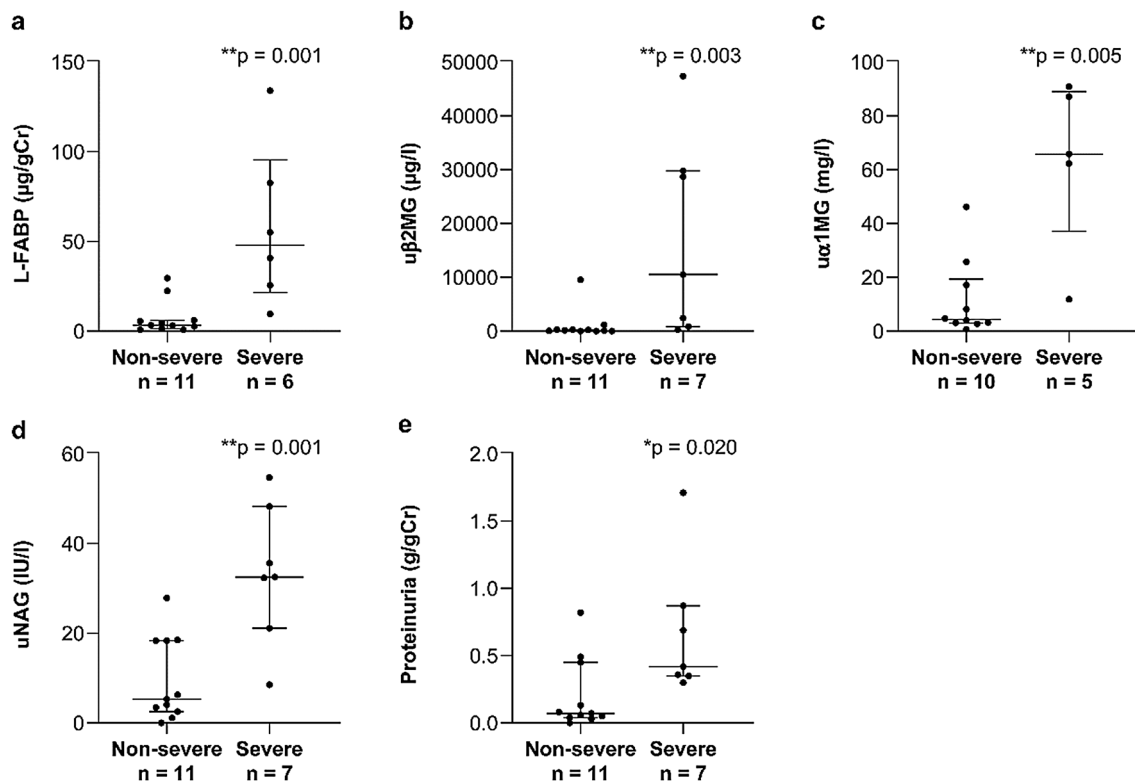


Fig. 1 The levels of L-FABP, u β 2MG, u α 1MG, uNAG, proteinuria in patients with COVID-19 on admission. * $p \leq 0.05$, ** $p \leq 0.01$. L-FABP liver-type fatty acid binding protein, u β 2MG β 2-microglobulin, u α 1MG α 1-microglobulin, uNAG N-acetyl- β -D-glucosaminidase

positively correlated with CRP ($r = 0.9004$, $p < 0.0001$), neutrophil-to-lymphocyte ratio (NLR) ($r = 0.5459$, $p = 0.0191$), ferritin ($r = 0.7317$, $p = 0.0006$), lactate dehydrogenase (LDH) ($r = 0.6615$, $p = 0.0028$), and D-dimer ($r = 0.5971$, $p = 0.0089$); IL-6 levels were negatively correlated with lymphocyte counts ($r = -0.515$, $p = 0.0287$) (Fig. 2).

Serum levels of IL-6 significantly correlated with the levels of L-FABP on admission ($r = 0.89$, $p < 0.0001$), u β 2MG ($r = 0.71$, $p = 0.001$), u α 1MG ($r = 0.79$, $p = 0.0008$), uNAG ($r = 0.74$, $p = 0.0005$), and proteinuria ($r = 0.66$, $p = 0.003$) (Fig. 3a–e); the changes in serum IL-6 (Δ IL-6) from baseline to 7 days after admission also significantly correlated with Δ L-FABP ($r = 0.67$, $p = 0.005$) and Δ u β 2MG ($r = 0.79$, $p = 0.003$) (Fig. 4a–e). Serum IL-6 and Δ IL-6 were significantly higher in the patients who exhibited the urinary cast at admission and on the seventh day (Fig. 3f, 4f).

Discussion

The salient findings in this study were as follows: (1) our subjects had an 11% AKI incidence during hospitalization; (2) even if they did not have evident AKI, patients with severe COVID-19 had significantly elevated tubular injury markers, such as L-FABP, u β 2MG, u α 1MG, and uNAG; (3)

urinary epithelial casts, which may indicate tubular injury, were also observed in cases with severe COVID-19 infections; and (4) IL-6 was significantly associated with tubular markers, and its reduction correlated to a decrease in L-FABP and u β 2MG. These observations suggest that severe systemic inflammatory condition, such as cytokine storms could be involved in tubular injury, and L-FABP and u β 2MG levels could be useful early diagnostic markers for COVID-19 associated renal injury.

The incidence of evident AKI in our COVID-19 patients during hospitalization was similar to the findings in previous reports [2]. In a previous paper reporting proximal tubular injury in COVID-19, 67% of the 49 patients had elevated urinary levels of u β 2MG, and 85% had an increase in proteinuria [18]. In our study, 18 COVID-19 patients had abnormalities in the following markers: proteinuria, 56% (10); u β 2MG, 61% (11); L-FABP; 47% (8 of 17), uNAG 61% (11); and u α 1MG, 53% (8 of 15). Consistent with our report, elevated tubular markers were observed even in cases without evident AKI in both studies. These observations may suggest that the elevation of tubular markers, such as L-FABP and u β 2MG, could reflect even the modest tubular injury and be early detection markers for COVID-19-mediated renal injury. Additionally, the group of patients with severe disease and worsening respiratory status had high

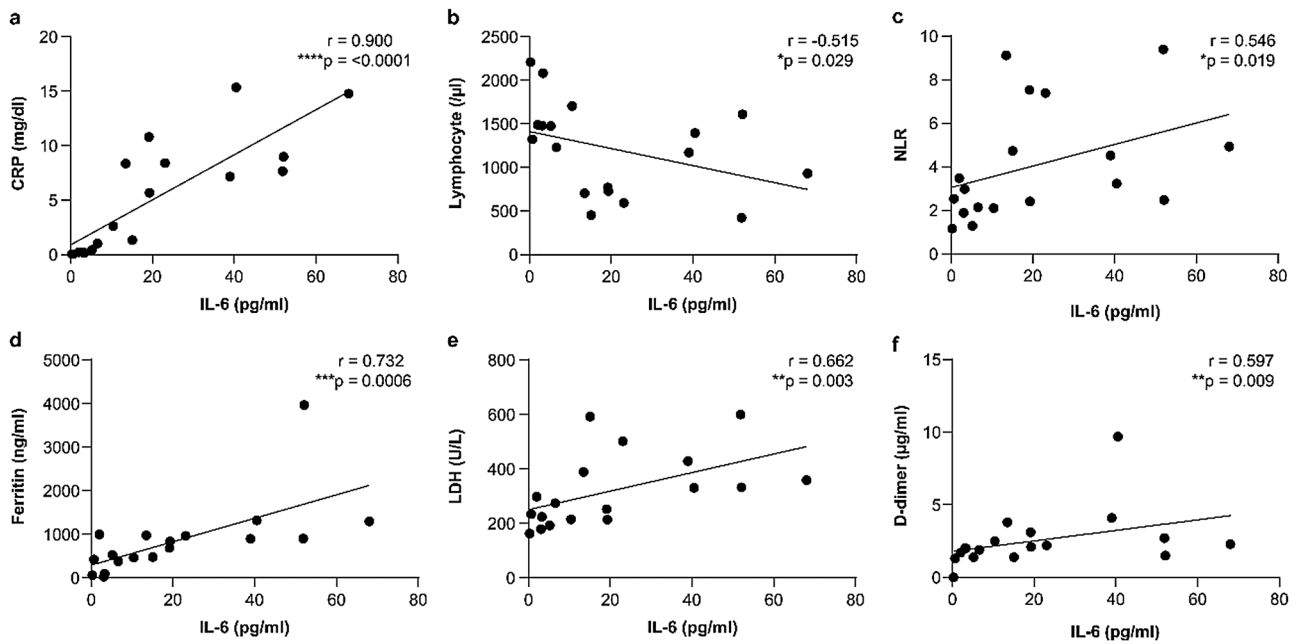


Fig. 2 Correlations between serum IL-6 levels and other variables. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. *IL-6* interleukin-6, *CRP* C-reactive protein, *NLR* neutrophil-to-lymphocyte ratio, *LDH* lactate dehydrogenase

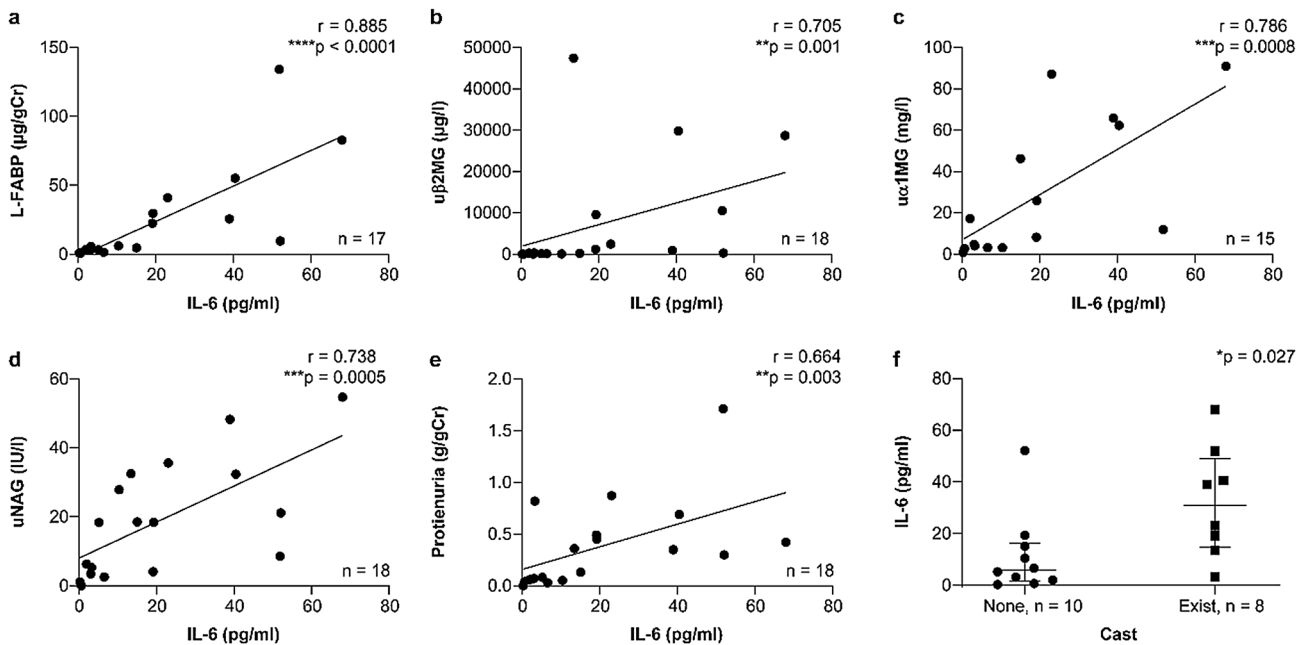


Fig. 3 Correlations between serum IL-6 levels and urinary biomarker values. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. *IL-6* interleukin-6, *L-FABP* liver-type fatty acid binding protein, *uβ2MG* β2-microglobulin, *uα1MG* α1-microglobulin, *uNAG* N-acetyl-β-D-glucosaminidase

levels of L-FABP, uβ2MG, uα1MG, uNAG, proteinuria, the presence of urinary casts, mainly epithelial cast, and serum levels of IL-6.

We confirmed a correlation between IL-6, CRP, NLR, ferritin, LDH, and D-dimer, consistent with earlier reports [19,

20], which is considered a parameter of disease aggravation. Interestingly, serum levels of IL-6 significantly correlated with urinary biomarker values, such as L-FABP, uβ2MG, uα1MG, uNAG, proteinuria, and urinary cast. Changes in L-FABP and uβ2MG had a strong positive correlation

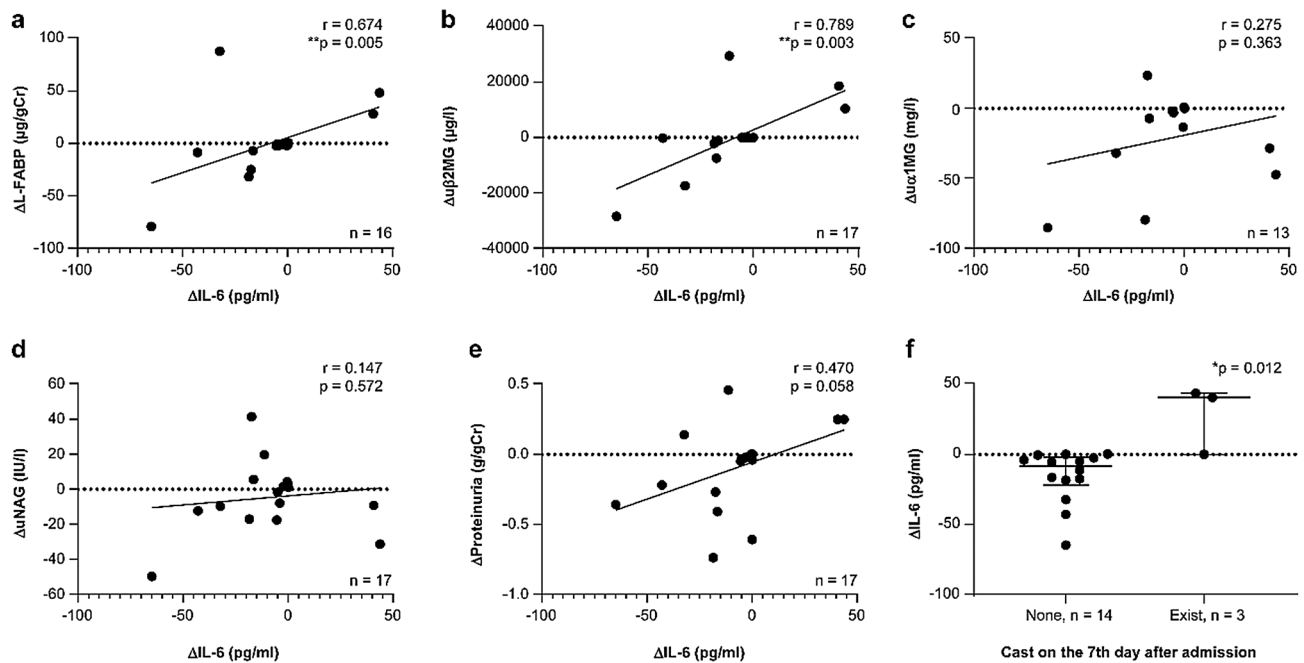


Fig. 4 Correlation of change from baseline in serum IL-6 levels and urinary biomarker values. * $p \leq 0.05$, ** $p \leq 0.01$. *IL-6* interleukin-6, *L-FABP* liver-type fatty acid binding protein, *uβ2MG* β2-microglobulin, *uα1MG* α1-microglobulin, *uNAG* *N*-acetyl-β-D-glucosaminidase

with the IL-6 changes. L-FABP, which is predominantly expressed in the proximal tubules, is known to be a predictive and sensitive early diagnostic biomarker for AKI [21]. Additionally, L-FABP can reflect defective renal microcirculation as opposed to other tubular markers, such as uNAG, uα1MG, and uβ2MG [21]. These results suggest that IL-6, one of key mediators for cytokine storm, could be strongly involved in developing our subjects' tubular injuries.

Most abnormalities in the urinary biomarker levels might be primarily due to acute proximal tubular damage; however, it is unclear whether proteinuria reflected tubular injury or glomerular impairment with podocyte damages. Aside from ACE2 expression in podocytes, the finding that IL-6 levels were significantly associated with the presence of nephritic sediments could indicate that SARS-CoV-2 might cause glomerular injury. Further study is needed to elucidate this issue.

The present study has several limitations. First, because we only studied patients from a single center, we had a small population; second, patients with severe COVID-19 were significantly older and more likely to have diabetes, which might confound the results; third, we lacked clinical and pathological data that would suggest decisive evidence for acute renal tubular damage; fourth, though there are various inflammatory cytokines, this study assessed only IL-6, known as one of the representative inflammatory cytokines; finally, our study could

not investigate the involvement of direct viral infection to the kidney cells. Large-scale studies are needed to focus on tubular damage and elucidate underlying mechanisms of renal complication in COVID-19 infection.

The present study suggests that acute tubular injury can occur depending on the severity of COVID-19 infection, and L-FABP and uβ2MG can be useful early diagnostic biomarkers for COVID-19-associated renal injury.

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Author contributions YF, HN, and YN wrote the original draft and contributed equally. MH, TN, MK, TG, SU, and YS reviewed and edited the manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study was approved by the ethics committee of Juntendo University Hospital (Process No. 20-036). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institution at which the studies were conducted and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all participants included in the study.

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