Divergence between serum creatine and cystatin C in estimating glomerular filtration rate of critically ill COVID-19 patients

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ABSTRACT

Background: The clinical use of serum creatine (sCr) and cystatin C (CysC) in kidney function evaluation of critically ill patients has been in continuous discussion. The difference between estimated glomerular filtration rate calculated by sCr (eGFRcr) and CysC (eGFRcysc) of critically ill COVID-19 patients were investigated in this study.

Methods: This is a retrospective, single-center study of critically ill patients with COVID-19 admitted to intensive care unit (ICU) at Wuhan, China. Control cases were moderate COVID-19 patients matched in age and sex at a ratio of 1:1. The eGFRcr and eGFRcysc were compared. The association between eGFR and death were analyzed in critically ill cases. The potential factors influencing the divergence between eGFRcr and eGFRcysc were explored.

Results: A total of 76 critically ill COVID-19 patients were concluded. The mean age was 64.5 ± 9.3 years. The eGFRcr (85.45 (IQR 60.58–99.23) ml/min/1.73m²) were much higher than eGFRcysc (60.6 (IQR 34.75–79.06) ml/min/1.73m²) at ICU admission. About 50% of them showed eGFRcysc < 60 ml/min/1.73 m² while 25% showed eGFRcr < 60 ml/min/1.73 m² (χ² = 10.133, p = 0.001). This divergence was not observed in moderate group. The potential factors influencing the divergence included serum interleukin-6 (IL-6), tumor necrosis factor (TNF-α) level as well as APACHEII, SOFA scores. Reduced eGFRcr (<60 mL/min/1.73 m²) was associated with death (HR = 1.939, 95%CI 1.078–3.489, p = 0.027).

Conclusions: The eGFRcr was generally higher than eGFRcysc in critically ill COVID-19 cases with severe inflammatory state. The divergence might be affected by inflammatory condition and illness severity. Reduced eGFRcr predicted in-hospital death. In these patients, we advocate for caution when using eGFRcysc.

Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a worldwide pandemic. Over 176 million cases and 3.8 million deaths were reported all over the world [1]. Besides alveolar damage, the involvements of other organs including kidney [2] have also been widely observed, especially in critically ill patients. The incidence of COVID-19 associated acute kidney injury (AKI) was reported as high as 36.6–46% in large cohorts of hospitalized patients [3–5], and this proportion was even higher in patients admitted to ICUs [6,7]. What’s more, the prevalence of kidney disease on admission and the kidney involvement during hospitalization in COVID-19 patients were associated with in-hospital mortality [8,9]. Therefore, correct estimation of kidney damage plays a very important role in improving prognosis by prompt intervention, appropriate dosing of drugs and adjustment of therapeutic strategies. In clinical practice, serum creatine (sCr) and cystatin C (CysC) were common biomarkers used to evaluate the glomerular filtration function. However, their performance in critically ill patients was not universally agreed [10–15]. Some studies [10,16–18] considered sCr highly misleading in ICU patients because of muscle mass loss and volume overload in many critically ill patients. CysC is also influenced by several factors such as age [19,20], corticosteroids administrations [21–23], inflammation [19,24], and diabetes status [25,26]. So far, little is...
known about the performance of sCr and CysC for glomerular filtration rate (GFR) estimation in critically ill patients with COVID-19. We conducted a retrospective observational study in critically ill patients with COVID-19 to explore the difference of sCr and CysC in GFR estimation and their relevance with prognosis.

Method

Study design and participants

This is a single-center, retrospective study conducted in ICU designated for critically ill patients with COVID-19 at the Sino-French New City Campus of Tongji Hospital in Wuhan, China. All of the patients in the ICU who met the criteria of a critically ill case of COVID-19 and had sCr as well as CysC tested at the same time between January 29 and March 20, 2020 were included. Moderate COVID-19 patients matched in age and sex at a ratio of 1:1 from non-ICU wards on the corresponding period were selected as control cases. The diagnosis and classification standards are as follows.

All confirmed patients were diagnosed according to the Guideline of Chinese National Health Commission (Fifth Trial Edition) [27]. The clinical diagnosis criteria were as follows: (1) fever or respiratory symptoms, (2) leukopenia or lymphopenia, (3) computerized tomography scan showing radiographic abnormalities in lung. Patients with two or more clinical diagnosis criteria and a positive result to high-throughput sequencing or RT-PCR assay of SARS-CoV-2 were defined as confirmed case with COVID-19. Severity of the disease was staged into mild, moderate, severe, and critical types. Critically ill COVID-19 cases were defined as including at least one of the following: septic shock, respiratory failure requiring mechanical ventilation, and a combination of other organ failures and admission to ICU. A severe case was defined as (1) respiratory rate > 30 breaths/min; (2) oxygen saturation ≤93%, or (3) PaO2/FiO2 ratio ≤ 300 mm Hg. A moderate case was defined as clinical symptoms of fever and cough, with radiographic evidence of pneumonia but did not meet the criteria of severe cases. A mild case was defined as mild clinical symptoms without radiographic evidence of pneumonia by chest CT scan.

This study was approved by the PUMCH Institutional Review Board (ZS-2328, SK-1197).

Data collection and definitions

Presence of comorbidities, laboratory data, treatment regimens, and clinical outcomes was collected from the electronic medical records, laboratory results, and medical order lists. The sCr was measured by enzyme colorimetry while CysC was measured by particle-enhanced immunonephelometric assay with nephelometer. The eGFR was calculated at ICU admission. Fever was defined as axillary temperature of at least 37.3 °C. Sepsis and septic shock were defined according to the 2016 Third International Consensus Definition for Sepsis and Septic Shock [28]. Hypoalbuminemia was defined as serum albumin < 30 g/L. Leukocytosis was defined as white blood cell (WBC) count > 9.5 × 10^9/L. Lymphocytopenia was defined as lymphocyte < 1.1 × 10^9/L. D-dimer levels were classified into four categories: < 0.5 as category 1, 0.5–5.0 as category 2, 5.0–21.0 as category 3, and > 21.0 as category 4. Elevated sCr was defined as > 104 μmol/L in men and > 84 μmol/L in women. Declined sCr was defined as < 59 μmol/L in men and < 45 μmol/L in women. Elevated CysC was defined as > 1.55 mg/L. Declined CysC was defined as < 0.6 mg/L. Reduced eGFR was defined as eGFR < 60 mL/min/1.73 m². The primary outcome was death in hospital before March 20th. Disease course was defined as time from illness onset to death or transference out from ICU.

Evaluation of glomerular filtration rate

The estimated glomerular filtration rate (eGFR) was calculated according to CKD-EPI equations as follows (Table 1).

The divergence between eGFRcr and eGFRcysc

To explore the factors influencing the divergence between eGFR-cr and eGFRcysc in critically ill group, patients were sub-grouped by the divergence degree between eGFRcr and eGFRcysc. The divergence degree between eGFRcr and eGFRcysc was measured by the difference ratio (ΔeGFRcr-cysc%)defined as ΔeGFRcysc divided by mean of eGFRcr and eGFRcysc. That is, ΔeGFRcr-cysc% = (eGFRcr−eGFRcysc)/(eGFRcr+eGFRcysc)/2. Group 1, group 2 and group 3 represented patients with ΔeGFRcr-cysc% 0–25 %, 25 %–45 %, and > 45 %, respectively [1]. Clinical features among sub-groups were analyzed. The association between eGFR and death were explored.

Statistical analysis

Categoric variables were expressed as frequency and percentage and continuous variables were expressed by mean ± SD (for data that were normally distributed), or median and inter-quartile range (IQR) (for data that were not normally distributed). When the data were normally distributed, independent t tests were used to
compare the means of continuous variables. Otherwise, the Mann–Whitney test was used. The \( \chi^2 \) test was used to compare the differences of categoric variables. Cox proportional hazards models were used to analyze the risk factors related to death. Kruskal–Wallis H test and ordinal multi-categorical logistic regression was used to identify the potential factors influencing the divergence. All statistical analyses were performed using SPSS version 22.0 software. A \( p \)-value of 0.05 is statistically significant.

**Results**

**Clinical characteristics of critically ill cases and moderate cases with COVID-19**

A total of 76 critically ill patients and 76 moderate cases matched with age and sex were included. The mean age of the critically ill patients was 64.5 ± 9.3 years and the male: female ratio was 49:27 (Table 2). In critically ill patients, the median Acute Physiology and Chronic Health Evaluation II (APACHEII) and Sequential Organ Failure Assessment (SOFA) scores were 13 (IQR, 10–18.75) and 5 (IQR, 4–8), respectively at admission to ICU. Vasopressors were needed in 63.2% (48/76) of patients, and 78.9% (60/76) of patients received invasive mechanic ventilation. Compared with moderate cases, significant elevation of inflammatory markers such as high-sensitivity C-reactive protein, IL-6, and ferritin were also observed in these patients (Table 2).

**Renal functions in critically ill patients and moderate patients**

The median sCr and eGFRcr of critically ill patients were 76.5 (IQR 53.25–104.25)μmol/L and 85.45 (IQR 60.58–99.23) ml/min/1.73m², which was comparable with moderate group (Table 2). The sCr of critically ill patients had a larger extent of dispersion with a coefficient of variation of 1.189 while the distribution was relatively concentrated in moderate cases with a coefficient of variation of 0.304 (Figure 1; Table 2).

The level of median sCysC of critically ill patients was much higher than that in the moderate group (1.17 (IQR, 0.99–1.78)mg/L vs. 0.99 (IQR, 0.88–1.09) mg/L, \( p < 0.001 \)) and the eGFRcysc was significantly lower (60.6 (IQR, 34.75–79.06) ml/min/1.73 m² vs 74.55 (IQR, 65.58–91.19) ml/min/1.73 m², \( p < 0.001 \)) (Table 2). In critically ill patients, eGFRcysc was significantly higher in group 2 and 3 than in group 1 (17 (IQR, 10.5–20) vs. 14 (IQR, 12–20) vs. 10 (IQR, 8–13), \( p = 0.001 \)) (Table 5, Figure 4). Ordinal multi-categorical logistic regression indicated a positive correlation between the \( \Delta \)eGFRcysc% and TNF-\( \alpha \) level (OR = 9.49, 95%CI 1.45–62.05, \( \chi^2 = 5.52, p = 0.019 \)) (grouped by quartile).

**Factors influencing the divergence between eGFRcr and eGFRcysc**

In the critically ill patients with COVID-19, higher eGFRcr than eGFRcysc was present in 86.8% (66/76). No significant difference in age, gender, plasma albumin level, plasma calcium level etc was found among three subgroups graded by the divergence degree between eGFRcr and eGFRcysc (\( \Delta \)eGFRcr-eGFRcysc %). Compared with group 1, patients in group 3 had significantly higher inflammation factor levels including IL-6 97.72 (IQR, 38.44–290.65) pg/ml vs. 30.21 (IQR, 12.46–44.92) pg/ml, \( p = 0.005 \) and TNF-\( \alpha \)13.1 (IQR, 8.6–20.4) pg/ml vs. 7.6 (IQR, 6.3–11.6) pg/ml, \( p = 0.022 \) (Table 5, Figure 4). Meanwhile, the APACHEII scores were higher in groups 2 and 3 than in group 1 (17 (IQR, 10.5–20) vs. 14 (IQR, 12–20) vs. 10 (IQR, 8–13), \( p = 0.001 \)) (Table 5, Figure 4). The associations between eGFR and outcome

**The associations between eGFR and outcome**

In critically ill group, a total of 56 (73.7%) patients died in hospital. The median time from illness onset and ICU admission to death was 29 (21–38) days and 9 (5–18) days, respectively (Table 2). Compared with survivors, non-survivors had higher levels of APACHEII, SOFA scores, D-dimer category and inflammatory markers, including WBC, IL-6, IL-8 and hsCRP (Table 3). Univariate analysis indicated a positive correlation between the \( \Delta \)eGFRcysc% and TNF-\( \alpha \) level (OR = 9.49, 95%CI 1.45–62.05, \( \chi^2 = 5.52, p = 0.019 \)) (grouped by quartile).

### Table 1. Description of the formulas used.

|                | CKD-EPI creatinine equation | CKD-EPI cystatin C equation |
|----------------|----------------------------|----------------------------|
| Female         | sCr ≤ 62: 144*(sCr/62)^−0.329* + 0.993\(^{\text{Age}}\) | CysC ≤ 0.8: 133*(CysC/0.8)^−0.499* + 0.996\(^{\text{Age}}\) + 0.932 |
|                | sCr > 62: 144*(sCr/62)^−1.209* + 0.993\(^{\text{Age}}\) | CysC > 0.8: 133*(CysC/0.8)^−1.328* + 0.996\(^{\text{Age}}\) + 0.932 |
| Male           | sCr ≤ 80: 141*(sCr/80)^−0.411* + 0.993\(^{\text{Age}}\) | CysC ≤ 0.8: 133*(CysC/0.8)^−0.499* + 0.996\(^{\text{Age}}\) |
|                | sCr > 80: 141*(sCr/80)^−1.209* + 0.993\(^{\text{Age}}\) | CysC > 0.8: 133*(CysC/0.8)^−1.328* + 0.996\(^{\text{Age}}\) |

The units of sCr and CysC were umol/L and mg/L, respectively.
Table 2. Demographic, clinical characteristics, and laboratory findings of critically ill and moderate patients.

|                        | Critically ill | Moderate | p Value |
|------------------------|---------------|----------|---------|
| **Demographic**        |               |          |         |
| Age (years)            | 64.5 ± 9.3    | 62.9 ± 9.3 | 0.182   |
| Male                   | 49 (64.5 %)   | 49 (64.5 %) | 1       |
| **Clinical characteristics** |             |          |         |
| Death, n (%)           | 56 (73.7 %)   | 0        |         |
| Disease course (days)  | 29 (21–38)    | 41 (32–50) | <0.001  |
| Time of hospitalization (days) | 17 (9–27) | 16 (8.25–22) | 0.269   |
| Time of ICU (days)     | 9 (5.25–18)   | –        |         |
| Time from illness to ICU (days) | 16.5 (11–25) | –        |         |
| **Comorbidity, n (%)** |             |          |         |
| Hypertension           | 35 (46.1 %)   | 30 (39.5 %) | 0.412   |
| Diabetes mellitus      | 18 (23.7 %)   | 15 (19.7 %) | 0.555   |
| Coronary heart disease | 16 (21.1 %)   | 8 (10.5 %)  | 0.075   |
| Current smoker         | 11 (15.1 %)   | 7 (9.3 %)   | 0.286   |
| Cerebrovascular disease| 5 (6.6 %)     | 2 (2.6 %)   | 0.246   |
| **Laboratory findings**|             |          |         |
| White blood cell count (× 10^9/L) | 11.57 (8.04–16.46) | 5.35 (4.27–6.46) | <0.001 |
| Neutrophils count (× 10^9/L) | 10.24 (7.37–15.12) | 3.25 (2.54–4.16) | <0.001 |
| Lymphocytes (× 10^9/L) | 0.56 (0.40–0.78) | 1.15 (0.95–1.76) | <0.001 |
| Lymphocytopenia         | 71 (93.4 %)   | 32 (42.7 %) |         |
| Hemoglobin (g/L)       | 108 (123.5–138) | 125 (112–137) | 0.607   |
| Anemia                 | 36 (47.4 %)   | 30 (39.5 %) | 0.361   |
| Platelets (× 10^9/L)   | 165 (101.25–220.25) | 220 (184–259) | <0.001 |
| Thrombocytopenia       | 25 (32.9 %)   | 32 (42.7 %) |         |
| Serum albumin (g/L)    | 28.5 (26.28–32.05) | 38.3 (35.6–41.7) | <0.001 |
| sCR (µmol/L)           | 76.5 (53.25–104.25) | 72.5 (61.25–82.25) | 0.359   |
| Elevated sCR, n (%)    | 19 (25 %)     | 5 (6.6 %)   | 0.002   |
| Declined sCR, n (%)    | 13 (17.1 %)   | 3 (9.9 %)   | 0.008   |
| eGFR-Cr (ml/min/1.73m²) | 85.45 (60.58–99.23) | 92.04 (80.45–97.81) | 0.119   |
| Reduced eGFR-Cr        | 19 (25 %)     | 5 (6.6 %)   | 0.002   |
| CysC (mg/L)            | 1.17 (0.99–1.78) | 0.99 (0.88–1.09) | <0.001 |
| Elevated CysC, n (%)   | 24 (31.6 %)   | 4 (5.3 %)   | <0.001  |
| Declined CysC, n (%)   | 0             | 0          |         |
| eGFR-CysC (ml/min/1.73m²) | 60.60 (34.75–79.06) | 74.55 (65.58–91.19) | <0.001 |
| Reduced eGFR-CysC      | 38 (50 %)     | 11 (14.5 %) | <0.001  |
| IL-6 (pg/ml) (<7)      | 54.88 (20.76–169.35) | 6.81 (3.12–16.31) | <0.001 |
| Ferritin (mg/ml) (15–150) | 1302.9 (730.45–2327.88) | 357.4 (350.8–580.8) | <0.001 |
| D-dimer (µg/ml FEU) (<0.5) |             |          |         |
| <0.5                   | 0             | 0         |         |
| 0.5–5.0                | 0             | 28 (37.3 %) |         |
| 5.0–21.0               | 40 (54.8 %)   | 15 (20 %)  | <0.001  |
| >21.0                  | 33 (45.2 %)   | 32 (42.7 %) |         |
| hsCRP (mg/L) (<1)      | 103.05 (59.58–153.8) | 3.50 (0.93–12.65) | <0.001 |

Figure 1. Comparison of sCr and CysC between critically ill patients and moderate patients. (a) The SCr of critically ill patients has an equivalent median with moderate patients but distributed more dispersedly; (b) The median of CysC was significantly higher in critically ill patients than in moderate patients.
showed differences in the same 76 critical ill patients.

Elevated 19 (25 %) 24 (31.6 %) 0.368
Reduced eGFR 19 (25 %) 38 (50 %) 0.001

- defined as
was defined as
CysC was defined as

Demographic, clinical characteristics, and laboratory findings of nonsurvivors and survivors in critically ill patients.

| Demographic | Non-survivors | Survivors | p Value |
|-------------|--------------|-----------|---------|
| Age (year)  | 65.4 ± 7.7   | 62.0 ± 12.7 | 0.274 |
| Male, n (%) | 38 (67.9 %)  | 11 (55 %)  | 0.302 |

| Clinical characteristics | Non-survivors | Survivors | p Value |
|--------------------------|--------------|-----------|---------|
| APACHEII                 | 14 (10–20)   | 11 (9.25–13.0) | 0.021 |
| SOFA                     | 6 (4–8.75)   | 4 (3–5)    | 0.002 |
| Invasive ventilation     | 50 (89.3 %)  | 10 (50 %)  | 0.002 |
| Glucocorticoids          | 48 (85.7 %)  | 15 (75 %)  | 0.739 |
| Vasopressors             | 44 (78.6 %)  | 4 (20 %)   | <0.001 |
| Death, n (%)             | 56 (100 %)   | 0          |        |
| Disease course           | 26 (20–35)   | 35.5 (27–49) | 0.016 |
| Time of hospitalization (days) | 13.5 (9–23) | 16 (8.25–22) | 0.007 |
| Time of ICU (days)       | 8.5 (5–13)   | 14 (7–27)  | 0.015 |

Comorbidity

- Hypertension
- Diabetes mellitus
- Coronary heart disease
- Current smoker
- Cerebrovascular disease

Laboratory findings

| Laboratory findings | Non-survivors | Survivors | p Value |
|---------------------|--------------|-----------|---------|
| White blood cell count (×10^9/L) | 12.11 (9.23–18.86) | 9.32 (6.25–12.7) | 0.017 |
| Neutrophil count (×10^9/L) | 10.94 (8.11–17.27) | 8.38 (5.05–11.08) | 0.017 |
| Lymphocytes (×10^9/L) | 0.53 (0.36–0.73) | 0.65 (0.41–0.83) | 0.242 |
| Hemoglobin (g/L) | 126 (109–140) | 114 (93.75–133.25) | 0.155 |
| Platelets (×10^9/L) | 157 (77–217) | 181 (134.5–270.8) | 0.075 |
| Serum albumin (g/L) | 27.75 (25.08–31.23) | 30.40 (28.1–33.1) | 0.035 |
| Serum calcium (mmol/L) (2.15–2.50) | 2.21 (1.95–2.31) | 2.07 (1.91–2.20) | 0.115 |
| Serum inorganic phosphorus (mmol/L) (0.81–1.45) | 0.99 (0.78–1.15) | 0.98 (0.86–1.37) | 0.591 |
| Serum uric acid (mmol/L) (142.8–339.2) | 214.0 (134.0–302.8) | 214.0 (161.3–334.8) | 0.483 |
| Urea (mmol/L) (2.6–7.5) | 8.25 (5.43–11.0) | 8.80 (6.05–15.15) | 0.286 |
| sCr (µmol/L) | 78.5 (54.75–115.25) | 74.0 (45.75–90.75) | 0.190 |
| Elevated sCr, n (%) | 15 (26.8 %) | 4 (20 %) | 0.547 |
| Declined sCr, n (%) | 6 (10.7 %) | 7 (35 %) | 0.033 |
| eGFR-Cr (ml/min/1.73m²) | 85.85 (55.78–98.0) | 84.85 (70.83–107.85) | 0.392 |
| eGFR-Cr < 60 | 16 (28.6 %) | 5 (15 %) | 0.229 |
| CysC (mg/L) | 1.21 (0.95–1.83) | 1.13 (1.05–1.50) | 0.972 |
| Elevated CysC, n (%) | 20 (35.7 %) | 4 (20 %) | 0.194 |
| Declined CysC, n (%) | 0 | 0 | |
| eGFR-CysC (ml/min/1.73m²) | 57.51 (30.51–81.54) | 60.86 (43.55–67.96) | 0.972 |
| eGFRCr-cysc (ml/min/1.73m²) | 68.69 (41.72–91.23) | 74.70 (57.66–89.63) | 0.663 |
| eGFRCr-cysc < 60 | 24 (42.9 %) | 5 (25 %) | 0.158 |
| IL-6 (pg/ml) | 116.5 (37.15–220.4) | 29.76 (19.16–38.38) | <0.001 |
| IL-8 (pg/ml) (≤62) | 29.65 (15.30–63.85) | 22.9 (9.55–31.25) | 0.045 |
| IL-10 (pg/ml) (≤9.1) | 13.5 (7.9–20.3) | 7.8 (6.05–12.45) | 0.057 |
| TNF-α (pg/ml) (≥8.1) | 10.55 (7.18–19.33) | 9.8 (6.95–13.8) | 0.459 |
| Ferritin (mg/ml) | 1427.1 (829.15–2483.15) | 867.7 (649.7–1852.5) | 0.057 |
| D-dimer (mg/ml FEU), n (%) | 0 | 0 | |
| 0.5–5.0 | 16 (28.6 %) | 12 (60 %) |
| 5.0–21.0 | 12 (21.4 %) | 3 (15 %) | 0.048 |
| >21.0 | 27 (48.2 %) | 5 (25 %) |
| hsCRP (mg/L) | 110.20 (64.53–162.55) | 60.25 (31.43–117.35) | 0.009 |

Table 3. Demographic, clinical characteristics, and laboratory findings of nonsurvivors and survivors in critically ill patients.

Table 4. Renal function estimation using sCr and CysC showed differences in the same 76 critical ill patients.

| SCR     | CysC | p Value |
|---------|------|---------|
| eGFR  | 85.45 (60.58–99.23) | 60.6 (34.75–79.06) | <0.001 |
| Reduced eGFR | 19 (25 %) | 38 (50 %) | 0.001 |
| Elevated CysC | 19 (25 %) | 24 (31.6 %) | 0.368 |
| Declined CysC | 13 (17.1 %) | 0 | <0.001 |

Reduced eGFR defined as eGFR < 60 mL/min/1.73 m². Elevated sCr was defined as > 104 µmol/L in men and >84 µmol/L in women. Declined sCr was defined as ≤ 59 µmol/L in men and ≤ 45 µmol/L in women. Elevated CysC was defined as > 1.55 mg/L. Declined CysC was defined as ≤ 0.6 mg/L.

Discussion

To the best of our knowledge, this study is the first to compare the difference between sCr and CysC in the GFR estimation in critically ill patients with COVID-19.
We reported a striking divergence between eGFRcr and eGFRcysc which might be affected by the inflammatory condition. In critically ill patients with COVID-19, multi-organ damages were observed including renal involvement [2,5,29]. However, systematic assessment of the kidney function evaluation biomarkers has not been carried out so far.

The ability to accurately quantify GFR in critically ill patients remains challenging [30]. In bedridden critically ill patients who have a continuing loss of muscle mass [31], a parallel decline in sCr may lead to an overestimation of true GFR. In the contrast, Carlier et al. [32] reported that CysC systematically underestimated inulin clearance in critically ill patients. In two independent studies of mixed heterogeneous ICU patients [33] and critically ill children [34], CysC was found to be a poor biomarker for diagnosing AKI. Several studies comparing the performance of sCr and CysC in renal function estimation have gotten conflicting results in ICU patients [15,35,36]. A recent study carried by Sangla et al. [11] compared eGFR using different equations with the measured GFR and found that all equations displayed poor accuracy in the mixed ICU population.

Our data showed a significant divergence up to 24.85 mL/min/1.73 m² between the median eGFRcr and eGFRcysc in critically ill COVID-19 patients. Twice as many patients had GFR less than 60 mL/min/1.73 m² when estimated from CysC compared with GFR estimated from sCr. This finding seemed in line with a previous study carried out in a general ICU which reported this divergence as 44 versus 26 % [36]. They observed that during ICU admission, sCr progressively fell, whereas CysC rose at the same time. Compared with their report, we chose the timepoint of comparison between eGFRcr and eGFRcysc at ICU admission while

**Figure 2.** Proportion of different definition of “renal dysfunction” including elevated sCr, elevated CysC, eGFRcysc<60, eGFRcysc<60 in critically ill patients and moderate patients. In critically ill group, eGFRcysc less than 60 mL/min/1.73 m² presented in 50 % patients and eGFRcr less than 60 mL/min/1.73 m² presented in 25 % (p = 0.001). This divergence was not obvious in the moderate group (14.5 % vs 6.6 %, χ² = 2.515, p = 0.113). The proportion of elevated sCr and elevated CysC was 25 % and 31.6 % (p = 0.368) in critically ill patients. In moderate group, the proportion of elevated sCr was equal to elevated CysC (20 %).

**Table 5.** Differences of IL-6, TNF-α and APACHEII among three subgroups graded by the gap between eGFRcr and eGFRcysc.

| Median (IQR) | Group 1 | Group 2 | Group 3 | p Value (overall) | p Value (1 vs 2) | p Value (1 vs 3) | p Value (2 vs 3) |
|-------------|---------|---------|---------|------------------|-----------------|-----------------|-----------------|
| IL6 (pg/ml) | 30.21 (12.46–44.92) | 56.36 (28.43–203.98) | 97.72 (38.44–290.65) | 0.006 | 0.007 | 0.005 | 1.000 |
| TNF-α (pg/ml) | 7.6 (6.3–11.6) | 11.5 (7.3–16.7) | 13.1 (8.6–20.4) | 0.027 | 0.27 | 0.022 | 0.936 |
| APACHEII | 10 (8–13) | 14 (12–20) | 17 (10.5–20) | 0.001 | 0.009 | 0.001 | 1.000 |
| SOFA | 4 (1–5) | 6 (4–8) | 6.5 (4–8.75) | 0.002 | 0.006 | 0.005 | 1.000 |
| Death | 11 (57.9 %) | 19 (82.6 %) | 16 (66.7 %) | 0.205 | – | – | – |
| Glucocorticoid | 16 (84.2 %) | 17 (73.9 %) | 20 (83.3 %) | 0.632 | – | – | – |

*All patients: n = 66, Group 1 = 19, Group 2 = 23, Group 3 = 24.

**Table 6.** Different multivariate Cox proportional hazards models for risk factors of in-ICU death.

| MODEL1 | Albumin (Alb) | eGFRcysc < 60 | DD categories | p Value |
|--------|--------------|--------------|---------------|---------|
| HR     | 95% CI       | p Value      |               |         |
| MODEL2 | HR           | 95% CI       | p Value       |         |
| MODEL3 | HR           | 95% CI       | p Value       |         |
| MODEL4 | HR           | 95% CI       | p Value       |         |

*p Value

Groups I, II, and III represented patients with ΔeGFRcr-cysc%<25 %, 25 %–45 %, and >45 %, respectively.

ΔeGFRcr-cysc% was defined as ΔeGFRcr-cysc/mean of eGFRcr and eGFRcysc × 100 %. ΔeGFRcr-cysc%= |eGFRcr–eGFRcysc| / (eGFRcr+cGFRcysc)/2.
they made it at ICU discharge. During the initial outbreak of COVID-19, our patients were admitted to ICU at a relatively late phase with critical conditions and rapid progressions because the medical resources as well as the understanding of the newly discovered disease were both in shortage. So it could be explainable why our patients had shown significant divergence between eGFRcr and eGFRcysc at ICU admission.

Inflammatory cytokines including IL-6, TNF-α as well as APACHEII, SOFA scores were potential influencing factors of the divergence between eGFRcr and eGFRcysc in our study. It has been proved serum CysC could act as an inflammation marker in chronic kidney disease [24] and chronic obstructive pulmonary disease (COPD) patients [37]. Stevens et al. [19] indicated that higher levels of serum CysC were associated with hsCRP levels and WBC counts. Recently, Chen et al. [38] found the relationship between high CysC levels and severe inflammatory conditions in COVID-19 thus concluded that CysC could act as a potential inflammatory biomarker in COVID-19 patients. Based on these evidences indicating the association between CysC level and inflammation, it is reasonable that inflammatory state increased the divergence between eGFRcr and eGFRcysc. Patients with higher APACHEII scores were suffering more serious conditions and had higher probabilities of inflammatory cytokine storm thus the divergence may be elevated. There were inconsistent results regarding the associations between glucocorticoid and CysC. Risch et al. [39] concluded that glucocorticoid therapy was associated with increased concentration of CysC. Nevertheless, Hüsing et al. [40] observed no difference in CysC concentration among different serum cortisol levels in their ICU patients. In our study, there were no significant difference in glucocorticoid therapy among three groups divided by the divergence degree between eGFRcr and eGFRcysc.

As endogenous biomarkers of renal function, sCr and CysC both indirectly assess GFR. Since the striking difference between eGFRcr and eGFRcysc existed in

Figure 3. The Kaplan–Meier survival curves for critically ill patients divided by reduced eGFRcr (a), reduced eGFRcysc (b), elevated sCr(c) and elevated CysC (d). Reduced eGFRcr (<60 mL/min/1.73 m²) rather than reduced eGFRcysc was associated with death after ICU admission in critically ill patients with COVID-19. Both elevated sCr and elevated CysC were associated with death after ICU admission in critically ill patients with COVID-19.
critically ill patients with COVID-19, it is reasonable to speculate that one or both fail to reflect actual GFR. Evidence suggested that renal impairments were associated with worse prognosis [41–43]. Recently, the relationship between kidney injury and mortality in COVID-19 has also been reported [8,9,44,45]. To a certain extent, the prognosis values of eGFRcr and eGFRcysc could indirectly reflect the ability of renal function assessment. In our study, reduced eGFR-cr other than reduced eGFR-cysc showed significant relativity with death. In contrast to eGFRcysc, CysC itself could serve as a risk factor for mortality. This finding was also consistent with previous studies. In a study of a mixed ICU, Dyanne et al. verified the prognosis value of CysC in the illness severity in critically ill patients [46]. The predictive value of Cys C in the prognosis of COVID-19 patients has also been reported by Yan Li et al. [45] and Dan Chen et al. [38] separately. It seemed interesting that eGFRcysc is a poor predictive factor while CysC itself could be a good predictor of mortality in critically ill patients with COVID-19. Based on the association between inflammatory state and CysC, this result was explainable if we take the severe inflammation state of this special population into account. It is suggested that inflammatory factors such as IL-6 and TNF-α are positively correlated with disease severity in patients with COVID-19 [47,48]. Lots of patients with severe COVID-19 might suffer a cytokine storm syndrome which is a key factor in developing ARDS and extrapulmonary multiple-organ failure [49–51]. In our study, critically ill patients displayed obvious elevated levels of inflammatory cytokines including IL-6, ferritin, and hsCRP. Given all that, we may reasonably conclude that in critically ill patients with COVID-19 who were suffering a severe inflammation condition, CysC itself showed clinical significance in prognosis as an inflammatory marker but its value in estimating GFR is suspicious with the disturbance of severe inflammation state. For this population, CysC was not recommend to be used in eGFR calculating.

Our study does have several limitations. Being a single center study, the numbers of enrolled patients were limited and our findings would need confirmation in larger groups as well as other age groups. Moreover, we were unable to acquire the measured GFR through iohexol or inulin clearance procedure as a golden standard. On account of the relationship between kidney injury and mortality in COVID-19, we conducted a comparison between the prognostic values of eGFRcr and eGFRcys. In addition, the tests of tubular functions
Conclusions

In conclusion, we reported a noticeable divergence between the estimated GFR based on sCr and CysC in critically ill patients with COVID-19. The divergence might be affected by the illness severity and inflammatory condition. In critically ill COVID-19 patients with severe inflammatory state, we advocate for caution when using CysC based estimated GFR equations.

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Author contribution

P. X, Y.L, and Y. Q designed this study. P. X, Y. L drafted this manuscript. P. X and Y.L did all the statistics and draw the figures. P. X, J. M, W.C, Z.L, and Y.Q cared the patients enrolled in this study and provide the original clinical records. P. X and Y.L extracted the clinical data. X. L and Y.Q reviewed the manuscript. All authors reviewed the manuscript and approved the manuscript in its final form.

Disclosure statement

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