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Critical Illness and Systemic Inflammation Are Key Risk Factors of Severe Acute Kidney Injury in Patients With COVID-19

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Introduction: Acute kidney injury (AKI) is an important complication in COVID-19, but its precise etiology has not fully been elucidated. Insights into AKI mechanisms may be provided by analyzing the temporal associations of clinical parameters reflecting disease processes and AKI development.

Methods: We performed an observational cohort study of 223 consecutive COVID-19 patients treated at 3 sites of a tertiary care referral center to describe the evolvement of severe AKI (Kidney Disease: Improving Global Outcomes stage 3) and identify conditions promoting its development. Descriptive statistics and explanatory multivariable Cox regression modeling with clinical parameters as time-varying covariates were used to identify risk factors of severe AKI.

Results: Severe AKI developed in 70 of 223 patients (31%) with COVID-19, of which 95.7% required kidney replacement therapy. Patients with severe AKI were older, predominantly male, had more comorbidities, and displayed excess mortality. Severe AKI occurred exclusively in intensive care unit patients, and 97.3% of the patients developing severe AKI had respiratory failure. Mechanical ventilation, vasopressor therapy, and inflammatory markers (serum procalcitonin levels and leucocyte count) were independent time-varying risk factors of severe AKI. Increasing inflammatory markers displayed a close temporal association with the development of severe AKI. Sensitivity analysis on risk factors of AKI stage 2 and 3 combined confirmed these findings.

Conclusion: Severe AKI in COVID-19 was tightly coupled with critical illness and systemic inflammation and was not observed in milder disease courses. These findings suggest that traditional systemic AKI mechanisms rather than kidney-specific processes contribute to severe AKI in COVID-19.

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COVID-19, the disease caused by the pandemic SARS-CoV-2 virus, remains a major global health threat with continuously rising case numbers.[1] While the disease is primarily affecting the respiratory system, there is increasing evidence for the importance of kidney involvement. Early data from China suggested that acute kidney injury (AKI) occurred only in a small

minority of patients, with rates of AKI ranging from 0.5% to 5.1% and that kidney replacement therapy (KRT) was required only in 0.8%.[2,3] In contrast, reports from New York of patients treated during the surge in March and April 2020 showed that AKI may be very frequent.[4,5] In the Mount Sinai health care system, 43% of hospitalized COVID-19 patients developed AKI. AKI requiring KRT developed in 8.6% of all patients and in 34% of intensive care unit (ICU) patients.[6] Administrative data from Germany show that KRT was required in 22.8% of hospitalized COVID-19 patients.[7] These numbers suggest the kidney to be the second most affected organ in patients with COVID-19.[8–10]

The precise etiology of AKI in COVID-19 has not been fully elucidated.[11,12] Mild kidney abnormalities, such as hematuria or proteinuria, have been reported early in the course of COVID-19, suggesting direct renal effects of SARS-CoV-2.[13] In severely ill patients with COVID-19, direct renal tropism of SARS-CoV-2 has been reported and may impact kidney function and contribute to the severity of AKI.[14–16]

Nevertheless, other studies showed no evidence of viral tropism and indicated that acute tubular necrosis is the predominant injury pattern.[17–22] In addition, large retrospective cohort studies indicated that the severe stages of AKI occurred almost exclusively in critically ill patients with respiratory failure treated in ICUs.[4–6,23–25] These latter observations would be consistent with a critical illness–associated pathophysiology that frequently underlies non–COVID-associated forms of AKI.[26–29]

We reasoned that the detailed clinical courses of patients with COVID-19 may provide additional important clues to the pathophysiology of COVID-associated AKI. In particular, the association of AKI with COVID-19 disease severity and its temporal association with the disease course might be informative. Previous studies of AKI in COVID-19 had focused on the relationship of clinical baseline characteristics at hospital admission with AKI development.[4,5,22] However, because COVID-19 is characterized by a highly dynamic disease process, admission characteristics may only incompletely capture the association of clinical risk factors with AKI. We therefore present here a longitudinal analysis of laboratory and clinical parameters in relation to AKI development in 223 consecutive COVID-19 patients hospitalized in one of Europe's largest tertiary care centers during the first wave of COVID-19.

METHODS

Study Design

This retrospective observational cohort study was approved by the local ethics committee (EA4/013/20). We identified adult patients ($n = 236$) with symptomatic COVID-19 disease who were admitted to any of the 3 hospitals of the Charité–Universitätsmedizin Berlin between 1 March 2020 and 3 June 2020. Outcome data for death, discharge status, and ICU and hospital lengths of stay were collected up to 18 August 2020.

Inclusion and Exclusion Criteria

Patients had to be at least 18 years old, show signs or symptoms consistent with COVID-19, and required a positive reverse-transcriptase polymerase chain reaction test result from an oropharyngeal swap, sputum, or bronchioalveolar lavage fluid. Patients admitted for other diagnoses who tested positive for SARS-CoV2 in the screening swab upon hospital admission were not included if no COVID-19–related symptoms occurred during their hospital stay. We excluded patients with preexisting dialysis-dependent chronic kidney disease (CKD), patients in whom KRT for AKI was initiated in another hospital before transfer to Charité, and patients in whom medical therapy was palliatively limited on the day of admission.

Data Collection and Data Preparation

Data on the disease course, patient demographics, preexisting conditions and medications, laboratory and clinical parameters, and outcomes were extracted from electronic health records. Data cleaning and data handling was done using Excel (Microsoft, Redmond, WA) and Python 3 (Python Software Foundation, Wilmington, DE) using the Pandas library. All parameters were assessed regarding plausibility by 2 independent clinicians. Values for parameters with upper quantification limits (procalcitonin, D-dimer, interleukin 6) were approximated with upper limit + 1 unit. The limits were as follows: procalcitonin, 100 μg/l; D-dimer, 20 mg/l; and interleukin 6, 50 ng/ml. Very few data points were above these limits. In 3 patients in the severe AKI group, procalcitonin and interleukin 6 levels were above the upper limit. D-dimer values above the upper detection limit were found in 10 patients in the AKI stage 3 group and in 2 patients in the group not at AKI stage 3.

AKI Adjudication

AKI was defined and staged using the Kidney Disease: Improving Global Outcomes (KDIGO) AKI criteria:

- Stage 1: Increase in serum creatinine by 0.3 mg/dl within 48 hours or a 1.5- to 1.9-fold increase in serum creatinine from baseline within 7 days or urine output < 0.5 ml/kg/h for 6 to 12 hours
- Stage 2: 2.0- to 2.9-fold increase in serum creatinine from baseline within 7 days or urine output of < 0.5 ml/kg/h for more than 12 hours

- Stage 3: 3-fold or higher increase in serum creatinine within 7 days, or an increase in serum creatinine >4 mg/dl, or initiation of KRT or urine output of <0.3 ml/kg/h for >24 hours or anuria for >12 hours.[30]

Urine output data were only available for all patients admitted to the ICU.

Two clinicians independently adjudicated the creatinine baseline values and the AKI diagnosis and stage. The following guiding principles were applied in the adjudication process. When preadmission creatinine values were available, the lowest of these values was chosen. When no preadmission creatinine values were available, but the admission creatinine value corresponded to an estimated GFR (eGFR; CKD-Epidemiology Collaboration) $\geq$ 75 ml/min per 1.73 m^2, this value was chosen. When no preadmission creatinine values were available and the admission creatinine value corresponded to an eGFR (CKD-Epidemiology Collaboration) $<$ 75 ml/min per 1.73 m^2, the lowest stable creatinine level after recovery of the index AKI was chosen. If none of these conditions applied, the baseline creatinine value was reported as missing (this applied to 11 patients). In patients for whom the adjudicated baseline creatinine differed by $\leq$ 0.2 mg/dl between the 2 adjudicating clinicians, the mean of the 2 values was used. When values differed by $>$ 0.2 mg/dl, the baseline creatinine values were defined after a joint discussion.

The urine output data were extracted in the form of time-series data from the ICU information system (COPRA System GmbH, Berlin, Germany). The data were preanalyzed by calculating moving averages of the urine volumes divided by the respective body-weight over 6-, 12-, and 24-hour windows and were checked for the AKI criteria. The detected AKIs were validated through a manual medical record review.

Outcomes

Severe AKI (stage 3 AKI according to KDIGO criteria) was defined as the primary outcome, and the combination of stage 2 or stage 3 as the secondary outcome.[30] Follow-up time was censored on 3 June 2020 for the primary and secondary outcomes. Death and discharge status were followed-up until 18 August 2020.

Definitions of Preexisting Conditions

Preexisting conditions, with the exception of CKD, were recorded as documented by the treating physicians in the electronic medical record. The presence of significant CKD was defined as a baseline eGFR of $<$ 60 ml/min per 1.73 m^2, estimated from the adjudicated baseline creatinine with the CKD-Epidemiology Collaboration formula, assuming that the reduced GFR had persisted for at least 3 months.[31]

Statistical Analysis

Absolute and relative frequencies are reported for categorical variables, median along with interquartile ranges (IQR) for ordinal and continuous variables for all patients, and by primary outcome. Parameters measured longitudinally before the event are summarized with their minima or maxima and median per patient.

Time-to-event analyses were based on a time-to-first-event approach for the primary and the secondary outcome. Competing-risk analysis was applied to consider that the outcome and death without prior occurrence of the outcome were competing risks. Cumulative incidence curves were used to graphically assess time to AKI stage 3 or death without AKI stage 3. Cause-specific hazard ratios (HRs) of potential risk factors were estimated based on multivariable Cox proportional hazards regression models with time-varying covariates. Parameters included into the model were selected based on their representation of potential AKI mechanisms and pathophysiological categories and based on their availability and completeness in longitudinal clinical data (for details refer to Supplementary Tables S1 and S2). In addition, the association of all parameters with the outcome was assessed in simplified models, only controlling for baseline characteristics of sex, age, body mass index (BMI), diabetes, hypertension, and baseline eGFR.

Incomplete data in baseline parameters and longitudinal clinical parameters were addressed by multiple imputation. The imputation model included baseline characteristics (sex, age, BMI, diabetes, hypertension, and baseline eGFR), longitudinal clinical parameters (bilirubin, aspartate amino transferase, alanine amino transferase, creatine kinase, myoglobin, hemoglobin, platelet counts, leukocyte counts, neutrophil counts, lymphocyte counts, C-reactive protein, serum procalcitonin, interleukin 6, ferritin, D-dimer, lactate dehydrogenase, intensive care admission, vasopressor use, mechanical ventilation, and extracorporeal membrane oxygenation), whether the patient was transferred from another hospital, time since hospital admission, and outcome (separately for the primary AKI stage 3 outcome or the secondary AKI stage 2 and 3 outcome). The imputation was based on a classification and regression tree algorithm with 100 imputed data sets and a maximum of 5 iterations, taking into account the clustered structure of the data set with multiple measurements per patient. As a sensitivity analysis, the described simplified competing-risk models were also assessed without imputation in a complete-case analysis.

Statistical analyses were performed using R software (R Foundation for Statistical Computing, Vienna,

Austria),[32] including R packages for data handling and plotting,[33] Cox regression,[34,35] and multiple imputation.[36]

RESULTS

Study Population

During the first wave of COVID-19 in Germany, from 1 March 2020 to 3 June 2020, 236 patients were admitted to 1 of 3 sites of the Charité–Universitätsmedizin Berlin for symptomatic COVID-19 disease, 223 of whom were included in this analysis. Reasons for exclusion are provided in the study diagram (Supplementary Figure S1). A high proportion of patients, 86 of 223 (38.6%), were referred from other hospitals, because the hospitals included in this study were designated as tertiary referral centers for particularly severe cases (Supplementary Table S3). There were 57 patients (25.6%) referred from external ICUs (secondary ICU transfers). Before arrival at Charité hospitals, 58 patients (26%) had been intubated as part of their emergency care or within external ICUs. Table 1 summarizes baseline characteristics of the study cohort. Patients were a median age of 62 years (IQR, 51–75 years), 65.9% were men, and the median BMI was 27.4 kg/m^2 (IQR, 24.1–31.5 kg/m^2). The most common comorbidities were hypertension (53.3%), diabetes (23.3%), and coronary artery disease (18.4%). CKD stage G3–5 was present in 13.5% of patients. The median baseline eGFR was 86.9 ml/min per 1.73 m^2 (IQR, 70.7–100.5 ml/min per 1.73 m^2).

Incidence and Severity of AKI

Any stage of AKI developed in 117 patients (52.4%) (Supplementary Table S4). The maximum AKI severity was stage 1 in 24 patients, stage 2 in 23 patients, and stage 3 in 70 patients (Supplementary Table S4). AKI stage 1 patients were frequently treated in the non-ICU setting, but treatment for AKI stages 2 and 3 patients occurred almost exclusively in the ICU (Supplementary Table S4). For the purposes of this study, AKI stage 3 (severe AKI) was defined as the primary analysis outcome, because it is of established clinical importance in that it is closely coupled to the need for KRT and has the worst outcomes among AKI stages.

On the first day of a diagnosis of severe AKI, 18 patients (26%) showed creatinine criteria for AKI stage 3 (3-fold increase of creatinine or increase of creatinine to a value of > 4 mg/dl), 48 patients (69%) showed urinary output criteria of AKI stage 3 (<0.3 ml/kg/h for >24 hours or anuria for >12 hours), and 52 patients fulfilled the KRT criterion of AKI stage 3. The AKI stage 3 diagnosis was driven by KRT alone in 20 patients (29%) (Supplementary Table S5). All severe AKI cases developed throughout the first 20 days of hospitalization, with a wide range of time spans to severe AKI development (Supplementary Figure S2). Patients with severe AKI were older, more frequently

Table 1. Baseline characteristics of the study cohort

Variable	Total (N = 223)	No severe AKI (n = 153)	Severe AKI (n = 70)
Demographics			
Age, y	62.0 (51.0–75.0)	61.0 (47.0–75.0)	64.5 (56.0–74.8)
Male sex	147 (65.9)	95 (62.1)	52 (74.3)
Body mass index, kg/m^2	27.4 (24.0–31.7)	26.0 (22.9–29.7)	30.8 (26.3–34.6)
Missing	40 (17.9)	35 (22.9)	5 (7.1)
BMI > 30 kg/m^2	62 (27.8)	29 (19.0)	33 (47.1)
Missing	40 (17.9)	35 (22.9)	5 (7.1)
Weight, kg	83.0 (75.0–96.7)	80.0 (70.0–88.4)	90.0 (80.0–110.0)
Missing	32 (14.3)	31 (20.3)	1 (1.4)
Comorbidities			
Comorbidities, No.	1.0 (0.0–3.0)	1.0 (0.0–3.0)	2.0 (1.0–3.0)
Hypertension	120 (53.8)	70 (45.8)	50 (71.4)
Diabetes	52 (23.3)	30 (19.6)	22 (31.4)
Chronic kidney disease ≥ G3	30 (13.5)	18 (11.8)	12 (17.1)
Baseline			
Creatinine, mg/dl	0.9 (0.7–1.0)	0.8 (0.7–1.0)	1.0 (0.8–1.1)
eGFR ml/min per 1.73m^2	86.9 (70.7–100.5)	90.6 (74.3–103.1)	76.7 (62.8–93.1)
Missing	11 (4.9)	1 (0.7)	10 (14.3)
Creatinine on admission, mg/dl	1.0 (0.8–1.4)	0.9 (0.8–1.2)	1.4 (1.0–2.2)
Coronary artery disease	41 (18.4)	30 (19.6)	11 (15.7)
Myocardial infarction	33 (14.8)	23 (15.0)	10 (14.3)
Heart failure	19 (8.5)	16 (10.5)	3 (4.3)
Atrial fibrillation	29 (13.0)	21 (13.7)	8 (11.4)
Stroke/TIA	20 (9.0)	14 (9.2)	6 (8.6)
Peripheral vascular disease	8 (3.6)	4 (2.6)	4 (5.7)
COPD	17 (7.6)	7 (4.6)	10 (14.3)
Asthma	14 (6.3)	9 (5.9)	5 (7.1)
Obstructive sleep apnea	11 (4.9)	5 (3.3)	6 (8.6)
History of smoking	43 (19.3)	31 (20.3)	12 (17.1)
Active malignancy	8 (3.6)	6 (3.9)	2 (2.9)
Permanent medications			
Medications, No.	1.0 (0.0–3.0)	1.0 (0.0–3.0)	1.0 (0.0–3.5)
Missing	5 (2.2)	2 (1.3)	3 (4.3)
ACE-I	41 (18.4)	25 (16.3)	16 (22.9)
ARB	42 (18.8)	30 (19.6)	12 (17.1)
β-Blocker	55 (24.7)	36 (23.5)	16 (22.9)
Diuretics	46 (20.6)	30 (19.6)	16 (22.9)
Inhalers for asthma or COPD	16 (7.2)	9 (5.9)	7 (10.0)
Calcium channel blockers	29 (13.0)	18 (11.8)	11 (15.7)
Antidiabetics	41 (18.4)	22 (14.4)	19 (27.1)
Antiplatlets	39 (17.5)	24 (15.7)	15 (21.4)
Anticoagulants	31 (13.9)	25 (16.3)	6 (8.6)
Immunosuppressants	16 (7.2)	13 (8.5)	3 (4.3)
Statins	50 (22.4)	36 (23.5)	14 (20.0)

ACE-I, angiotensin-converting enzyme inhibitor; AKI; acute kidney injury, ARB; angiotensin II receptor blocker; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; TIA, transient ischemic attack.
Data are displayed as median (interquartile range) or n (%).

male, and had a higher BMI. Hypertension, diabetes, preexisting CKD, and chronic obstructive pulmonary disease were more frequent in patients with severe AKI (Table 1). KRT was initiated in 67 of 70 patients (95.7%) during the course of severe AKI. Continuous KRT was used in the initial phase, and intermittent hemodialysis was also used later during the course of the disease.

Disease Course, Time-Varying Clinical Parameters, and Their Association With AKI

Overall, 138 patients (61.9%) were admitted to an ICU at or during the course of hospitalization. Of these, 96 (69.6%) were mechanically ventilated, and 32 (23.2%) received extracorporeal membrane oxygenation. Severe AKI developed predominantly in patients who were mechanically ventilated (68 of 70 of severe AKI cases [97.1%]) and who received vasopressor support (66 of 70 of severe AKI cases [94.3%]). In mechanically ventilated patients, AKI occurred mostly after intubation (67 of 68 patients), with a median time lag of 4 days (IQR, 1–9 days) (Supplementary Figure S3).

We collected time-varying clinical parameters throughout the disease course and examined their association with severe AKI development. These parameters included laboratory tests and treatment parameters, including the need for mechanical ventilation and for vasopressor therapy (for selected key parameters see Table 2; for a full list of parameters see Supplementary Table S1). Consistent with the observation that severe AKI was confined to patients treated in ICUs, mechanical ventilation and vasopressors were more frequently used in these patients. Similarly, deviations of laboratory parameters from normal values were usually more pronounced in patients with severe AKI (Table 2).

To identify risk factors for COVID-19–associated severe AKI, we analyzed baseline parameters (static covariates) together with longitudinal parameters (time-varying covariates) in an explanatory multivariable risk model with severe AKI as the main outcome. Death without prior severe AKI, which occurred primarily during the later disease course (Supplementary Figure S2), was considered as a competing risk. We constructed a main model using 6 baseline and 11 time-dependent parameters and a reduced model with 5 baseline and 8 time-dependent parameters (Figure 1). The approach was hypothesis driven, in that parameters selected for analysis were chosen based on their putative contribution to the pathophysiology of AKI and on their availability across the entire cohort (Supplementary Tables S1 and S2). The main fully

Table 2. Time-varying parameters in COVID-19 patients with and without severe AKI

Variable	No severe AKI (n = 153)	Severe AKI (n = 70)
Mechanical ventilation		
No. (%)	29 (19.0)	67 (95.7)
Need for vasopressors[a]		
No. (%)	29 (19.0)	66 (94.3)
Hemoglobin, g/dl		
Median	12.3 (10.2–13.5)	10.6 (9.5–12.4)
Min	11.6 (9.1–12.7)	9.9 (8.7–11.7)
n	153	70
Thrombocyte count, count/nl		
Median	258.5 (202.0–333.0)	238.6 (159.8–327.9)
Min	187.0 (149.0–234.0)	194.0 (134.8–264.8)
n	153	70
Leucocyte count, count/nl		
Median	6.6 (5.3–7.6)	10.6 (7.4–14.3)
Max	8.2 (6.3–11.5)	14.3 (9.9–17.7)
n	152	70
Neutrophil count, count/nl		
Median	4.5 (3.3–5.8)	8.2 (5.6–10.4)
Max	6.1 (4.2–8.9)	10.2 (7.3–12.8)
n	151	52
Lymphocyte count, count/nl		
Median	1.2 (0.9–1.5)	0.9 (0.6–1.1)
Min	0.9 (0.7–1.1)	0.7 (0.5–1.0)
n	151	52
Neutrophil/lymphocyte ratio		
Median	6.6 (5.3–7.6)	10.6 (7.4–14.3)
Max	8.2 (6.3–11.5)	14.3 (9.9–17.7)
n	152	70
Total bilirubin, mg/dl		
Median	0.4 (0.3–0.6)	0.6 (0.4–1.0)
Max	0.6 (0.4–0.8)	0.8 (0.6–1.5)
n	149	70
AST, U/l		
Median	44.0 (30.0–59.0)	69.0 (56.4–113.5)
Max	59.0 (37.0–103.0)	90.0 (65.8–134.2)
n	125	62
ALT, U/l		
Median	38.0 (24.5–74.2)	40.1 (28.5–55.2)
Max	63.0 (31.5–105.0)	49.5 (32.2–72.8)
n	127	62
Creatine kinase, U/l		
Median	65.5 (40.4–97.8)	326.8 (164.2–1064.8)
Max	110.0 (64.2–288.2)	629.5 (262.2–1945.5)
n	144	68
Myoglobin, µg/l		
Median	46.0 (32.0–92.5)	429.0 (140.0–1520.0)
Max	71.0 (38.0–216.5)	799.0 (174.0–2358.0)
n	37	47
LDH, U/l		
Median	311.5 (274.5–370.0)	487.0 (409.0–602.0)
Max	397.0 (329.0–507.5)	570.0 (486.0–697.0)
n	151	69
D-dimer,[b] mg/l		
Median	1.6 (0.9–2.9)	3.3 (1.4–8.4)
Max	2.7 (1.1–6.1)	4.2 (1.5–12.2)
n	69	59

(Continued on following page)

Table 2. (Continued)

Variable	No severe AKI (n = 153)	Severe AKI (n = 70)
CRP, mg/dl		
Median	51.0 (18.6–82.4)	229.9 (135.5–334.6)
Max	100.8 (42.5–182.6)	330.3 (188.9–419.0)
n	153	70
Procalcitonin,[b] µg/l		
Median	0.1 (0.0–0.1)	1.1 (0.5–2.7)
Max	0.1 (0.1–0.5)	2.1 (0.8–5.0)
n	148	69
Interleukin-6,[b] ng/l		
Median	18.8 (6.5–37.7)	262.3 (102.2–679.5)
Max	48.5 (15.9–108.1)	507.8 (216.8–1402.8)
n	138	50
Ferritin, µg/l		
Median	654.4 (399.6–1149.7)	1530.8 (879.6–2591.8)
Max	871.5 (453.1–1526.6)	1910.3 (980.8–2909.9)
n	141	56

AKI, acute kidney injury; ALT, alanine Aminotransferase; AST, aspartate aminotransferase; CRP; C-reactive protein; LDH, lactate dehydrogenase.
[a]Includes norepinephrine and vasopressin.
[b]When data points exceeded the upper detection limit. these values were imputed as upper limit + 1 unit.
Displayed are medians (interquartile range) of individual patient's median and individual patient's extreme values (maximum or minimum, as appropriate) during the in-hospital disease course, and n indicates the group size. For parameters in the AKI stage 3 group, only data preceding AKI stage 3 were considered.

adjusted model and the reduced model (Figure 1) revealed that the need for mechanical ventilation or vasopressors, blood leucocyte counts, and serum procalcitonin levels were significant time-dependent risk factors of severe AKI.

Separate analyses of longitudinal parameters were conducted in simplified, minimally adjusted models (adjustment for sex, age, hypertension, diabetes, eGFR, and BMI) (Supplementary Figure S4) and confirmed these risk factors. To confirm that these results were specific to AKI, we conducted a sensitivity analysis, repeating the risk modeling with a combined AKI stage 2/3 outcome, which was defined by the earliest occurrence of AKI stage 2 or stage 3. This analysis yielded a virtually identical model (Supplementary Figure S5).

We next visualized the time courses of the independent risk factors in relation to the development of severe AKI (Figure 2, Supplementary Figures S6–S8). This confirmed that mechanical ventilation (Supplementary Figure S6) and the need for vasopressor therapy (Supplementary Figure S7) frequently preceded the onset of severe AKI. In addition, heat map visualizations indicated that there was a particularly close temporal association between increasing serum procalcitonin levels and the onset of severe AKI (Supplementary Figure S2). A similarly close temporal association was observed for increasing blood leucocyte counts and severe AKI (Supplementary Figure S8).

Outcomes of Patients With Severe AKI

Among patients with severe AKI, 47.1% died during the hospital stay, 45.7% were discharged, and 7.1% continued to be hospitalized (Table 3). In contrast, among patients without severe AKI, only 6.5% died, and the remaining 93.5% were discharged.

DISCUSSION

This analysis of a heterogeneous cohort of COVID-19 patients consecutively hospitalized in 3 sites of a large tertiary care center provides a longitudinal clinical characterization of COVID-19 patients and its association with AKI. Severe AKI was restricted to ICU patients. Measures of COVID-19–associated critical illness (the need for vasopressors and mechanical ventilation) and increasing blood levels of markers of systemic inflammation (serum procalcitonin and blood leucocytes) emerged as key time-varying risk factors for severe AKI. A particularly close temporal association was observed for increasing markers of systemic inflammation with the onset of severe AKI. Procalcitonin and blood leucocytes have both been previously described as predictive of adverse disease courses and mortality in COVID-19 and indicate an exaggerated inflammatory response.[37] D-dimer and lactate dehydrogenase, additional established risk factors of severe COVID-19 courses,[38–40] were not independently associated with severe AKI, although they were predictive in simplified models. A noteworthy finding is that we did not observe severe AKI outside the ICU setting, confirming the close association of AKI with the overall COVID-19 disease severity and arguing against an isolated development of severe AKI in COVID-19 patients who are not critically ill. Our data are consistent with Hirsch *et al.*,[4] who identified mechanical ventilation and vasopressor use as important AKI determinants in COVID-19. However, their analysis was confined to admission data and did not consider time-varying clinical parameters.

The frequencies of AKI (52.4%) and of KRT (30%) in our cohort are markedly higher than in a recently published German nationwide observational study of 10,021 hospitalized patients based on administrative records and in cohorts from New York during the surge of COVID-19.[4,6,7] A likely explanation for this is that our study was conducted in a tertiary care center with a large proportion of secondary referrals of critically ill patients. Our cohort consistently displayed high rates of ICU admission (62.2%) and mechanical ventilation (43.5%). This rate of mechanical ventilation was also markedly higher than the reported nationwide mechanical ventilation rate of 17% for COVID-19 patients in Germany.[7]

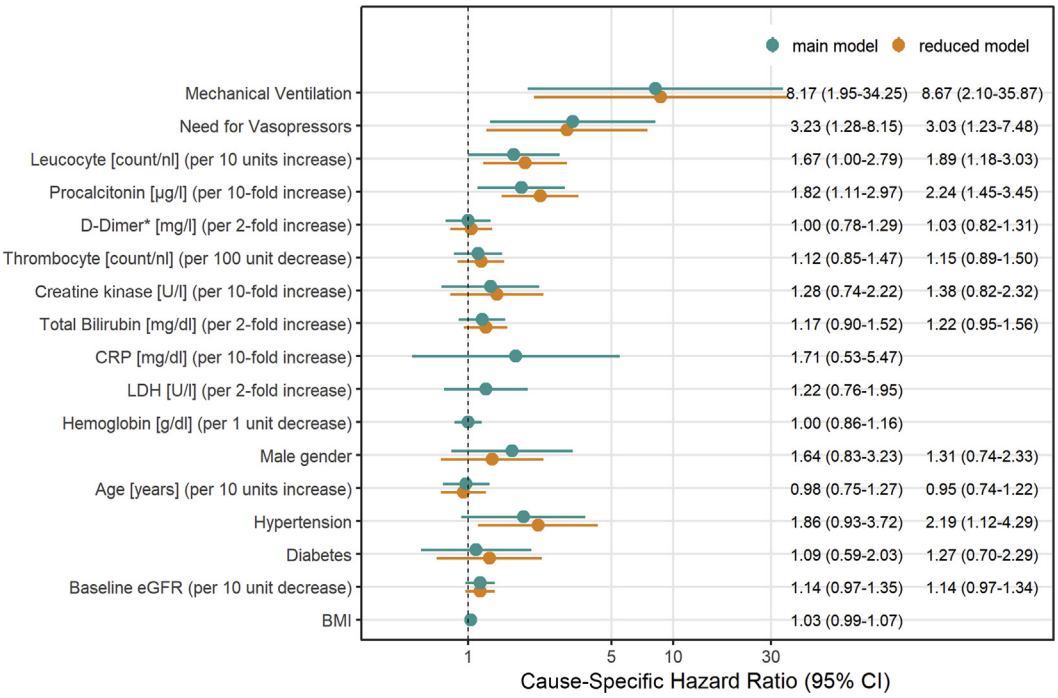

Figure 1. Longitudinal risk factors of severe acute kidney injury (AKI) during the COVID-19 disease course. Multivariable Cox proportional hazards regression with time-varying covariates was used to estimate hazards of potential risk factors for time to severe AKI, along with 95% confidence intervals (CI). Estimates were derived from 2 multivariable competing-risk models with time-varying covariates, adjusted for all parameters displayed. Missing data were addressed by multiple imputation. A main multivariable model (including 11 time-varying risk factor covariates) and a reduced model (including 8 time-varying risk factor covariates) were fitted to account for partially overlapping clinical information content of the time-dependent risk factors of severe AKI. Dots indicate hazard ratio estimates, and the horizontal bars indicate 95% CIs. *When data points exceeded the upper detection limit these values were imputed as upper limit + 1 unit. BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase.

It is important to note that health care resources in Germany, including those in the catchment area of this study, were not overwhelmed during the first wave of the pandemic, with sufficient ICU capacity to manage severely ill patients. Nevertheless, normal ward capacities were reduced during the surge of the pandemic, and mild and moderate courses of COVID-19 were treated outside the hospital setting. This would be consistent with our observation that most of the cases of AKI in our cohort were KDIGO stage 3, in sharp contrast to a previous retrospective analysis of AKI cases in the same health care setting before the onset of the pandemic, where less severe AKI stages were substantially more frequent compared with stage 3.[41]

Although mechanical ventilation and vasopressor use emerged as risk factors of severe AKI, the start of mechanical ventilation or initiation of vasopressors frequently preceded severe AKI development by several days. In contrast, increasing serum procalcitonin levels and blood leucocyte counts frequently coincided with start of AKI. One might speculate, on the basis of these data, that respiratory and circulatory failure in COVID-19 may create a permissive situation during which kidney function may initially be preserved before an exaggerated systemic inflammation precipitates organ failure.

An obvious candidate scenario causing decompensation may be a superimposed bacterial infection. Bacterial infections are common in other viral pneumonias, particularly influenza, and serum procalcitonin levels associate with bacterial infections, particularly bacterial pneumonia.[42] Nevertheless, recent studies have highlighted a lack of specificity of procalcitonin for bacterial infections,[43] and elevated procalcitonin release has been observed in various disease processes, including liver failure, cardiogenic shock, major burns, or major surgery.[44–47] In fact, SARS-CoV2 itself may directly cause systemic immune activation and thereby precipitate a hyperinflammatory clinical picture, with high procalcitonin and white blood cell elevation. The reasons for the sometimes abrupt increases in these markers in patients who had already been treated in ICUs for several days are currently unknown. Experimental evidence suggests that kidney injury may not only be the consequence of respiratory failure, but also conversely aggravate lung injury.[10] It is therefore possible that interorgan cross talk might contribute to the observed temporal associations.

Our findings imply that AKI is strongly linked to the overall COVID-19 disease course rather than being an independent complication. Whether direct viral infection of the kidney occurs in patients with COVID-19 is

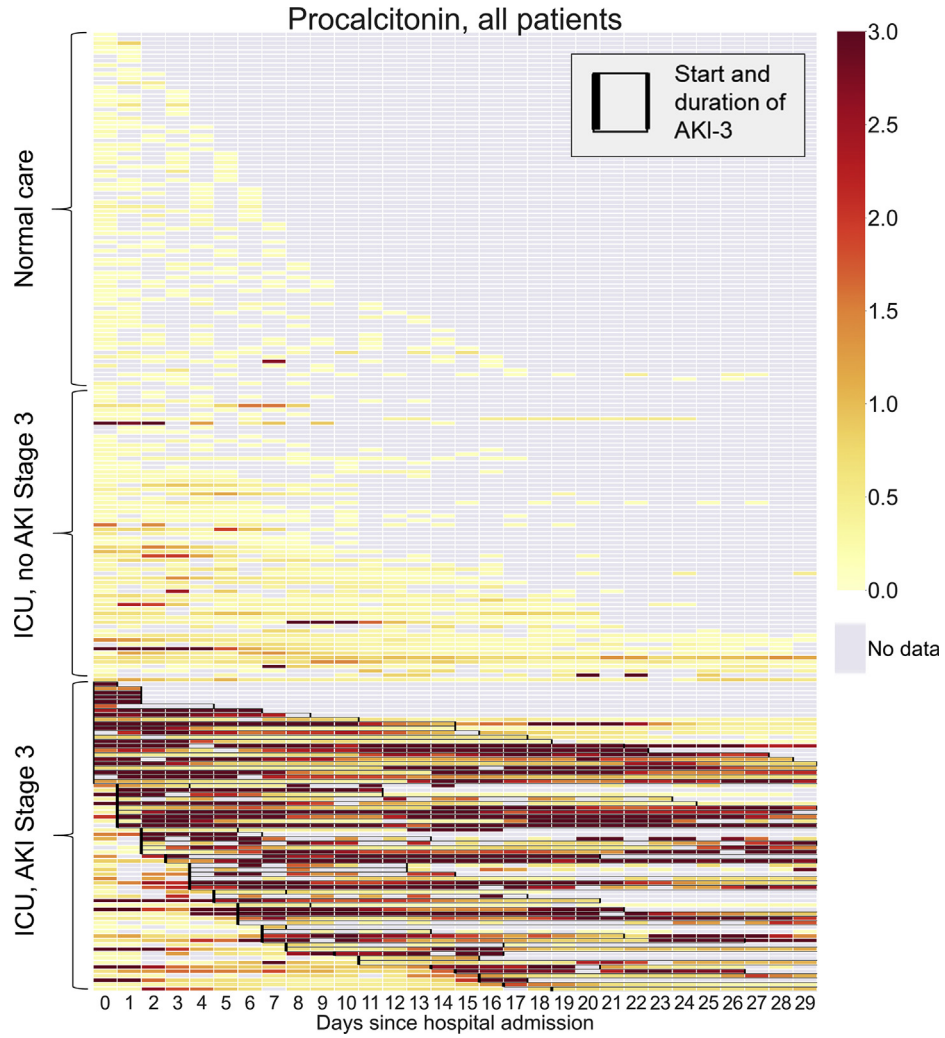

Figure 2. Heat map visualization of serum procalcitonin levels over time and their relationship to severe AKI (AKI stage 3) in all 223 patients included in the study. Displayed are procalcitonin levels in ng/l over time (grey cells indicating missing data). Days with AKI stage 3 are labeled by black boxes. Every row represents 1 patient and each column 1 day of hospitalization. Day 0 refers to the day of admission to Charité university hospitals. Patients are sorted on the basis of whether they were ever admitted to an intensive care unit (ICU). Patients ever admitted to the ICU were then sorted by AKI stage 3 status and by the start and duration of AKI stage 3.

a matter of current debate.[15,16,18,19,48] If it does occur during the early phases of the disease, it does not seem to be sufficient on its own to cause severe AKI. Our findings are consistent with recent histopathologic studies of kidney tissue from autopsies of COVID-19 patients showing predominantly acute tubular necrosis, as in patients with AKI and critical illness of other etiology.[18,19]

Our study has strengths and limitations. We were fortunate that our health care system was not overwhelmed, allowing us to assess the disease course independently of the impact of resource limitations, such as very late referrals or triaging. Further strengths include the availability of high-quality time-resolved clinical data.

We achieved comprehensive identification of different stages of AKI based on clinical adjudication, which included urinary output data and high-resolution creatinine dynamics. The availability of baseline creatinine data was high (95.1%), contrasting markedly with <20% complete data in previous studies.[4]

The size of this cohort (223 patients) is relatively small, and analyses were restricted to routine clinical data. Nevertheless, the high incidence rate of AKI in this

Table 3. Outcomes of the study cohort split by severe AKI status

General outcome	Total (N = 223)	No severe AKI (n = 153)	Severe AKI (n = 70)
Death during hospitalization	43 (19.3)	10 (6.5%)	33 (47.1%)
Ongoing hospitalization	5 (2.2)	0 (0%)	5 (7.1%)
Ongoing ICU stay	4 (1.8)	0 (0)	4 (5.7)
Discharge acute care	175 (78.5)	143 (93.5)	32 (45.7)
Total hospital stay, d	17.5 (9.0–34.0)	14 (8.0–23.0)	40 (22.25–60.75)
Total ICU stay, d	20.5 (7.0–43.0)	9 (5.0–20.0)	34 (21.0–56.0)

AKI, acute kidney injury; ICU, intensive care unit.
Data are presented as n (%) or median (interquartile range).

population added substantial power to the statistical modeling approaches. Because the hospitals involved in this study were the highest level referral centers for COVID-19 patients, our cohort includes several severely ill patients referred from external ICUs. Therefore, general conclusions regarding the frequency of critical illness, AKI, and KRT in COVID-19 patients cannot be derived from our cohort. In addition, data available from external ICUs were limited to the date of symptom onset and the dates of initiation of mechanical ventilation or KRT. We therefore cannot exclude that a subset of patients experienced non–KRT-dependent AKI at external hospitals before admission at Charité.

In addition, urinary output data were restricted to ICU patients, which may potentially bias AKI stage 3 adjudication to the ICU setting.

Owing to the retrospective study design, longitudinal clinical data had variable levels of completeness, but analysis with and without multiple imputation approaches indicated that the predictors of severe AKI remained consistent. Previous studies have highlighted that high serum interleukin 6, interleukin 8, and tumor necrosis factor-α levels and high neutrophil/lymphocyte ratios were strong and independent predictors of disease severity and patient survival in COVID-19.[49–52] However, the limited longitudinal availability or absence of these parameters in routine clinical data prevented us from assessing the impact of these parameters on severe AKI development.

CONCLUSION

Severe forms of AKI in COVID-19 are confined to critically ill patients and intricately linked to overall disease severity. Markers of critical illness and systemic inflammation were key time-varying risk factors of severe AKI development. In contrast, severe AKI did not occur uncoupled from overall COVID-19 severity.

DISCLOSURE

None of the authors declared a conflict of interest with respect to this manuscript.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Study flowchart.

Figure S2. Cumulative incidence AKI stage 3 and death without AKI stage 3 in COVID-19 patients.

Figure S3. Temporal relation of AKI stage 3 onset and intubation

Figure S4. Individual minimally adjusted risk model of AKI stage 3 risk factors with time-varying covariates

Figure S5. Competing risk model with time-varying covariates of AKI Stage 2 or Stage 3 risk factors

Figure S6. Visualization of mechanical ventilation over time and its relationship to AKI Stage 3 in all 223 patients included into the study

Figure S7. Visualization of vasopressor use over time and its relationship to AKI Stage 3 in all 223 patients included into the study

Figure S8. Heat map visualization white blood cell levels over time and their relationship to AKI stage 3 in all 223 patients included into the study

Table S1. Parameters screened and included in analysis

Table S2. Longitudinal parameters overview of missing values

Table S3. Route of admission

Table S4. AKI frequency

Table S5. Overview of the individual components of the KDIGO diagnosis criteria for stage 3 AKI per patient

CODE AVAILABILITY

The code (written in R) used for the statistical modeling will be made available upon request.

REFERENCES

1. Johns Hopkins Coronavirus Resource Center. COVID-19 Dashboard, 2020. Available at: https://coronavirus.jhu.edu/map.html. Accessed September 11, 2020.
2. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708–1720.
3. Cheng Y, Luo R, Wang K, et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int*. 2020;97:829–838.
4. Hirsch JS, Ng JH, Ross DW, et al. Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int*. 2020;98:209–218.
5. Cummings MJ, Baldwin MR, Abrams D, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. *Lancet*. 2020;395:1763–1770.
6. Chan L, Chaudhary K, Saha A, et al. AKI in hospitalized patients with COVID-19. *J Am Soc Nephrol*. 202132:151–160.
7. Karagiannidis C, Mostert C, Hentschker C, et al. Case characteristics, resource use, and outcomes of 10021 patients with COVID-19 admitted to 920 German hospitals: an observational study. *Lancet Respir Med*. 2020;8:853–862.
8. Gabarre P, Dumas G, Dupont T, et al. Acute kidney injury in critically ill patients with COVID-19. *Intensive Care Med*. 2020;46:1339–1348.

9. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020;8:475–481.
10. Rabb H. Kidney diseases in the time of COVID-19: major challenges to patient care. *J Clin Invest*. 2020;130:2749–2751.
11. Batlle D, Soler MJ, Sparks MA, et al. Acute kidney injury in COVID-19: emerging evidence of a distinct pathophysiology. *J Am Soc Nephrol*. 2020;31:1380–1383.
12. Siew ED, Birkelo BC. COVID-19–associated acute kidney injury: an evolving picture. *Clin J Am Soc Nephrol*. 2020;15:1383–1385.
13. Gross O, Moerer O, Weber M, et al. COVID-19-associated nephritis: early warning for disease severity and complications? *Lancet*. 2020;395:e87–e88.
14. Werion A, Belkhir L, Perrot M, et al. SARS-CoV-2 causes a specific dysfunction of the kidney proximal tubule. *Kidney Int*. 2020;98:1296–1307.
15. Puelles VG, Lütgehetmann M, Lindenmeyer MT, et al. Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med*. 2020;383:590–592.
16. Braun F, Lütgehetmann M, Pfefferle S, et al. SARS-CoV-2 renal tropism associates with acute kidney injury. *Lancet*. 2020;396:597–598.
17. Kudose S, Batal I, Santoriello D, et al. Kidney biopsy findings in patients with COVID-19. *J Am Soc Nephrol*. 2020;31:1959–1968.
18. Golmai P, Larsen CP, DeVita MV, et al. Histopathologic and ultrastructural findings in postmortem kidney biopsy material in 12 patients with AKI and COVID-19. *J Am Soc Nephrol*. 2020;9:1944–1947.
19. Sharma P, Uppal NN, Wanchoo R, et al. COVID-19–associated kidney injury: a case series of kidney biopsy findings. *J Am Soc Nephrol*. 2020;31:1948–1958.
20. Santoriello D, Khairallah P, Bomback AS, et al. Postmortem kidney pathology findings in patients with COVID-19. *J Am Soc Nephrol*. 2020;31:2158–2167.
21. Xia P, Wen Y, Duan Y, et al. Clinicopathological features and outcomes of acute kidney injury in critically ill COVID-19 with prolonged disease course: a retrospective cohort. *J Am Soc Nephrol*. 2020;31:2205–2221.
22. Mohamed MMB, Lukitsch I, Torres-Ortiz AE, et al. Acute kidney injury associated with coronavirus disease 2019 in urban New Orleans. *Kidney360*. 2020;1:614–622.
23. Ng JH, Hirsch JS, Hazzan A, et al. Outcomes among patients hospitalized with COVID-19 and acute kidney injury. *Am J Kidney Dis*. 2021;77(2):204–215.e1.
24. Gupta S, Coca SG, Chan L, et al. AKI treated with renal replacement therapy in critically ill patients with COVID-19. *J Am Soc Nephrol*. 2021;32:161–176.
25. Fisher M, Neugarten J, Bellin E, et al. AKI in hospitalized patients with and without COVID-19: a comparison study. *J Am Soc Nephrol*. 2020;31:2145–2157.
26. Bellomo R, Ronco C, Mehta RL, et al. Acute kidney injury in the ICU: from injury to recovery: reports from the 5th Paris International Conference. *Ann Intensive Care*. 2017;7:49.
27. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. *Lancet*. 2019;394:1949–1964.
28. Mehta RL. Critical care nephrology in 2016: managing organ dysfunction in critical care. *Nat Rev Nephrol*. 2017;13:71–72.
29. Desanti De Oliveira B, Xu K, et al. Molecular nephrology: types of acute tubular injury. *Nat Rev Nephrol*. 2019;15:599–612.
30. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury *Kidney. Int Suppl*. 2012;2:1–138.
31. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl*. 2013;3:1–150.
32. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2019. https://www.R-project.org/.
33. Wickham H, Averick M, Bryan J, et al. Welcome to the Tidyverse. *J Open Source Softw*. 2019;4:1686.
34. Therneau TM, Grambsch PM. *Modeling Survival Data: Extending the Cox Model*. New York: Springer; 2000.
35. Therneau TM. A Package for Survival Analysis in R, 2015. Available at: https://CRAN.R-project.org/package=survival. Accessed September 27, 2020.
36. Buuren S, Groothuis-Oudshoorn C. MICE: multivariate imputation by chained equations in R. *J Stat Softw*. 2011;45:1–65.
37. Zhang JJY, Lee KS, Ang LW, et al. Risk factors of severe disease and efficacy of treatment in patients infected with COVID-19: a systematic review, meta-analysis and meta-regression analysis. *Clin Infect Dis*. 2020;71:2199–2206.
38. Yu B, Li X, Chen J, et al. Evaluation of variation in D-dimer levels among COVID-19 and bacterial pneumonia: a retrospective analysis. *J Thromb Thrombolysis*. 2020;50:548–557.
39. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395:1054–1062.
40. Chen XY, Huang MY, Xiao ZW, et al. Lactate dehydrogenase elevations is associated with severity of COVID-19: a meta-analysis. *Crit Care*. 2020;24:459.
41. Khadzhynov D, Schmidt D, Hardt J, et al. The incidence of acute kidney injury and associated hospital mortality. *Dtsch Arztebl Int*. 2019;116:397–404.
42. Klein EY, Monteforte B, Gupta A, et al. The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. *Influenza Other Respir Virus*. 2016;10:394–403.
43. Kamat IS, Ramachandran V, Eswaran H, et al. Procalcitonin to distinguish viral from bacterial pneumonia: a systematic review and meta-analysis. *Clin Infect Dis*. 2019;70:538–542.
44. Rule JA, Hynan LS, Attar N, et al. Procalcitonin identifies cell injury, not bacterial infection, in acute liver failure. *PLoS One*. 2015;10:e0138566.
45. Picariello C, Lazzeri C, Chiostri M, et al. Kinetic of procalcitonin in patients with cardiogenic shock following acute myocardial infarction: preliminary data. *HSR Proc Intensive Care Cardiovasc Anesth*. 2010;2:201–207.
46. Cheng Z-B, Chen H. Higher incidence of acute respiratory distress syndrome in cardiac surgical patients with elevated

serum procalcitonin concentration: a prospective cohort study. *E Eur J Med Res*. 2020;25:11.

47. Ammori BJ, Becker KL, Kite P, et al. Calcitonin precursors in the prediction of severity of acute pancreatitis on the day of admission. *Br J Surg*. 2003;90:197–204.
48. Su H, Yang M, Wan C, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int*. 2020;98:219–227.
49. Liu Y, Du X, Chen J, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *J Infect*. 2020;81:e6–e12.
50. Herold T, Jurinovic V, Arnreich C, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol*. 2020;146:128–136.e124.
51. Chen R, Sang L, Jiang M, et al. Longitudinal hematologic and immunologic variations associated with the progression of COVID-19 patients in China. *J Allergy Clin Immunol*. 2020;146:89–100.
52. Del Valle DM, Kim-Schulze S, Huang H-H, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med*. 2020;26:1636–1643.