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Evaluation of Coronavirus Disease 2019 Severity Using Urine Biomarkers

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Subjects: Early detection of coronavirus disease 2019 in patients likely to develop severe manifestations enables appropriate interventions, including rapid ICU admission. This study was conducted to determine whether noninvasive urine biomarkers can predict the clinical severity of coronavirus disease 2019.

Interventions: Not applicable.

Measurements and Main Results: This is single-center study, national center hospital designated for infectious disease. Fifty-eight patients who tested positive for severe acute respiratory syndrome coronavirus 2 in respiratory specimens through real-time reverse transcription-polymerase chain reaction were retrospectively studied. Urinary β2-microglobulin, liver-type fatty acid-binding protein were serially measured. Serum interferon-γ and monocyte chemotactic protein-1 were also evaluated. The 58 patients were assigned into three groups. Patients requiring intensive care were assigned to the severe group (n = 12). Patients treated with oxygen were assigned to the moderate group (n = 13). Other patients were assigned to the mild group (n = 33). Urine tests revealed that low β2-microglobulin and liver-type fatty acid-binding protein levels were associated with mild disease, whereas high levels were associated with severe disease. In severe cases, liver-type fatty acid-binding protein tended to be persistently high. The resulting cutoff values were β2-microglobulin; severe versus moderate + mild: 2,457 μg/dL (specificity 76.9% and sensitivity 90.0%, area under the receiver operating characteristic curve 85.9%), liver-type fatty acid-binding protein; severe versus moderate + mild: 22.0 μg/gCre (specificity 84.6% and sensitivity 90%, area under the receiver operating characteristic curve 91.8%). Urinary β2-microglobulin and serum interferon-γ/monocyte chemotactic protein-1 showed a similar trend.

Conclusions: Evaluating urinary biomarkers such as β2-microglobulin and liver-type fatty acid-binding protein may allow determination of coronavirus disease 2019 patients with active cytokines and recognition of patients likely to become critically ill and requiring careful observation and early intervention.

Key Words: acute kidney injury; coronavirus disease 2019; cytokine; liver-type fatty acid-binding protein; urinary biomarker; β2-microglobulin

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Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and causes symptoms ranging from asymptomatic to severe illness. Early recognition of severe manifestations facilitates rapid and appropriate intervention, including admission to ICUs and intensive care therapy, such as mechanical ventilation. However, limited data exists for predicting the clinical course of COVID-19. Initially, elderly patients were reported to be at higher risk of severe illness. However, it has been determined that even young patients without a history of illness can become severely ill. It is currently impossible to predict which patients will have worsening respiratory conditions. Cytokine involvement in COVID-19 pathogenesis has attracted a great deal of attention (1). To date, there have been no reports on the use of urine biomarkers to assess COVID-19 severity. This study was conducted to determine whether noninvasive urinalysis is useful in assessing and predicting the severity or clinical course of COVID-19.

MATERIALS AND METHODS

Patient Population

We included patients who tested positive for SARS-CoV-2 in respiratory specimens through real-time reverse transcriptase-polymerase chain reaction and were admitted to the National Center for Global Health and Medicine between January 30 and April 10. Patients under 20 years old, with end-stage renal disease, or without urinalysis data during hospitalization were excluded. We obtained demographic data, information on clinical symptoms, and laboratory data. The study protocol was approved by the Institutional Review Board (approval number: NCGM-G-003472-02), and written informed consent was obtained from each patient.

Study Definitions and Urinary Biomarker Measurement

The primary outcome was set as ventilator use and the need to administer oxygen. Patient data were censored at the time of data cutoff on April 19. To assess COVID-19 severity, we adopted the assessment method used in previous reports (**Supplementary methods**, Supplemental Digital Content 1, http://links.lww.com/CCX/A250) (2). According to their clinical course, 58 patients were assigned to three groups. Patients requiring intensive care therapy such as mechanical ventilation were assigned to the severe group. Patients treated with oxygen but not requiring mechanical ventilation were assigned to the moderate group. The remaining patients were assigned to the mild group. For each patient, 2–4 urine samples were sequentially obtained. Urine obtained at admission was defined as being collected within 72 hours of admission.

Statistical Analysis

Data were expressed as the median (maximum and minimum). The baseline characteristics and clinical findings of patients at admission were presented. Urinary biomarker performance was determined using receiver operating characteristics (ROCs) curve analysis. All analyses were conducted using R (Version 3.5.1.; R Foundation for Statistical Computing, Vienna, Austria). For additional methods, see Supplementary methods (Supplemental Digital Content 1, http://links.lww.com/CCX/A250).

RESULTS

Cohort Demographics

Fifty-eight patients were studied retrospectively, of which 47 (81.0%) were male and 11 (19.0%) were female. Baseline characteristics are shown in **Supplementary Table 1** (Supplemental Digital Content 2, http://links.lww.com/CCX/A251). Mean age was 51 years old (range, 21–83 yr old). Mean body mass index was 25.6 (range, 16.6–35.5). The time from onset to admission was 6 days (range, 1–16 d). Thirteen patients (22.4%) had coexisting hypertension. All patients had fever (> 37.5°C). Thirty-eight patients (65.5%) had COVID-related pneumonia observed by CT scan. Four patients (6.9%) required venovenous extracorporeal membrane oxygenation, and one patient (1.7%) required continuous renal replacement therapy (CRRT) at the ICU. There were 12 (20.7%), 13 (22%), and 33 (56.9%) patients in the severe, moderate, and mild groups, respectively.

Variation of Urinary Biomarkers in Response to Disease

During treatment, severity-related events, such as initiation of oxygen and ventilator initiation, occurred within a week of admission in all patients. Only one patient developed severe acute kidney injury (AKI) and required CRRT. In other patients, serum creatinine generally remained below 1 mg/dL. However, tubular biomarker urinary liver-type fatty acid-binding protein (L-FABP) was persistently high in severe cases, whereas urinary β2-microglobulin (β2MG) was highly variable (**Fig. S1**, Supplemental Digital Content 3, http://links.lww.com/CCX/A252; **legend**, Supplemental Digital Content 6, http://links.lww.com/CCX/A255).

COVID-19 Severity Assessment Using Urine Specimens Within 10 Days of Onset

We examined the correlation between urine biomarkers and the subsequent clinical course of patients, from whom urine samples at admission and which were obtained within 10 days of symptom onset (n = 49, 84.5%). Urinary β2MG and L-FABP tended to be higher in accordance to severity (**Fig. 1*A*–*D***). Scatter plots were prepared presenting the urinary β2MG and L-FABP levels. A panel of biomarkers allowed for clearer delineation of severity (**Fig. 1*E***). The ROC curve was drawn to find the cutoff value with the highest area under the ROC curve. The resulting cutoff values were: β2MG; severe versus moderate + mild: 2,457 μg/dL, L-FABP; severe versus moderate + mild: 22.0 μg/gCre (**Fig. 2*A*–*D***). Those who had higher biomarkers at admission were more likely to have increased severity, requiring oxygen or ventilator placement over the course of treatment (**Fig. 2**, ***E*** and ***F***). Cutoff values to detect mild and severe cases are shown in **Figure S2** (Supplemental Digital Content 4, http://links.lww.com/CCX/A253; legend, Supplemental Digital Content 6, http://links.lww.com/CCX/A255). Furthermore, we examined the time series correlation between urinary β2MG and interferon-γ (IFN-γ) and monocyte chemotactic protein-1 (MCP-1) in four patients in the severe and moderate groups. With the exception of one moderate patient, urinary β2MG and IFN-γ/MCP-1 showed a similar trend (**Fig. S3**, Supplemental Digital Content 5, http://links.lww.com/CCX/A254;

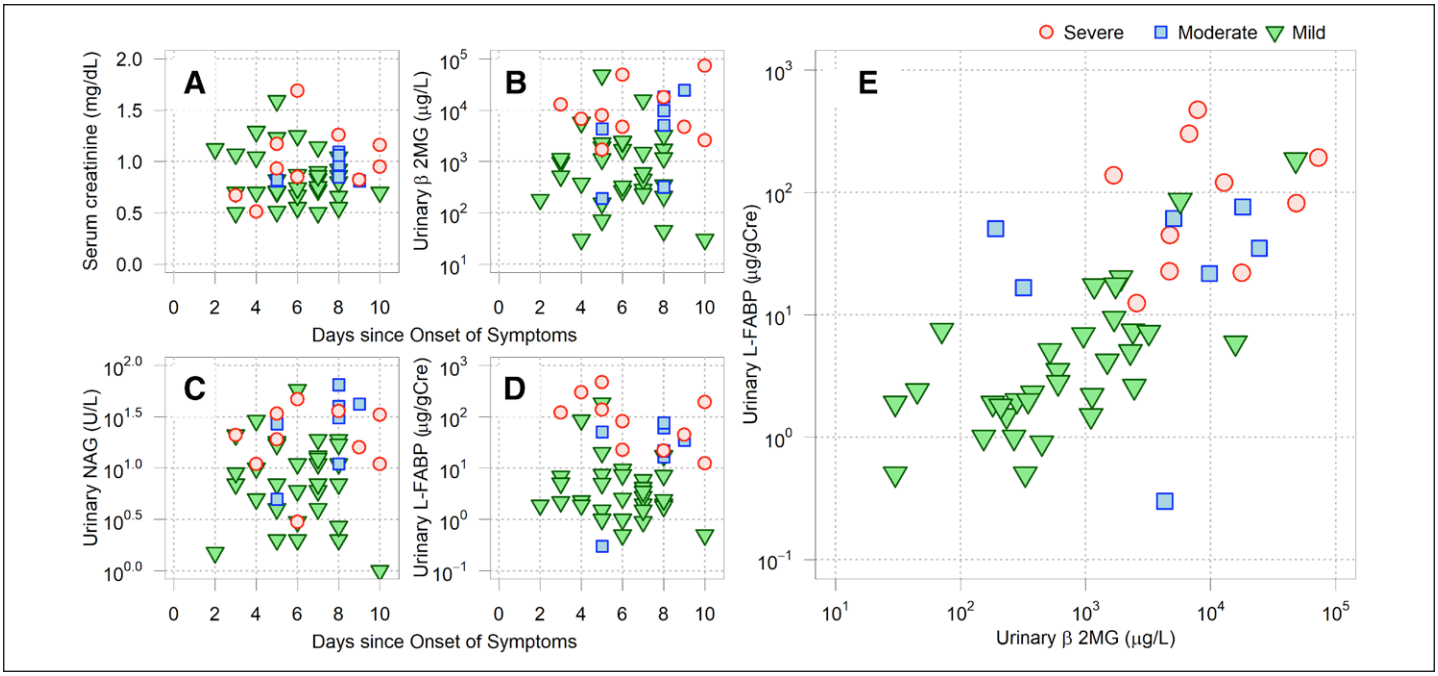

Figure 1. Assessment of coronavirus disease 2019 severity using urine specimens within 10 d of onset. The distribution and clinical severity of sCre (**A**), urinary β2-microglobulin (β2MG) (**B**), urinary NAG (**C**), and urinary liver-type fatty acid-binding protein (L-FABP) (**D**) were presented in patients for whom we were able to obtain urine specimens within 10 d of disease onset ($n = 49$). *Scatter plots* plotting urinary β2MG on the horizontal axis and urinary L-FABP on the vertical axis allowed us to distinguish between severe, moderate, and mild illness (**E**). Patients with moderate disease but mild acute kidney injury at admission, or uncontrolled HIV, tended to have relatively high levels of β2MG and L-FABP. NAG = N-acetyl-β-d-glucosaminidase, sCre = serum creatinine.

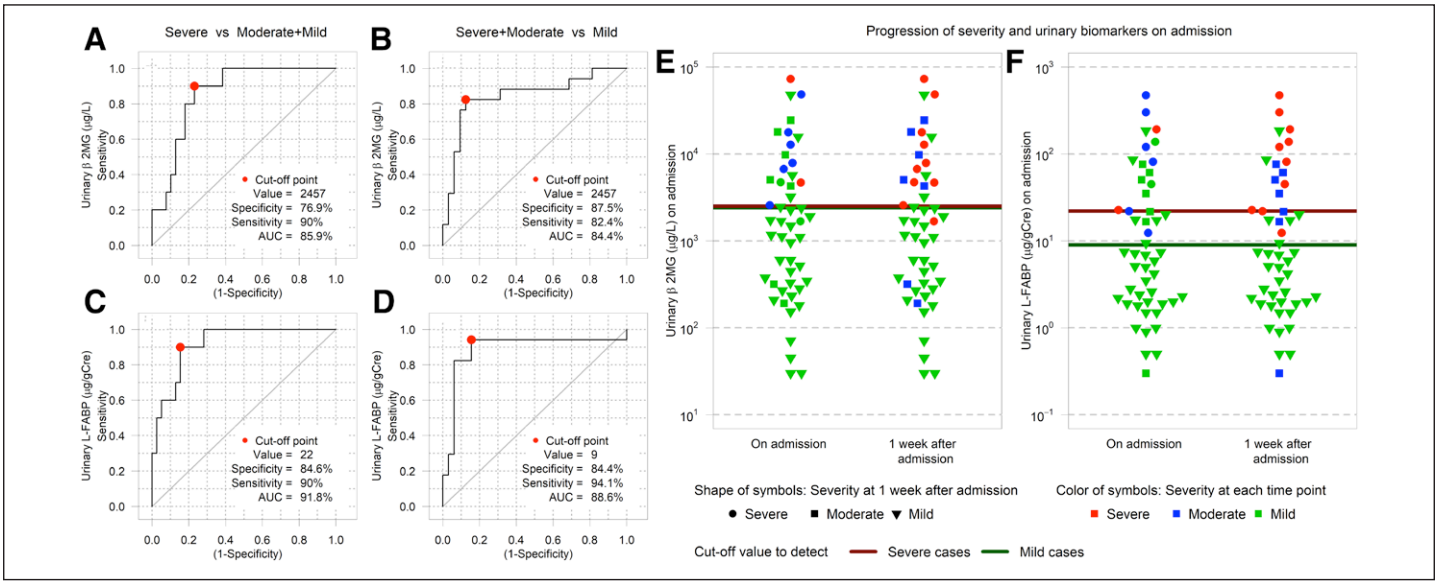

Figure 2. Receiver operating characteristic (ROC) curves for detecting mild or severe cases in coronavirus disease 2019. **A–D**, ROC curve analysis was performed to detect the mild and severe groups using urine tests. The resulting cutoff values were β2-microglobulin (β2MG); severe versus moderate + mild (**A**): 2,457 μg/dL (specificity 76.9% and sensitivity 90.0%, area under the receiver operating characteristic curve [AUC] 85.9%), severe + moderate versus mild (**B**): 2,457 μg/dL (specificity 87.5% and sensitivity 82.4%, AUC 84.4%), liver-type fatty acid-binding protein (L-FABP); severe versus moderate + mild (**C**): 22.0 μg/gCre (specificity 84.6% and sensitivity 90.0%, AUC 91.8%), severe + moderate versus mild (**D**): 9.0 μg/gCre (specificity 84.4% and sensitivity 94.1%, AUC 88.6%). **E** and **F**, Progression of disease severity and urinary biomarkers at admission. The same patient is plotted in the same position at both time points. Shape of symbols indicates the severity 1 wk later (severe, *circle*; moderate, *square*; mild, *triangle*). Symbol color is determined by the severity at each time point (severe, *red*; moderate, *blue*; mild, *green*). The *green* and *red lines* are the cutoff values of the biomarkers calculated from the ROC analysis.

legend, Supplemental Digital Content 6, http://links.lww.com/CCX/A255).

DISCUSSION

Some patients (surrounded by COVID-19 patients with mild illnesses), whose respiratory condition deteriorates rapidly around 10 days after onset, required both a ventilator and an artificial lung (3). However, it is currently unknown which patients should be carefully observed, making the provision of appropriate therapeutic interventions challenging. Older age, higher Sequential Organ Failure Assessment score, and high D-dimer levels were related to COVID-19 mortality (4). Another study reported older age as a risk factor of acute respiratory distress syndrome development and mortality (5). During the first 3 months of the COVID-19 outbreak in Japan, all COVID-19-positive patients (including those with mild illnesses) were required to be hospitalized, according to administrative policy. Our institution is a tertiary referral national hospital for infection disease. We were therefore in an environment where changes in the severity of the illness were easily monitored for the duration. In our treatment of COVID-19 patients, we noted that many critically ill patients had very high urine biomarkers in their urine samples at the time of admission, even if they did not present high serum creatinine. To the best of our knowledge, there have been no reports of urinary biomarkers being used to evaluate COVID-19 disease activity, rather than evaluating the renal function of COVID-19 patients. Our findings suggest that urinary biomarkers, especially β2MG and L-FABP, may assist with predicting severity at an early stage.

β2MG is an 11,870 Da single-chain polypeptide present on almost all nucleated cells. It is a cytokine-inducible protein that is elevated by an increase in IFN-γ released by T helper cell type 1 cells. IFN-γ causes human leukocyte antigen-class 1 overexpression and an increase in the H and L chains, but the L chain that fails to connect to the H chain (= β2MG) leaks into the urine. Since both IFN-γ and MCP-1 are associated with urinary β2MG (6, 7), the extreme rise in urinary β2MG was thought to reflect cytokine storm (8). L-FABP is expressed in renal proximal tubules and is shed into urine with hypoxia (9). L-FABP is used clinically to detect AKI more accurately than serum creatinine, but can also be used to predict severity and mortality in ICUs (10). N-acetyl-β-d-glucosaminidase exists in the lysosomes of proximal tubule epithelial cells, and its clinical relevance as a specific biomarker for renal disease is established. In our study, L-FABP levels were significantly higher among severe patients, even if they did not satisfy the AKI criteria of the Kidney Disease Improving Global Outcomes. A higher urinary β2MG (> 2,457 μg/dL) or L-FABP (> 22.0 μg/gCre) within 10 days of onset thus indicates a high risk of developing severe disease. Since these patients may become seriously ill, they can be transferred to a hospital with adequate medical resources or prepared for ICU admission.

There are several limitations to our study. First, only a limited number of patients were examined. Therefore, to evaluate the usefulness of urinalysis in addition to patient severity predictors (such as lactate dehydrogenase or D-dimer, which have already been reported in COVID-19), a larger cohort study must to be considered. Second, patients in chronic kidney disease stage 4 and 5 sometimes show higher baseline β2MG and L-FABP, which may preclude the judgment of the cutoff value. The current study did not include such patients.

CONCLUSIONS

Noninvasive urinalysis can be used to screen patients requiring careful observation. We suggest that observing β2MG and L-FABP may provide a timely assessment of the cytokine status and disease severity in COVID-19 patients.

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Drs. Katagiri and Ishikane designed the study. Drs. Kinoshita, Ota, Moriyama, Ide, Nakamura, Nakamoto, Nomoto, Akiyama, Miyazato, Suzuki, Okuhama, Kanda, Wakimoto, Morioka, Saito, Yamamoto, Ujiie, Hayakawa, Kustuna, Yanagawa, Terada, Takasaki, Izumi, and Hojo obtained consent from the patients. Dr. Asai created the figures. Drs. Sugiyama and Mizokami carried out cytokine experiments. Drs. Katagiri, Ishikane, Hinoshita, Noiri, Ohmagari, and Sugiyama drafted and revised the article. All authors approved the final version of the article.

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