Kidney and Blood Pressure Research

Research Article

Kidney Blood Press Res 2023;48:424–435
DOI: 10.1159/000529690

Received: October 6, 2022
Accepted: February 1, 2023
Published online: June 26, 2023

Urinary N-Terminal Pro-Brain Natriuretic Peptide Predicts Acute Kidney Injury and Severe Disease in COVID-19

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Keywords
COVID-19 · N-terminal pro-brain natriuretic peptide · Acute kidney injury · Urinary biomarkers

Abstract

Introduction: The ongoing COVID-19 pandemic is placing an extraordinary burden on our health care system with its limited resources. Accurate triage of patients is necessary to ensure medical care for those most severely affected. In this regard, biomarkers could contribute to risk evaluation. The aim of this prospective observational clinical study was to assess the relationship between urinary N-terminal pro-brain natriuretic peptide (NT-proBNP) and acute kidney injury (AKI) as well as severe disease in patients with COVID-19. ***Methods:*** 125 patients treated with an acute respiratory infection in the emergency department of the University Hospital Regensburg were analyzed. These patients were divided into a COVID-19 cohort (n = 91) and a cohort with infections not caused by severe acute respiratory syndrome-coronavirus-2 (n = 34). NT-proBNP was determined from serum and fresh urine samples collected in the emergency department. Clinical endpoints were the development of AKI and a composite one consisting of AKI, intensive care unit admission, and in-hospital death. ***Results:*** 11 (12.1%) COVID-19 patients developed AKI during hospitalization, whereas 15 (16.5%) reached the composite endpoint. Urinary NT-proBNP was significantly elevated in COVID-19 patients who suffered AKI or reached the composite endpoint (each $p <$ 0.005). In a multivariate regression analysis adjusted for age, chronic kidney disease, chronic heart failure, and arterial hypertension, urinary NT-proBNP was identified as independent predictor of AKI (p = 0.017, OR = 3.91 [CI: 1.28–11.97] per standard deviation [SD]), as well as of the composite endpoint (p = 0.026, OR 2.66 [CI: 1.13–6.28] per SD). ***Conclusion:*** Urinary NT-proBNP might help identify patients at risk for AKI and severe disease progression in COVID-19.

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Introduction

Disease severity of an infection with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) ranges from mild upper respiratory symptoms to severe pneumonia, acute respiratory distress syndrome, and multiple organ failure. While milder forms of 2019 coronavirus disease (COVID-19) can be treated as an outpatient, many patients require inpatient care. In addition to pulmonary manifestations, it became apparent during the course of the pandemic that renal involvement in COVID-19 in the form of acute kidney injury (AKI) is not only common but also leads to a dramatically increased mortality [1]. Due to the varying severity and course of COVID-19, accurate triage of patients in early stages of disease is necessary to ensure appropriate medical care and to allocate our limited medical resources to the patients who need them the most. For this purpose, in addition to various clinical parameters, biomarkers measured in the laboratory can contribute importantly to exact risk evaluation and disease prognosis. For example, the use of N-terminal pro-brain natriuretic peptide (NT-proBNP) in acute and chronic heart failure (CHF) is already part of everyday clinical practice and is also recommended by the European Society of Cardiology in the current guidelines [2]. In contrast, the ideal use of biomarkers in COVID-19 is still the subject of current research and requires further investigation.

NT-proBNP is a peptide hormone produced mainly by cardiac myocytes during cardiac wall stress [3]. In addition to its general use as a serum biomarker in heart failure, it has also been shown to be a useful tool for risk assessment in COVID-19 [4]. Because excretion of NT-proBNP is mainly renal, it can also be found and measured in urine [3]. In previous studies, urinary NT-proBNP has been shown to be of value for diagnosis and prognosis in heart failure [5, 6]. Unlike other urinary biomarkers such as KIM-1 or proteinuria, which have already demonstrated their utility for risk assessment and prognosis in COVID-19, the use of urinary NT-proBNP in COVID-19 remains largely unexplored [7, 8]. The aim of this study was to assess the value of urinary NT-proBNP as a biomarker of disease severity and AKI in COVID-19. Therefore, a cohort of COVID-19 patients was analyzed in this prospective observational clinical study.

Methods

Study Population

For this prospective cohort study, adult patients treated with symptoms of a suspected acute respiratory infection in the emergency department of University Hospital Regensburg were included between March 2020 and June 2021. Prespecified exclusion criteria were ages under 18 years and the absence of written consent. Every patient was tested for SARS-CoV-2 using RT-PCR analysis. Samples were obtained by pharyngeal lavage or nasopharyngeal swab. Depending on the test result, patients were divided into a COVID-19 and a non-COVID-19 group (defined as either bacterial or viral respiratory infection). If acute respiratory infection could not be confirmed at final discharge (n = 21) or the patient was unable to deliver urine (n = 102), the case was excluded from analysis. A STARD flow diagram of the trial design is displayed in Figure 1 [9].

Baseline data, vital signs, and clinical examination findings were collected and documented for each patient. Clinical endpoints assessed were development of AKI, admission to the intensive care unit (ICU), and in-hospital death. Further, a combination of AKI, ICU admission, and death (defined as an event) was evaluated. AKI was diagnosed according to the 2012 KDIGO criteria [10].

Sample Processing and Biochemical Analysis

Immediately after patient admission, blood and fresh spot urine samples were obtained and sent to the central laboratory. All biomarkers displayed in the study except KIM-1 were measured on the same day together with parameters of routine patient care. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula [11]. Urinary and serum NT-proBNP were measured by Roche cobas pro e801 with the Elecsys proBNP II Immunoassays (Roche Holding, Basel, Switzerland). For the later analysis of KIM-1, urine samples were centrifuged, aliquoted, and frozen at −80°C within 24 h. The ELISA Duo kit and the corresponding ancillary reagent kit (R&D Systems, Minneapolis, MN, USA) were used for the measurement of KIM-1 as described elsewhere [8]. All values of urinary NT-proBNP and KIM-1 were normalized to urinary creatinine to preclude dilutional bias.

Statistics

Descriptive data are presented as mean and standard deviation (SD) for normally distributed data and as median and interquartile range for non-normally distributed data. Variables were tested for normal distribution via Kolmogorov-Smirnoff test. Student's T-test was performed for normally distributed continuous variables, Mann-Whitney U-test for non-normally distributed continuous values, and χ^2 test for categorical variables. Correlation coefficients were calculated according to Spearman. Differences in biomarker concentrations are analyzed and visualized using boxplots. To evaluate the predictive value of the biomarkers, receiver operating curves (ROC) were performed and the area under the curve (AUC) was estimated. Sensitivity and specificity were calculated, and possible cutoff values were defined according to the highest Youden index. ROC comparison was performed using DeLongs method [12]. Further, univariate and multivariate regression analysis were performed for both endpoints. To address potential overfitting in the multivariate regression models, a bootstrapping procedure with 3,000 iterations was performed. All statistical analyses were performed using SPSS 28 (SPSS Inc., Chicago, IL, USA).

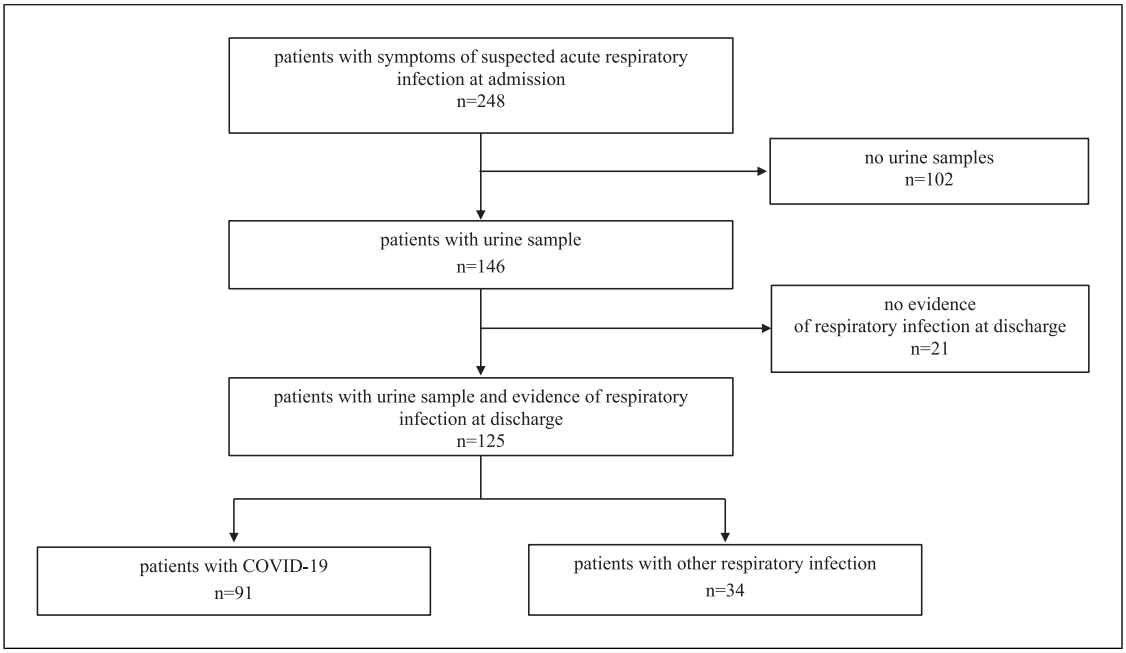

Fig. 1. STARD flow diagram of the study design.

Results

Study Population

In total, 125 patients were enclosed in the current study (for more information, see Table 1). There was evidence of SARS-CoV-2 infection for 91 subjects, whereas 34 subjects served as the SARS-CoV-2-negative group. The average age was 57.4 years and did not differ between both groups (p = 0.12). Men were more prevalent in both cohorts. Coronary artery disease, chronic obstructive pulmonary disease, and chronic kidney disease (CKD) occurred more frequently in the non-COVID-19 cohort (each $p < 0.05$). The COVID-19 cohort suffered more often from anosmia ($p = 0.04$), while other symptoms, vital signs, and baseline medication did not differ between both groups (each $p > 0.05$).

11 (12.1%) Patients in the COVID-19 cohort and 8 (23.5%) non-COVID-19 patients suffered from AKI during hospitalization. In the SARS-CoV-2 group, 12 (13.2%) patients had to be transferred to ICU and 7 (7.7%) people died due to the infection. In contrast, 3 (8.8%) patients in the non-COVID-19 group were admitted to the ICU and 2 (5.9%) deaths occurred. In total, 15 (16.5%) patients with COVID-19 and 10 (29.4%) patients in the non-COVID-19 cohort reached the composite endpoint.

Urinary and Serum Markers

There were no differences in urinary NT-proBNP (p = 0.063), serum creatinine (p = 0.078), eGFR (p = 0.13), C-reactive protein (CRP, p = 0.99), and serum NT-proBNP (p = 0.004). Interleukin-6 (IL-6, p = 0.038) and the white blood cell count (WBC, $p < 0.001$) were significantly higher in the non-COVID-19 cohort than in the SARS-CoV-2 cohort. More information regarding biomarker levels in both cohorts can be found in Table 2.

In the COVID-19 cohort, urinary NT-proBNP was significantly correlated with serum NT-proBNP ($\rho = 0.86$, $p < 0.001$) and IL-6 ($\rho = 0.5$, $p < 0.001$), but not with serum creatinine ($\rho = 0.19$, $p = 0.066$). Further, urinary NT-proBNP was significantly negatively correlated with eGFR ($\rho = -0.45$, $p < 0.001$). Scatterplots of the correlation between urinary NT-proBNP and serum NT-proBNP as well as serum creatinine can be found in Figure 2. In the non-COVID-19 cohort, urinary NT-proBNP was significantly correlated with serum NT-proBNP ($\rho = 0.81$, $p < 0.001$), serum creatinine ($\rho = 0.45$, $p = 0.008$), and IL-6 ($\rho = 0.43$, $p = 0.12$). Additionally, urinary NT-proBNP was significantly negatively correlated with eGFR ($\rho = -0.57$, $p < 0.001$) in the non-COVID-19 group.

Detection of AKI and the Composite Endpoint in the COVID-19 Cohort

In the COVID-19 cohort, urinary NT-proBNP was significantly elevated in patients who developed AKI compared to those who did not ($p < 0.001$, Fig. 3a).

Table 1. Baseline characteristics

	Overall cohort	COVID-19	Controls	*p* values
Baseline characteristics				
N	125	91	34	
Age,† years	57.4±16.6	56.3±16.1	60.1±17.9	0.27[b]
Sex, % male (*n*)	62.4 (78)	58.2 (53)	73.5 (25)	0.12[a]
Smokers (continued), % (*n*)	8.8 (11)	5.5 (5)	17.6 (6)	0.033[a]
Intrahospital death, % (*n*)	7.2 (9)	7.7 (7)	5.9 (2)	0.73[a]
ICU admission, % (*n*)	12 (15)	13.2 (12)	8.8 (3)	0.50[a]
AKI, % (*n*)	15.2 (19)	12.1 (11)	23.5 (8)	0.11[a]
CT scan, % (*n*)	88.8 (111)	91.2 (83)	82.4 (18)	0.16[a]
CT scan with CM, % (*n*)	34.4 (43)	35.2 (32)	32.4 (11)	0.77[a]
CM volume in CT scan,‡ mL	70 (70–70)	70 (70–70)	70 (70–85)	0.27[b]
Baseline medication, % (*n*)	72 (90)	72.5 (66)	70.6 (24)	0.83[a]
Immunosuppressants, % (*n*)	10.4 (13)	7.7 (7)	17.6 (6)	0.23[a]
Steroids, % (*n*)	7.2 (9)	5.5 (5)	11.8 (4)	0.31[a]
ACE-/AT-1 inhibitors, % (*n*)	30.4 (38)	29.7 (27)	32.4 (11)	0.77[a]
Beta-blockers, % (*n*)	27.2 (34)	24.2 (22)	35.3 (12)	0.21[a]
Diuretics, % (*n*)	23.2 (29)	19.8 (18)	32.4 (11)	0.14[a]
DOAK, % (*n*)	9.6 (12)	6.6 (6)	17.6 (6)	0.062[a]
ASS, % (*n*)	21.6 (27)	19.8 (18)	26.5 (9)	0.42[a]
Pre-existing diseases				
Coronary artery disease, % (*n*)	16 (20)	6.6 (6)	41.2 (14)	<0.001[a]
CHF, % (*n*)	5.6 (7)	2.2 (2)	14.7 (5)	0.07[a]
aHT % (*n*)	45.6 (57)	41.8 (38)	55.9 (19)	0.16[a]
Diabetes mellitus, % (*n*)	18.4 (23)	17.6 (16)	20.6 (7)	0.70[a]
Obesity, % (*n*)	28 (35)	27.5 (25)	29.4 (10)	0.83[a]
COPD, % (*n*)	3.2 (4)	0 (0)	11.8 (4)	<0.001[a]
Asthma, % (*n*)	5.6 (7)	6.6 (6)	2.9 (1)	0.43[a]
CKD, % (*n*)	17.6 (22)	12.1 (11)	32.4 (11)	0.008[a]
Symptoms				
Cough, % (*n*)	57.6 (72)	56 (51)	61.8 (21)	0.61[a]
Dyspnea, % (*n*)	58.4 (73)	54.9 (50)	67.6 (23)	0.20[a]
Fever, % (*n*)	63.2 (79)	60.4 (55)	70.6 (24)	0.30[a]
Chills, % (*n*)	43.2 (54)	39.4 (36)	52.9 (18)	0.18[a]
Fatigue, % (*n*)	76 (95)	80.2 (73)	64.7 (22)	0.07[a]
Anosmia, % (*n*)	12.8 (16)	16.5 (15)	2.9 (1)	0.04[a]
Dysgeusia, % (*n*)	30.4 (38)	35.2 (32)	17.6 (6)	0.06[a]
Vital signs				
Heart rate,† bpm	89±18	87.5±16	93±22.5	0.12[c]
Systolic blood pressure,† mm Hg	130±18.5	130.5±18	128.5±20	0.60[c]
Diastolic blood pressure,† mm Hg	89±12.5	80.5±12	77.5±14	0.20[c]
Oxygen demand, % (*n*)	44.8 (56)	47.3 (43)	38.2 (13)	0.89[a]
Temperature,† °C	37.3 (36.7–38.1)	37.3 (36.8–38)	37.4 (36.8–38.2)	0.65[b]
Respiratory rate,† /min	22 (17–26)	22 (17–28)	21.5 (17.75–25)	0.88[b]
Biomarkers				
Urinary NT-proBNP,‡ pg/mg UCr	52.9 (15–269)	45.7 (13.4–231)	113 (36.2–676.8)	0.063[b]
Serum NT-proBNP,‡ pg/mL	141 (50–665.5)	104 (50–448)	574.5 (63–1,553.5)	0.004[b]
Serum creatinine,‡ mg/dL	0.94 (0.76–1.3)	0.91 (0.75–1.24)	1.06 (0.8–1.44)	0.078[b]
eGFR,‡ mL/min per m^2	84 (55–98)	87 (62–100)	76.5 (44.5–94)	0.13[b]
IL-6,‡ pg/mL	37.5 (15.05–79.35)	32.9 (15–69.4)	59.2 (21.5–184.3)	0.038[b]
CRP,‡ mg/dL	48.7 (20.5–90.5)	42.8 (21–82.5)	56 (8.78–95.6)	0.99[b]
White blood cell count,‡ *n*/nL	6.4 (4.33–9.34)	5.12 (4.1–7.53)	10.34 (7.32–14.15)	<0.001[b]

ACE, angiotensin-converting enzyme; ASS, acetylsalicylic acid; AT-1, angiotensin II receptor type I; CM, contrast media; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CT scan, computed tomography scan; DOAK, direct oral anticoagulants; eGFR, estimated glomerular filtration rate; ICU, intensive care unit; IL-6, interleukin-6; NT-proBNP, N-terminal prohormone of brain natriuretic peptide. [a]Fisher's exact test. [b]Mann-Whitney U test. [c]Student's *t* test. ‡Median (interquartile range). †Mean ± standard deviation.

Table 2. Median concentration of urinary and serum NT-proBNP in COVID-19 and non-COVID-19 cohort

	Urinary NT-proBNP, pg/mg UCr	Serum NT-proBNP, pg/mL
AKI versus no AKI		
COVID-19	4,480 (183–29,516) versus 23 (12.5–151)	2,040 (38–5,438) versus 74.9 (50–256)
Non-COVID-19	913 (217–1,439) versus 55 (31–163)	2,186 (1,363–4,720) versus 335 (50–962)
ICU/exitus/AKI versus no ICU/exitus/AKI		
COVID-19	526 (83–15,998) versus 22 (12–151)	1,191 (52–19,678) versus 20 (7–172)
Non-COVID-19	913 (202–2,772) versus 45 (30.5–150)	386 (180–1,552) versus 61 (5–144)

AKI, acute kidney injury; ICU, intensive care unit; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; UCr, urinary creatinine.

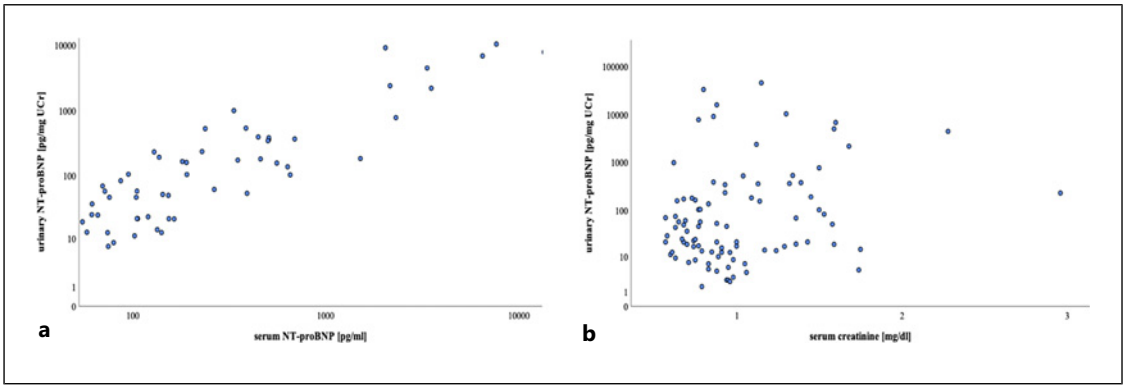

Fig. 2. Scatterplots showing the correlation between urinary NT-proBNP and serum NT-proBNP (**a**) and serum creatinine (**b**) in the COVID-19 cohort. **a** Urinary NT-proBNP is significantly correlated with serum NT-proBNP ($\rho = 0.86$, $p < 0.001$). **b** Urinary NT-proBNP is not significantly correlated with serum creatinine ($\rho = 0.19$, $p = 0.066$).

The same applies to serum NT-proBNP ($p < 0.001$, Fig. 3b). According to ROC analysis, urinary NT-proBNP was able to detect subsequent AKI in COVID-19 with an AUC of 0.91 and serum NT-proBNP with an AUC of 0.87 (Table 3, Fig. 4). Further, in COVID-19 cohort, IL-6 and serum creatinine showed significantly higher and eGFR significantly lower levels in patients with AKI compared to those without (each $p < 0.05$, Fig. 3c, d). In ROC analysis, creatinine showed an AUC of 0.69 and IL-6 an AUC of 0.85 for the detection of AKI in COVID-19 (Table 3; Fig. 4). In ROC comparison, urinary NT-proBNP ($p = 0.014$) but not serum NT-proBNP or IL-6 showed a significantly higher AUC than serum creatinine ($p > 0.05$). There was no significant difference between the AUC of urinary NT-proBNP, serum NT-proBNP, and IL-6 (each $p > 0.05$). In a univariate logistic regression analysis with AKI as dependent variable and one biomarker each as independent variable, only urinary NT-proBNP ($p = 0.006$) and IL-6 ($p = 0.016$) were identified as significant predictors of AKI, in contrast to serum NT-proBNP and serum creatinine (both $p > 0.05$, Table 4). To further analyze the diagnostic capabilities of those laboratory biomarkers that were found to be predictive of future AKI, multivariate stepwise logistic regression analyses were performed. In each case, one biomarker (either urinary NT-proBNP or IL-6) was examined together with the four clinical parameters of age, CKD, CHF, and arterial hypertension (aHT). Only urinary NT-proBNP ($p = 0.017$, OR = 3.91 [CI: 1.28–11.97] per SD) and IL-6 ($p = 0.015$, OR = 5.56 [CI: 1.34–22.09] per SD) were significant predictors of AKI. In contrast, age, CHF, CKD, and aHT did not reach statistical significance in either analysis (each $p > 0.05$). A bootstrap analysis with 3,000 resamples confirmed urinary NT-proBNP ($p = 0.008$; CI: 0.12–19.35) and IL-6 ($p = 0.002$, CI: 0.80–11.94) as independent predictors of AKI.

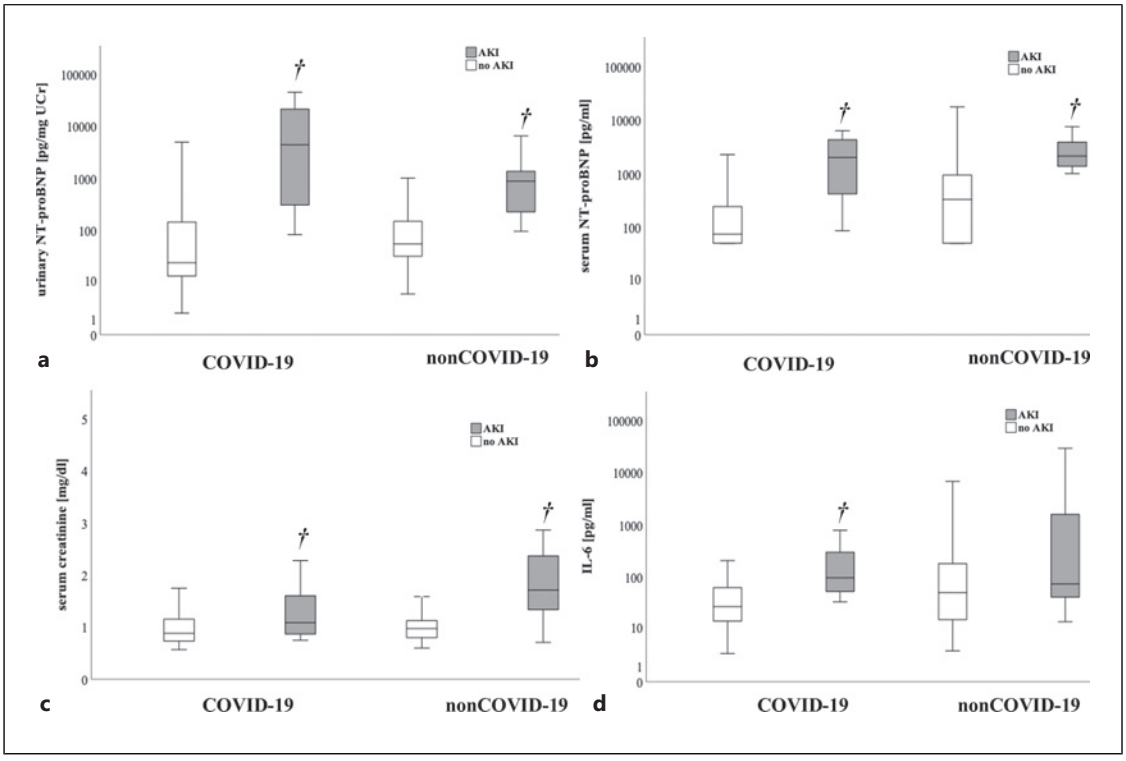

Fig. 3. Boxplots showing urinary and serum NT-proBNP, serum creatinine, and IL-6 of patients with and without AKI in the COVID-19 and the non-COVID-19 study cohort. **a–c** COVID-19/non-COVID-19: urinary and serum NT-proBNP as well as creatinine are significantly elevated in patients who suffer AKI ($^{\dagger}p < 0.05$ vs. no AKI). **d** COVID-19: IL-6 is significantly elevated in patients with versus without AKI ($^{\dagger}p < 0.05$ vs. no AKI). Non-COVID-19: IL-6 shows a trend toward higher values in patients with AKI versus without AKI.

Table 3. ROC analysis for urinary and serum NT-proBNP, serum creatinine, and IL-6 for detection of AKI in the COVID-19 cohort and the non-COVID-19 cohort

	COVID-19					Non-COVID-19					
	AUC	CI	cutoff value	sensitivity, %	specificity, %		AUC	CI	cutoff value	sensitivity, %	specificity, %
Urinary NT-proBNP	0.91	0.84–0.99	162 pg/mg UCr	91	77.5	Urinary NT-proBNP	0.85	0.71–0.98	201 pg/mg UCr	87.5	81
Serum NT-proBNP	0.87	0.77–0.96	172.5 pg/mL	91	71	Serum NT-proBNP	0.91	0.81–1.01	1,310 pg/mL	87.5	85
Serum creatinine	0.69	0.54–0.85	0.86 mg/dL	82	45	Serum creatinine	0.83	0.63–1.02	1.21 mg/dL	87.5	85
IL-6	0.85	0.74–0.95	40.2 pg/mL	82	64	IL-6	0.65	0.43–0.86	40.9 pg/mL	75	46

AKI, acute kidney injury; AUC, area under the curve; CI, 95% confidence interval; IL-6, interleukin-6; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ROC, receiver operating characteristic; UCr, urinary creatinine.

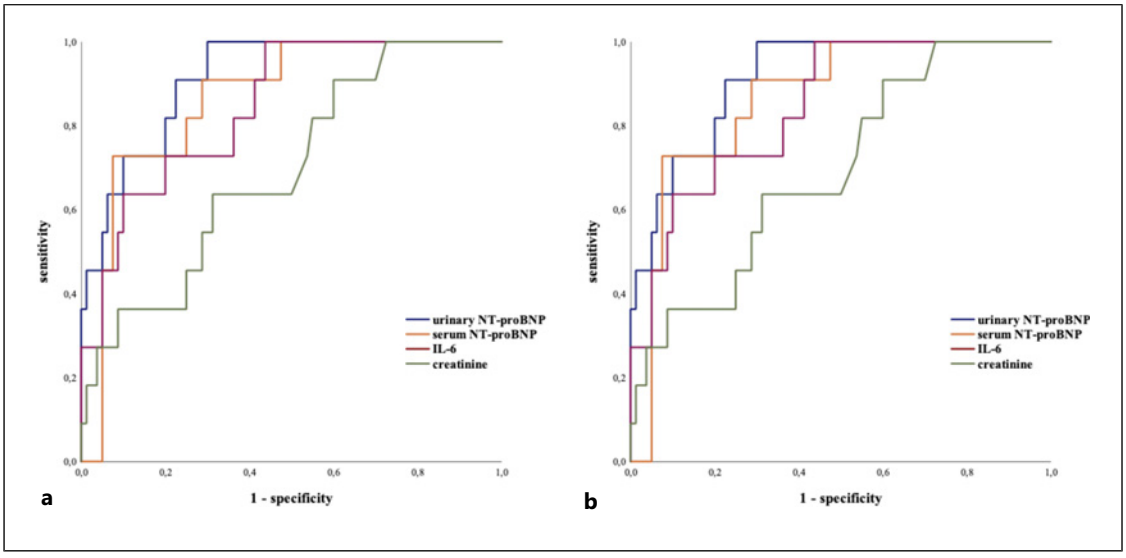

Fig. 4. Receiver operating characteristic (ROC) analysis of urinary and serum NT-proBNP, creatinine, and IL-6 in the COVID-19 cohort. **a** ROC for urinary and serum NT-proBNP, creatinine, and IL-6 for AKI versus no AKI in the COVID-19 cohort. **b** ROC for urinary and serum NT-proBNP, creatinine, and IL-6 for the composite endpoint in the COVID-19 cohort.

Table 4. Univariate logistic regression analysis of urinary and serum NT-proBNP, serum creatinine, and IL-6 for the detection of AKI and the composite endpoint in the COVID-19 cohort

	AKI		ICU/exitus/AKI	
	p value	OR per SD (CI)	*p* value	OR per SD (CI)
Urinary NT-proBNP	0.006	8.05 (1.83–35.44)	0.015	4.97 (1.37–18.04)
Serum NT-proBNP	0.142	1.41 (0.89–2.23)	0.26	1.29 (0.83–1.99)
Creatinine	0.125	6.28 (0.60–65.77)	0.34	2.85 (0.33–24.95)
IL-6	0.016	5.67 (1.39–23.19)	0.02	4.09 (1.24–13.45)

AKI, acute kidney injury; CI, 95% confidence interval, ICU, intensive care unit; IL-6, interleukin-6; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; OR, odds ratio; SD, standard deviation.

Regarding the composite endpoint, urinary as well as serum NT-proBNP appeared significantly higher in patients reaching the composite endpoint (each $p < 0.05$, Fig. 5a, b). Further, IL-6 showed significantly higher and eGFR significantly lower values in patients who reached the composite endpoint (each $p < 0.05$, Fig. 5d), in contrast to creatinine ($p = 0.15$). In ROC analysis, urinary NT-proBNP achieved an AUC of 0.81 and serum NT-proBNP an AUC of 0.79 (Table 5; Fig. 4). For the detection of the composite endpoint, IL-6 showed an AUC of 0.80 in ROC analysis.

Regarding ROC comparison, urinary NT-proBNP and IL-6 showed a significantly higher AUC than serum creatinine ($p = 0.049$ and $p = 0.023$), as opposed to serum NT-proBNP ($p = 0.071$). The AUCs of urinary NT-proBNP, serum NT-proBNP, and IL-6 did not differ significantly (each $p > 0.05$).

A univariate regression analysis revealed that NT-proBNP in urine ($p = 0.015$) and IL-6 ($p = 0.02$), but not serum NT-proBNP ($p = 0.26$) and creatinine ($p = 0.34$, Table 4), was predictive for the composite endpoint. Multivariate stepwise logistic regression analyses adjusted for age, CKD, CHF, and aHT were performed for urinary NT-proBNP on the one hand and IL-6 on the other. Hereby, urinary NT-proBNP ($p = 0.026$, OR 2.66 [CI: 1.13–6.28] per SD) as well as IL-6 ($p = 0.02$, OR 3.74 [CI: 1.24–11.30] per SD) were identified as independent predictors of the composite endpoint, in contrast to age, CKD, CHF, and aHT (each $p > 0.05$). After bootstrapping with 3,000 iterations, urinary NT-proBNP ($p = 0.007$,

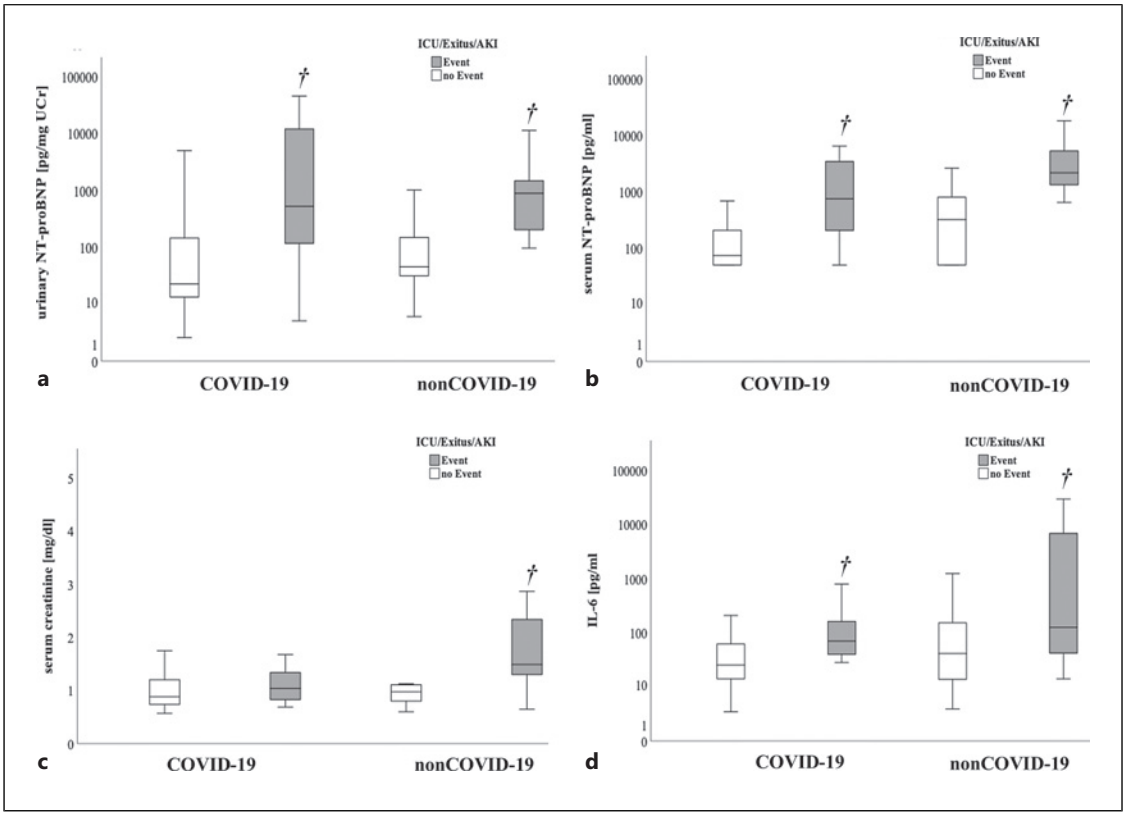

Fig. 5. Boxplots showing urinary and serum NT-proBNP, serum creatinine, and IL-6 of patients with and without an event in the COVID-19 and the non-COVID-19 study cohort. **a**, **b**, **d** COVID-19/non-COVID-19: urinary and serum NT-proBNP as well as IL-6 are significantly elevated in patients reaching the composite endpoint ($^{\dagger}p < 0.05$ vs. no event). **c** COVID-19: creatinine shows a trend toward higher values in patients reaching the composite endpoint. Non-COVID-19: creatinine is significantly elevated in patients reaching the composite endpoint ($^{\dagger}p < 0.05$ vs. no Event).

CI: −1.2 to 2.7) and IL-6 ($p = 0.02$, CI: 1.24–11.3) were confirmed as independent predictors of the composite endpoint.

Detection of AKI and the Composite Endpoint in the Non-COVID-19 Cohort

In the non-COVID-19 cohort, urinary as well as serum NT-proBNP were significantly higher in patients with AKI compared to those without ($p < 0.05$, Fig. 3a, b). According to ROC analysis, urinary NT-proBNP achieved an AUC of 0.85 and serum NT-proBNP an AUC of 0.91 (Table 3). Creatinine showed significantly higher and eGFR significantly lower values in patients with subsequent AKI in the non-COVID-19 group (each $p < 0.05$, Fig. 3c). ROC analysis indicated an AUC of 0.83 for creatinine. IL-6 showed a nonsignificant trend toward higher levels in patients with AKI compared to those without AKI ($p = 0.23$, Fig. 3d).

Urinary NT-proBNP, serum NT-proBNP, IL-6, and creatinine showed significantly higher and eGFR significantly lower values in patients who reached the composite endpoint (Fig. 5). In ROC analysis, urinary NT-proBNP achieved an AUC of 0.89 and serum NT-proBNP an AUC of 0.93 (Table 5). Further, ROC analysis provided an AUC of 0.74 for IL-6 and of 0.76 for creatinine (Table 5).

KIM-1 in a Subgroup of the COVID-19 Cohort

KIM-1 values were only available for 88 of the 91 COVID-19 patients. Therefore, it is shown here separately. KIM-1 showed significantly higher levels in patients with AKI compared to those without ($p = 0.002$). Further, it detected AKI with an AUC of 0.79 (Table 6).

KIM-1 was significantly elevated in patients who reached the composite endpoint ($p = 0.003$). For the detection of the composite endpoint, KIM-1 showed an AUC of 0.75 in ROC analysis (Table 6).

Table 5. ROC analysis for urinary and serum NT-proBNP, serum creatinine, and IL-6 for detection of the composite endpoint in the COVID-19 cohort and the non-COVID-19 cohort

	COVID-19					Non-COVID-19					
	AUC	CI	cutoff value	sensitivity, %	specificity, %		AUC	CI	cutoff value	sensitivity, %	specificity, %
Urinary NT-proBNP	0.81	0.68–0.94	162 pg/mg UCr	73	78	Urinary NT-proBNP	0.88	0.77–1.00	201 pg/mg UCr	80	83
Serum NT-proBNP	0.79	0.67–0.91	172.5 pg/mL	80	72	Serum NT-proBNP	0.93	0.84–1.01	1,310 pg/mL	80	87.5
Serum creatinine	0.62	0.47–0.77	0.86 mg/dL	73	61	Serum creatinine	0.76	0.54–0.98	1.21 mg/dL	80	87.5
IL-6	0.80	0.70–0.91	40.2 pg/mL	73	65	IL-6	0.74	0.56–0.93	40.9 pg/mL	80	50

AKI, acute kidney injury; AUC, area under the curve; CI, 95% confidence interval; IL-6, interleukin-6; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; ROC, receiver operating characteristic; UCr, urinary creatinine.

Table 6. ROC analysis for KIM-1 regarding the detection of AKI and the composite endpoint in the COVID-19 cohort

	AUC	CI	Cutoff value	Sensitivity	Specificity
AKI	0.79	0.68–0.89	1,622 ng/g UCr	82	70
Composite endpoint	0.75	0.64–0.85	1,622 ng/g UCr	67	70

AKI, acute kidney injury; AUC, area under the curve; CI, 95% confidence interval; KIM-1, kidney injury molecule-1; ROC, receiver operating characteristic; UCr, urinary creatinine.

Discussion

The present study is the first to investigate urinary NT-proBNP as a biomarker of AKI and severe disease progression in COVID-19 and other respiratory infections. Urinary as well as serum NT-proBNP were shown to be significantly elevated on the day of hospital admission in patients with COVID-19 who were experiencing AKI during hospitalization. In addition, significantly elevated values of urinary as well as of serum NT-proBNP were observed in COVID-19 patients who reached the composite endpoint consisting of AKI, ICU admission, and death. Both markers detected AKI and the composite endpoint, respectively, with good predictive values. Thereby, urinary NT-proBNP tended to have a higher AUC and a better specificity than serum NT-proBNP. Urinary, but not serum NT-proBNP, was found to be an independent predictor of AKI and the composite endpoint in a multivariate regression analysis, even after adjustment for age, CHF, CKD, and aHT. A bootstrapped analysis of the same regression model confirmed urinary NT-proBNP as an independent predictor. These findings implicate that urinary NT-proBNP might be a helpful tool regarding the detection of COVID-19 patients at risk of future AKI and severe course of disease. For this purpose, the measurement of NT-proBNP in urine might be superior to the one in serum.

Additionally, similar results were found in the non-COVID-19 cohort. This suggests that urine NT-proBNP may also be an important adjunct in risk stratification for respiratory diseases other than COVID-19.

Furthermore, serum creatinine and IL-6 showed significantly higher concentrations in COVID-19 patients suffering from AKI. Only IL-6, but not serum creatinine,

was significantly elevated in patients reaching the composite endpoint. However, urinary NT-proBNP tended to detect AKI and the composite endpoint with a better sensitivity and specificity than IL-6 and serum creatinine. Furthermore, urinary NT-proBNP showed a significantly higher AUC than serum creatinine in the ROC comparison for detection of subsequent AKI, in contrast to serum NT-proBNP and IL-6.

NT-proBNP and Its Clinical Value as a Biomarker

NT-proBNP is a 76 amino acid long peptide produced by atrial and ventricular cardiomyocytes when exposed to increased wall stress [3, 13]. During this process, the precursor hormone proBNP is cleaved into hormonally inactive NT-proBNP and the hormonally active BNP and excreted into the blood [3, 13]. Besides its well-known use as biomarker in heart failure, it has also been shown to be a useful tool for risk assessment in COVID-19 [4]. Wang et al. [14] found that NT-proBNP levels were significantly associated with the severity of COVID-19 pneumonia in a cohort of 91 COVID-19 patients. Caro-Codón et al. [4] analyzed a cohort of 396 COVID-19 patients and found NT-proBNP to be highly and independently associated with mortality, even after adjusting for acute and CHF.

Further, NT-proBNP was shown to be a predictor of AKI in patients undergoing cardiac and non-cardiac surgery as well as in patients treated in ICU [15–17]. Since the excretion of NT-proBNP occurs mainly through the kidney, it can also be measured in urine [3]. A strong positive correlation between serum and urinary NT-proBNP was found, which is consistent with the results of previous studies [6, 18]. A diagnostic and prognostic benefit of NT-proBNP in urine has been demonstrated multiple times in patients with heart failure [5, 6]. A recent study presented NT-proBNP in urine as a predictor of all-cause mortality and major adverse cardiac events in patients with acute chest pain [19]. Yamasaki et al. [18] found urinary NT-proBNP to be associated with a higher risk of cardiovascular diseases.

Until now, data regarding the value of urinary NT-proBNP in COVID-19 were missing. The findings of this study suggest urinary NT-proBNP as a biomarker of future AKI as well as of severe disease in COVID-19, even after adjusting for age, CHF, CKD, and aHT. For this purpose, urinary NT-proBNP might be superior to serum NT-proBNP. This is particularly interesting as the concentration of urinary NT-proBNP was found to be mainly determined by its plasma concentration [20]. One may speculate that a possible reason for this finding may be an impaired tubular processing (regional cleavage processes and/or absorbance) of NT-proBNP due to acute tubular damage, which is thought to play a role in COVID-19-induced AKI [1]. This may lead to a higher excretion of NT-proBNP and therefore to higher levels of NT-proBNP in the urine of patients with AKI. This hypothesis is supported by the fact that NT-proBNP ratio (urine/serum) is significantly higher in COVID-19 patients who suffered from AKI or reached the composite endpoint, respectively (data not shown). Nevertheless, these explanations are only speculative, so the cause of these findings remains unclear and requires further research. The relationship between kidney function and NT-proBNP clearance is controversial. Previous studies suggested that excretion depends on renal plasma flow rather than GFR [21]. In this study, urinary as well as serum NT-proBNP were significantly negatively correlated with eGFR in COVID-19 cohort.

Furthermore, urinary NT-proBNP outperformed serum creatinine regarding the detection of subsequent AKI, as it showed a significantly higher AUC. This is particularly interesting because renal function is usually assessed by serum creatinine. However, serum creatinine mainly represents glomerular filtration but cannot make a reliable statement about the acute (tubular) damage [22, 23]. Therefore, serum creatinine is considered to be a non-sensitive non-specific biomarker for AKI that indicates AKI only at an advanced stadium [22–24]. Urinary NT-proBNP may therefore be helpful in predicting AKI at times where serum creatinine is still unchanged.

Previously, we demonstrated the value of KIM-1 as a biomarker in COVID-19 in a subset of this cohort ($n = 54$, [8]). KIM-1 was also assessed in a subgroup of 88 COVID-19 patients in this study. Again, KIM-1 was found to be significantly elevated in COVID-19 patients who developed AKI or reached the composite endpoint. In this somewhat smaller cohort of 88 COVID-19 patients, urinary NT-proBNP showed nearly identical predictive values as in the entire COVID-19 cohort of 91 patients (data not shown). Hereby, urinary NT-proBNP showed a trend toward higher predictive values compared to KIM-1. However, to assess which marker is superior in predicting AKI and the composite endpoint, further studies contrasting both markers in a head-to-head comparison are needed.

Recently, the novel urinary proteomic biomarker COV50 was shown to be valuable regarding the prediction of death and disease progression in COVID-19 [25]. From this large multicenter study, it could be inferred that high COV50 levels may justify earlier drug treatment and thus lead to fewer hospital days and effective cost reduction in patient treatment [25]. This is especially interesting, as the idea of using urinary biomarker for early risk

prediction is the same for COV50 and urinary NT-proBNP. COV50 is measured via capillary electrophoresis coupled with mass spectroscopy, which is – compared to the measurement of NT-proBNP – much more expensive and may not be available in smaller laboratories. However, the authors focused mainly on the prediction of mortality and clinical deterioration as displayed by higher grades on the WHO scale. This study was conducted to evaluate the use of urinary NT-proBNP for the prediction of AKI and severe disease as displayed by the composite endpoint. Since Staessen et al. [25] were the first to investigate this promising biomarker, there are no data available regarding the value of COV50 for the prediction of AKI in COVID-19. For this reason, comparing the predictive values of urinary NT-proBNP and COV50 is currently limited. Further research should be conducted that directly compares both urinary biomarkers for their value in predicting mortality and AKI.

Conclusion

The results of the current study suggest that NT-proBNP measured in fresh spot urine may contribute importantly to the early identification of patients at high risk for AKI in COVID-19. These patients may profit from adjustments in their regimen, such as an enhanced monitoring of urine output and volume balance as well as by avoiding or discontinuing nephrotoxic medications.

Furthermore, high urinary NT-proBNP was found to be an independent predictor of a severe course of COVID-19. Considering the limited healthcare resources as a global problem in COVID-19 pandemic, it is critical to identify those high-risk patients to provide appropriate treatment to those who need it most. Consequently, measurement of NT-proBNP in urine may in the future represent a convenient and inexpensive test procedure that does not require venipuncture and can be performed in medical settings without in-house laboratories, such as general practice. However, further studies with larger cohorts are needed to confirm our results and to conclusively evaluate the value of NT-proBNP in COVID-19.

Limitations

The main limitation of our study comes from the relatively low number of patients included. An important reason for this is the large number of patients who had to be excluded because they were unable to pass urine due to infection-related hypovolemia. In addition, no distinction was made between the different SARS-CoV-2 variants, so no conclusion could be drawn about the possibly different benefits of urinary NT-proBNP in different virus subtypes.

Acknowledgments

We thank the physicians and nursing staff of the emergency department and the laboratory staff of the University Hospital Regensburg for their valuable participation in the execution of the study. Furthermore, we thank Roche Diagnostics for providing the test kits. We also thank Johannes Vogel and Alana Vogel for their expert advice on the linguistic revision and our statistician Florian Zeman for the proficient consultation.

Statement of Ethics

The study was approved by the Ethics Committee of the University of Regensburg (approval number 20-1765-101). Written informed consent was obtained from all participants of this study. It was conducted in accordance with the guidelines of Good Clinical Practice and the standards for experiments on humans set out in the Declaration of Helsinki.

Conflict of Interest Statement

The authors declare no conflict of interest.

Funding Sources

M.V. is funded by the Kaltenbach scholarship of the German Heart Foundation.

Author Contributions

Manuel J. Vogel, Julian Hupf, and Carsten G. Jungbauer collected and analyzed the data, performed statistical analysis, and wrote the manuscript. Stephan T. Staudner and Simon B. Leininger gathered data. Ute Hubauer, Stefan Wallner, Julian Mustroph, Frank Hanses, Markus Zimmermann, Petra Lehn, Ralph Burkhardt, and Lars S. Maier gathered data and revised the manuscript for critical intellectual content.

Data Availability Statement

Data are not publicly available due to ethical reasons. Further inquiries can be directed to the corresponding author.

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