OBSERVATIONAL STUDY

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Host Response Protein Biomarkers Indicative of Persistent Acute Kidney Injury in Critically Ill COVID-19 Patients

IMPORTANCE: Sepsis-related host-response anomalies contribute to acute kidney injury (AKI) duration. Data on the host-response specific to COVID-19-associated AKI (COVID-AKI) in critically ill patients is limited.

OBJECTIVES: We postulated that persistent COVID-AKI (> 48 hr) differs in host response from transient (< 48 hr) or no COVID-AKI.

DESIGN, SETTING, AND PARTICIPANTS: This prospective biomarker study observed patients with severe acute respiratory syndrome coronavirus 2 infection, without chronic kidney disease, in three ICUs from March 2020 to July 2020. AKI was assessed by hourly urine output and daily plasma creatinine.

MAIN OUTCOMES AND MEASURES: Luminex and enzyme-linked immunosorbent assay were used to analyze 48 plasma protein biomarkers across six pathophysiological domains, which were tested with mixed-effects models.

RESULTS: Of 177 included patients, 106 (59.9%) had AKI within the first 48 hours of admission, of whom 76 (71.7%) had persistent AKI and 30 (28.3%) transient AKI. Those with persistent AKI often had obesity, hypertension, and a higher Sequential Organ Failure Assessment score due to the renal component. Longitudinal analyses revealed that seven proteins were elevated in persistent AKI compared with no AKI. These were related to inflammation (triggering receptor expressed on myeloid cells 1, $p < 0.001$; tumor necrosis factor receptor 1, $p < 0.001$; procalcitonin, $p = 0.001$), complement activation (mannan-binding lectin serine protease-2, $p = 0.001$), kidney dysfunction (cystatin C, $p < 0.001$; neutrophil gelatinase-associated lipocalin, $p < 0.001$), and lung dysfunction (Clara cell secretory protein 16, $p < 0.001$). AKI (duration) was not associated with differences in the cytokine signaling, endothelial cell activation, or coagulation domains.

CONCLUSIONS AND RELEVANCE: In contrast with sepsis-associated AKI, primarily inflammation-related biomarker levels correlated with COVID-AKI persistence. This study offers insights into COVID-AKI and may guide approaches to mitigate its persistence.

KEYWORDS: acute kidney injury; COVID-19; host response; intensive care unit

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DOI: 10.1097/CCE.0000000000001222

COVID-19 is a disease with prominent pulmonary manifestations caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first described in Wuhan, China (1). Acute kidney injury (AKI) is recognized as a common complication of COVID-19, particularly in the ICU. Two recent studies conducted in New York described an AKI occurrence rate of 68–78% and an independent association of AKI with mortality in critically ill COVID-19 patients (2, 3). The clinical relevance of AKI in COVID-19 patients also resonates from the associated long-term implications. More so than other patients with AKI, patients with COVID-19-associated AKI (COVID-AKI) are at risk for persistently reduced renal function after discharge from the hospital (4).

KEY POINTS

Question: Is persistence of COVID-associated acute kidney injury (AKI) associated with distinct host-response pathway activation related to (systemic) inflammation, endothelial cell activation, coagulation activation, complement activation, and lung and kidney dysfunction?

Findings: Longitudinal analyses revealed that seven plasma proteins were elevated in persistent AKI compared with no AKI. These markers were related to increased inflammatory response, complement activation, and lung and kidney dysfunction.

Meanings: The inflammatory host-response to COVID might be involved in AKI persistence. This study offers insights into COVID-AKI and may guide approaches to mitigate its persistence through targeting immune response pathways.

Whether SARS-CoV-2 directly infects the kidneys of COVID-19 patients remains controversial, which leaves many questions about COVID-AKI unanswered (5, 6). Analyses of kidney samples taken from deceased patients with severe COVID-AKI have revealed that acute tubular injury is mainly characterized by mild focal acute tubular necrosis (7, 8). This uncoupling between the modest extent of histological injury and strong decline in kidney function is similar to what is known about sepsis-associated AKI (SA-AKI) (9, 10).

Current knowledge of SA-AKI and COVID-19 pathophysiology suggests a role for local and systemic inflammation, immune suppression, endothelial cell dysfunction, activation of the complement cascade, and coagulopathy (6, 11). In sepsis, the host-response anomalies have been, linked to the development and extent of AKI (12). Although data on the host-response to COVID-19 and the development of AKI are scarce, the protective effects of anti-inflammatory drugs—such as corticosteroids and interleukin (IL)-6 receptor blockers—which are associated with reduced dialysis requirement in patients with severe COVID-AKI (13, 14) suggest that inflammation is a key factor in the pathogenesis of COVID-AKI.

Based on previous findings in SA-AKI (12) and specific interaction of lung and kidney damage in COVID-19 (15), we selected a panel of 48 biomarkers that together represent six key pathophysiological pathways consist of (systemic) inflammation, endothelial cell activation, coagulation activation, complement activation, lung dysfunction, and kidney dysfunction. These biomarkers were measured in sequential plasma samples taken from critically ill COVID-19 patients admitted to three large medical centers in the Netherlands.

METHODS

Study Design and Population

Between March 1, 2020, and July 1, 2020, all patients with polymerase chain reaction confirmed SARS-CoV-2 infection, admitted to the ICUs of the academic Amsterdam University Medical Center (UMC) hospitals and the general hospital Flevoziekenhuis in Almere, were included in this study. Daily clinical parameters and patient outcome data were collected by the CovidPredict consortium (16) through which informed consent was extended to the present study. Readmissions and patients transferred from another ICU were excluded, except when patients were transferred to the ICU of a participating center within 48 hours of initial hospital presentation. Patients with known advanced stage of chronic kidney disease (CKD stage 3 or 4, or those on chronic dialysis) were also excluded (17, 18).

Ethics

This study was executed in accordance with the Helsinki Declaration of 1975 and with the ethical standards of the ethics committees of the Amsterdam UMCs. This study falls under the "CovidPredict" study protocol, which was approved by the medical ethics committee on March 18, 2020 (Amsterdam UMC; 20.131) (19). The Amsterdam UMC COVID-19 biobank study was approved by the biobank ethics committees of both Amsterdam UMC hospitals on March 20, 2020 (2020_065) (20).

Definitions

The presence of AKI was assessed using hourly measurements of urine output and daily measurements of plasma creatinine. The presence of AKI was assessed

using criteria based on the Kidney Disease: Improving Global Outcomes (KDIGO) criteria (21). Cases in the present study were labeled with AKI if they had: an increase of at least 1.5 times their baseline creatinine (if recorded within the 7 d before ICU admission) or an hourly urine output of less than 0.5 mL/kg/hr for at least 6 hours. Missing baseline creatinine values were estimated using the categories specified in the 2012 KDIGO guideline assuming a glomerular filtration rate of 75 mL/min/1.73 m^2 (21, 22). The ethnicity correction was used for patients of African-Surinamese and Ghanaian descent when labeled in the COVID-predict study (23). Cases of AKI were staged in accordance with the KDIGO guideline in which the original baseline creatinine was used, and the use of renal replacement therapy (RRT) always resulted in AKI stage 3 for that ICU day (21). **Table S6** (http://links.lww.com/CCX/B478) shows a contingency matrix of AKI stages based on creatinine or urine output per day in the ICU.

In accordance with expert consensus (24), AKI was classified in retrospect as "persistent" or "transient." A case of AKI was labeled "persistent" if one or more of the aforementioned KDIGO criteria for serum creatinine and urine output remained present after 48 hours from the start of AKI or if they normalized within 48 hours, but relapsed within the next 48 hours. Cases were labeled "transient" if serum creatinine and urine output normalized within 48 hours of AKI onset and remained normal for at least 48 hours. In some cases, 48-hour follow-up was not possible. This was due to early death or discharge from the ICU. In these cases, AKI was classified as persistent if patients met one of the modified KDIGO criteria at the end of follow-up. If the last available measurement of serum creatinine or urine output was normal, they were labeled as transient (24). The primary analyses were conducted with AKI episodes that occurred within the first 48 hours of ICU admission and were stratified by AKI duration (no AKI, transient AKI, or persistent AKI).

Biomarker Assays

In a subset of patients who were enrolled from March 23, 2020, to May 26, 2020, the levels of 48 plasma protein biomarkers were used in this study. During this period, serial blood samples were archived in the Amsterdam UMC COVID-19 biobank, which contains archive biomaterials from adult SARS-CoV-2-infected patients admitted to the two academic hospitals of Amsterdam UMC. Plasma biomarkers were quantified utilizing Luminex or enzyme-linked immunosorbent assay techniques (**Table S1**, http://links.lww.com/CCX/B478). Plate-to-plate variation was accounted for using negative and positive controls. Values below the detection limit were imputed by division of the lower limit of quantification by its square root. More specifications of the assays used in this study are described in the **Supplementary Data** (http://links.lww.com/CCX/B478) and have already been published elsewhere (20). Plasma was sampled at a minimum of four time points per patient (day 0, days 2–4, days 5–6, and days 9–10), although the specific timing of collections could vary, the between-patient variability was kept to a minimum. Sample collection days are presented per patient in **Supplementary Figure 2** (http://links.lww.com/CCX/B478).

Statistical Analyses

Baseline characteristics were compared with analysis of variance or independent *t* test for continuous variables meeting the normality assumption. Non-normally distributed continuous variables were compared using a Kruskal-Wallis or Mann-Whitney *U* test. Categorical data were analyzed using Fisher exact test. The primary outcome was the host-response to SARS-CoV-2 infection as represented by the prespecified 48 protein plasma biomarkers. Secondary outcomes were 12-week mortality and the need for RRT during ICU stay.

Differences in (log-transformed) plasma biomarker outcomes between AKI groups upon ICU admission were compared using linear regression with contrast dummy coding and are presented in volcano plots. Calculation of principal component analysis (PCA) plots was done by a singular value decomposition of the centered and scaled data matrix including the (logged) protein plasma biomarkers for each key immune pathway. Biomarker trajectories over time were analyzed using general mixed model analysis in which a linear regression model was fitted on logarithmically transformed data, taking group (no AKI, transient AKI, and persistent AKI), time (numeric), and their interaction as fixed effects, and patient-specific intercept and slope of time as random effects. Plasma biomarker analyses (48) were corrected for multiple

testing using the Benjamini-Hochberg 5% false discovery rate (FDR). Handling of missing data was done by a list-wise deletion approach, assuming missing at random. Data analyses were performed in R (v4.0.4; R Foundation for Statistical Computing, Vienna, Austria). Significance level was set to 5%.

RESULTS

Patient Characteristics and Outcome

During the study period, there were 185 admissions to the ICUs of the participating centers. Eight patients who suffered from CKD were excluded (see flow diagram, **Fig. S1**, http://links.lww.com/CCX/B478). Of the remaining 177 patients, 106 patients (59.9%) had AKI within the first 48 hours of admission. Measured preadmission baseline creatinine was available for 35 of 177 patients. Imputed values were used to determine AKI stage for the remaining patients. Of the 106 patients with AKI, 76 patients (71.7%) had a persistent AKI course and 30 patients (28.3%) had transient AKI. Obesity (body mass index > 30), hypertension, and non-Dutch ethnicity were more prevalent in the patients with AKI than in those without AKI (**Table S2**, http://links.lww.com/CCX/B478). Within the first 24 hours of admission, patients with AKI were subjected to treatment with diuretics more often than those without AKI. Although patients without AKI had slightly lower average disease severity—as indicated by the mean total Sequential Organ Failure Assessment (SOFA) score—the non-renal SOFA score was similar between AKI and no AKI. Differences in 12-week mortality between no AKI and AKI were statistically non-significant (31.0% vs. 34.9%; $p = 0.704$). More than half of patients (38/61 [61.3%]) without AKI at admission, developed ICU-acquired AKI at some point during ICU admission. **Figure 1** shows AKI occurrence rate during ICU stay. Of the total cohort, 149 patients (84.2%) had AKI at some point during their stay on the ICU (Table S2, http://links.lww.com/CCX/B478).

Patients with persistent AKI more often had obesity, hypertension, and a higher total SOFA score (mainly driven by the renal component)

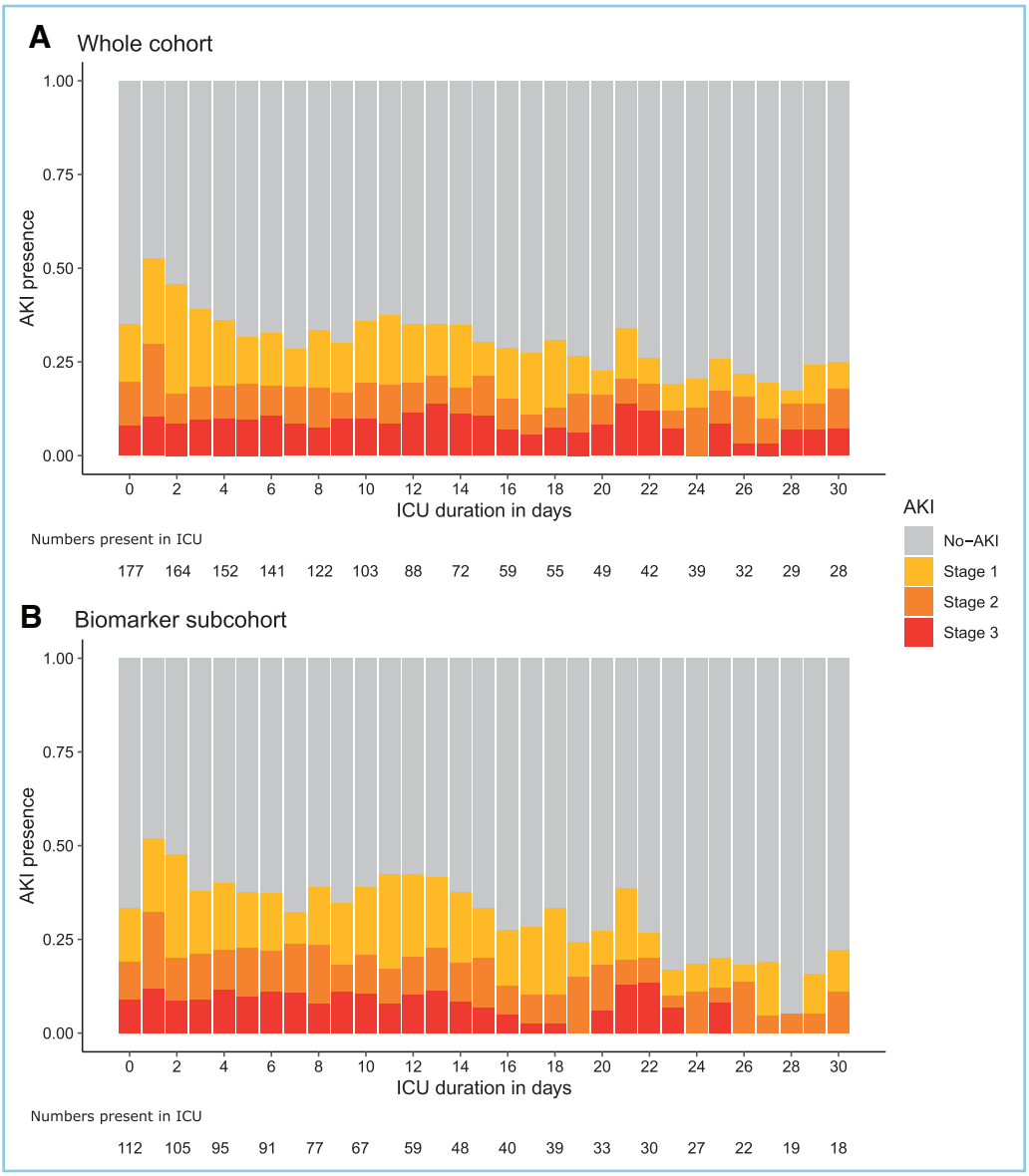

Figure 1. Relative acute kidney injury (AKI) presence during stay in the ICU, according to stage of AKI. **A**, Whole cohort ($n = 177$). **B**, Biomarker cohort ($n = 112$).

compared with both transient AKI and no AKI (**Table 1**). After 12 weeks of follow-up, group mortality rates showed no statistically significant differences between the no AKI, transient AKI and persistent AKI groups (33.3% vs. 26.7% vs. 38.2%; $p = 0.456$, respectively; Table 1). In the no AKI group, 38 patients (61.3%) developed ICU-acquired AKI.

Plasma Host-Response Biomarkers at ICU Admission

Serial plasma biomarkers, indicative of host-response pathways, were measured in a subgroup of the patients enrolled in the Amsterdam UMC COVID-19 biobank ($n = 112$, 63.3% of the cohort). The clinical characteristics of this subgroup were largely comparable to the cohort as a whole; however, there were differences, such as higher occurrence rate of invasive ventilation (87.2% vs. 78.4%, with similar median duration 12.0 d [7.0–18.8 d] vs. 12.0 d [7.0–18.0 d]) and more transferred patients (23.3% vs. 14.7%). **Tables S3** and **S4** (http://links.lww.com/CCX/B478) for the baseline characteristics. Sample collection days showed only modest between-patient variation (Supplementary Fig. 2, http://links.lww.com/CCX/B478). Minimum follow-up was less than 5 days in eight patients.

In **Figure 2**, three volcano plots (AKI vs. no AKI, transient AKI vs. no AKI, and persistent AKI vs. no AKI) show univariate associations of the 48 plasma biomarkers measured at ICU admission (day 0). No differences in plasma biomarkers were observed between transient AKI and no AKI, but five biomarkers were significantly higher in persistent AKI than in no AKI (Clara cell secretory protein 16 [CC16], cystatin C, neutrophil gelatinase-associated lipocalin [NGAL], triggering receptor expressed on myeloid cells 1 [TREM-1], and tumor necrosis factor receptor 1 [TNF-RI]). After correction for multiple testing, no differences were observed between transient and persistent AKI (data not shown). PCA were used to summarize admission biomarker levels in the three groups across the six pathophysiological domains of (systemic) inflammation, endothelial dysfunction, coagulation activation, complement activation, kidney dysfunction, and lung dysfunction (**Supplementary Fig. 3*A–G***, http://links.lww.com/CCX/B478). In particular, the inflammatory response pathway was more strongly activated in patients with persistent AKI compared with no AKI (principal component [PC] 1; $p < 0.008$) and transient AKI (PC1; $p = 0.031$). The lung dysfunction domain was also more strongly activated in persistent AKI compared with no AKI (PC1; $p = 0.013$) but not significantly so when compared with transient AKI (PC1; $p = 0.063$). Unsurprisingly, plasma levels of kidney dysfunction biomarkers (NGAL, cystatin C) were higher in persistent AKI compared with no AKI and transient AKI (PC1, $p = 0.017$; PC1, $p = 0.028$, respectively). Other domains were not different between groups.

Changes in Plasma Host-Response Biomarker Trajectories

The longitudinal mixed models revealed significant FDR-corrected between-group differences in TREM-1, TNF-RI, procalcitonin, mannan-binding lectin serine protease-2 (MASP-2), NGAL, cystatin C, and CC16 between groups. The full results are available in **Table S7** (http://links.lww.com/CCX/B478). We found no significant changes in trajectories between no AKI, transient AKI, and persistent AKI; all interactions were nonsignificant ($p > 0.10$). In the persistent AKI group, the plasma levels of the aforementioned markers remained elevated from baseline and relatively higher during the first 5 days of ICU admission compared with the no AKI group (TREM-1, $p < 0.001$; TNF-RI, $p < 0.001$; procalcitonin, $p = 0.001$; MASP-2, $p = 0.001$; and NGAL, $p < 0.001$; cystatin C, $p < 0.001$; and CC16, $p < 0.001$; **Fig. 3**). In the first 5 days, MAPS2, cystatin C, and NGAL were higher in persistent AKI compared with transient AKI ($p < 0.001$, $p = 0.005$, and $p = 0.005$, respectively). No differences between transient AKI and no AKI were observed. Biomarkers in the endothelial dysfunction and coagulation activation domains, as well as cytokine levels, demonstrated no statistically significant differences between any of the groups.

ICU-Acquired AKI Subgroup

A significant proportion (68.2%) of patients developed AKI more than 48 hours after admission to the ICU, as is shown in Table 1. Therefore, we explored biomarker trajectories in patients who did not show signs of AKI in the first 48 hours of admission but were at risk of developing AKI later during their stay in the ICU ($n =$

TABLE 1.

Comparative Baseline Characteristics of the COVID-19 Study Cohort: No Acute Kidney Injury Patients, Transient Acute Kidney Injury Patients, and Persistent Acute Kidney Injury Patients

Characteristic	Overall	No AKI	Transient AKI	Persistent AKI	*p*
n	177	71	30	76	
Demographics					
Sex, male (%)	130 (73.4)	53 (74.6)	23 (76.7)	54 (71.1)	0.804
Age, yr, mean (SD)	61.2 (10.9)	60.8 (11.4)	58.7 (11.2)	62.5 (10.2)	0.253
Ethnicity, non-Dutch (%)	60 (42.0)	19 (30.6)	14 (50.0)	27 (50.9)	0.056
Body mass index, weight (kg)/height^2 (m), mean (SD)	28.6 (5.7)	26.3 (4.4)	28.1 (7.1)	30.7 (5.4)	< 0.001
Obesity (%)	61 (35.7)	11 (15.9)	8 (28.6)	42 (56.8)	< 0.001
ICU transfer (%)	26 (14.7)	12 (16.9)	1 (3.3)	13 (17.1)	0.156
Comorbidities, *n* (%)					
Cardiovascular disease	33 (18.8)	13 (18.3)	4 (13.8)	16 (21.1)	0.690
Hypertension	74 (42.5)	21 (29.6)	13 (44.8)	40 (54.1)	0.011
Liver disease	7 (4.0)	4 (5.6)	1 (3.4)	2 (2.7)	0.649
Immune compromised	17 (9.8)	8 (11.3)	3 (10.0)	6 (8.2)	0.826
Malignant neoplasm	3 (1.7)	2 (2.9)	0 (0.0)	1 (1.3)	0.561
Diabetes	44 (25.1)	14 (20.0)	9 (30.0)	21 (28.0)	0.431
Medications, *n* (%)					
Diuretics	24 (13.6)	3 (4.2)	5 (16.7)	16 (21.1)	0.010
Angiotensin-converting enzyme inhibitor or angiotensin receptor blocker	38 (21.5)	14 (19.7)	5 (16.7)	19 (25.0)	0.577
Beta blockers	26 (14.7)	10 (14.1)	3 (10.0)	13 (17.1)	0.637
Nonsteroidal anti-inflammatory drug	8 (4.5)	5 (7.0)	0 (0.0)	3 (3.9)	0.283
Corticosteroids	6 (3.4)	2 (2.9)	2 (6.7)	2 (2.7)	0.563
Anticoagulants	17 (9.7)	4 (5.7)	4 (13.3)	9 (12.0)	0.338
Laboratory values < 24 hr ICU admission, mean (SD)					
Albumin, mmol/L	26.9 (6.5)	25.95 (6.11)	27.89 (7.51)	27.37 (5.27)	0.276
Creatinine, µmol/L	110.1 (114.8)	73.8 (23.8)	85.8 (39.3)	152.3 (161.6)	< 0.001
Urea, mmol/L	9.2 (8.0)	7.2 (3.6)	8.0 (7.2)	11.5 (10.4)	0.006
Bicarbonate, mmol/L	25.7 (4.5)	26.3 (4.9)	25.9 (4.1)	25.1 (4.2)	0.374
24 hr urine output, mL	1723.3 (1165.4)	2104.5 (1079.6)	1530.2 (722.5)	1446.0 (1298.9)	0.004
Severity < 24 hr ICU admission					
Nonrenal SOFA score, median (IQR)	5.0 (4.0–7.0)	5.5 (3.0–7.0)	5.0 (4.0–6.0)	5.0 (4.3–7.0)	0.288
SOFA score, median (IQR)	6.0 (5.0–8.0)	6.0 (4.0–7.0)	5.0 (4.0–6.0)	7.0 (5.0–8.0)	0.002
Shock (%)	49 (49.0)	15 (38.5)	7 (46.7)	27 (58.7)	0.174

(*Continued*)

TABLE 1. (*Continued*)

Comparative Baseline Characteristics of the COVID-19 Study Cohort: No Acute Kidney Injury Patients, Transient Acute Kidney Injury Patients, and Persistent Acute Kidney Injury Patients

Characteristic	Overall	No AKI	Transient AKI	Persistent AKI	*p*
Outcomes					
Renal replacement therapy (%)	27 (15.3)	5 (7.0)	1 (3.3)	21 (27.6)	< 0.001
Invasive ventilation (%)	80 (78.4)	29 (72.5)	14 (87.5)	37 (80.4)	0.423
Invasive ventilation duration, d, median (IQR)	12.0 (7.0–18.0)	12.0 (6.0–18.0)	11.0 (8.5–24.5)	11.5 (8.3–18.0)	0.725
Length of hospital stay, d, median (IQR)	22.0 (13.0–37.0)	24.0 (13.0–38.8)	19.0 (11.8–33.5)	22.0 (13.0–38.0)	0.709
Length of ICU stay, d, median (IQR)	13.0 (7.0–20.3)	12.5 (5.3–18.0)	11.0 (6.3–20.0)	14.0 (9.0–23.0)	0.183
Mortality at 12 wk (%)	59 (33.3)	22 (31.0)	8 (26.7)	29 (38.2)	0.456

AKI = acute kidney injury at least stage I, IQR = interquartile range, SOFA = Sequential Organ Failure Assessment.
Group differences were assessed with Fisher exact tests (categorical variables), or analysis of variance if continuous variables are presented as mean with SD, and with Kruskal-Wallis test if continuous variables are presented as median (IQR).
Nonrenal SOFA score was calculated without the renal component. Urea, creatinine, albumin, and bicarbonate were measured in blood plasma.

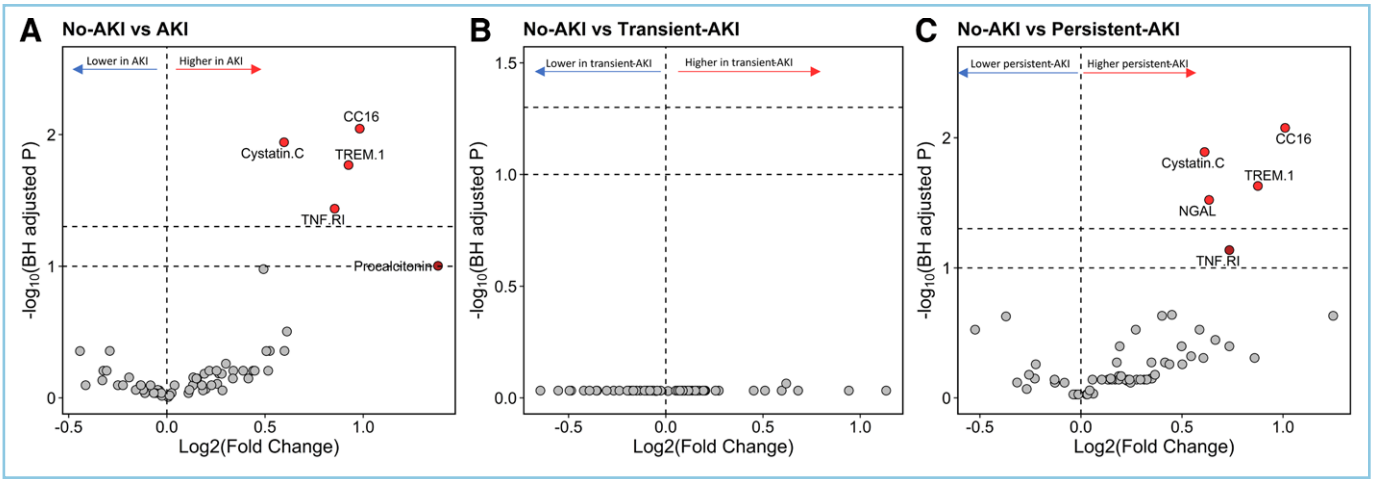

Figure 2. *Volcano plots* of the differentially expressed biomarkers across groups. Measured on the first day of ICU admission, comparing: 1) patients without acute kidney injury (AKI) to AKI (at least Kidney Disease: Improving Global Outcomes stage 1; **A**), 2) patients without AKI and transient AKI (**B**), and 3) patients without AKI and persistent AKI (**C**). Only significant Benjamini-Hochberg (BH) adjusted biomarkers are highlighted, denoted in *red* (upregulated) or *blue* (downregulated). *Gray dots* represent nonsignificantly different expressed biomarkers. Each comparison was tested for statistical significance with the BH multiple testing correction. CC16 = Clara cell secretory protein 16, NGAL = neutrophil gelatinase-associated lipocalin, TNF.RI = tumor necrosis factor receptor 1, TREM.1 = triggering receptor expressed on myeloid cells 1.

31, excluding 13 patients who died or were discharged within 48 hr). The median start of ICU-acquired AKI was 7 days from admission (IQR, 4–10 d). For sequential biomarker analyses, patients were followed-up until day 12. During this period, 19 patients developed ICU-acquired AKI and 12 patients did not. Although the difference was statistically nonsignificant, at ICU admission more patients in the ICU-acquired group met the criteria for shock (6, 31.5%) compared with the no ICU-acquired AKI group (1, 12.5%). Other baseline characteristics were comparable between groups (**Table S5**, http://links.lww.com/CCX/B478).

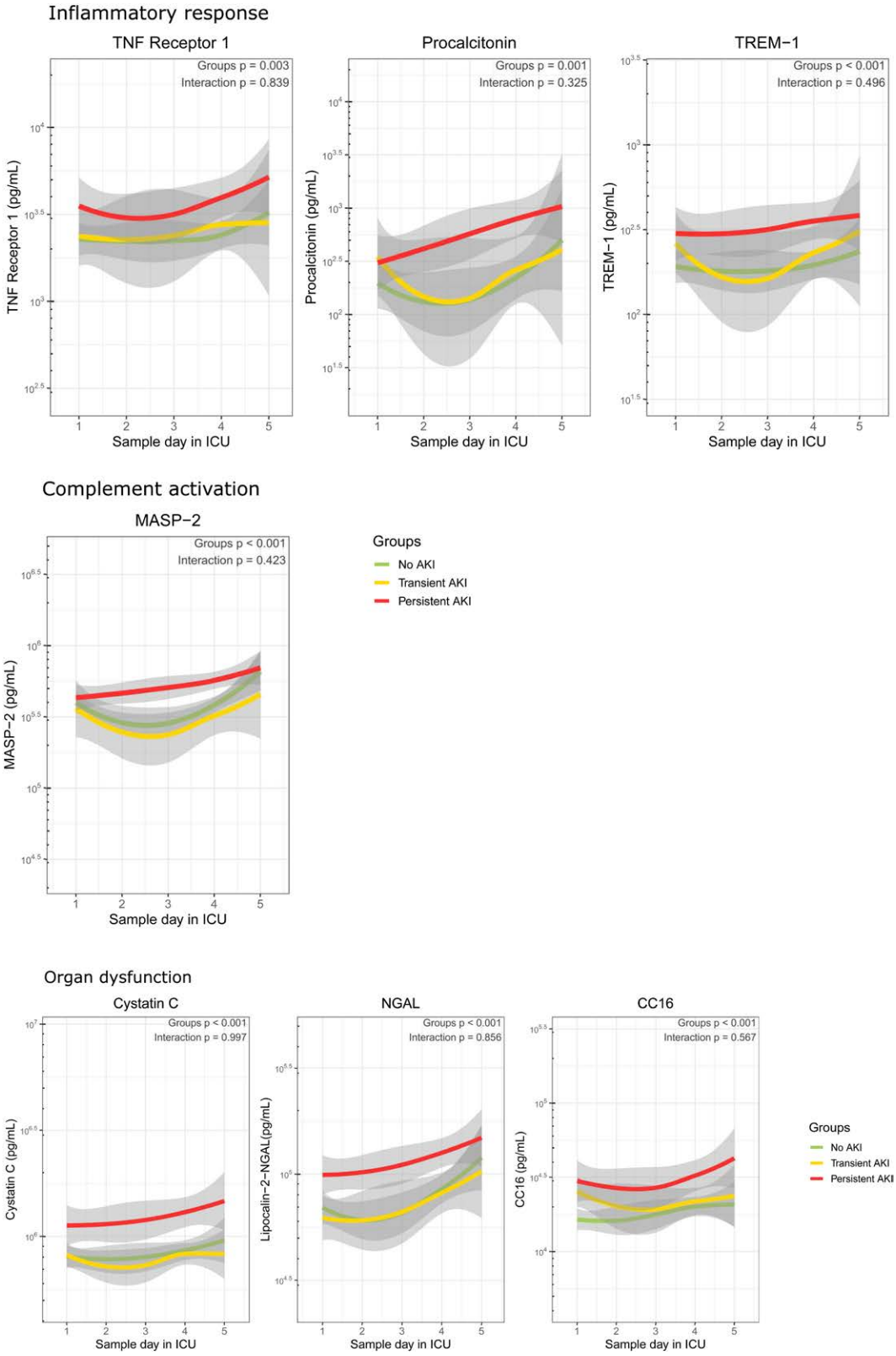

Figure 3. Seven biomarkers measured in the first 5 d after ICU admission, stratified into patients without acute kidney injury (AKI; in the first 48 hr), transient AKI, and persistent AKI. Day 1 represents ICU admission day. Overall, *p* values were derived from the linear mixed model, in which the group or the interaction of time × group (i.e., the trajectory) were defined as fixed effects and patient-specific intercept and slopes were defined as random effects. All 48 biomarker results were multiple testing adjusted with the Benjamini-Hochberg procedure. *Line plots* of observed biomarker values are shown with 95% CIs. CC16 = Clara cell secretory protein 16, NGAL = neutrophil gelatinase-associated lipocalin.

At baseline, before signs of kidney dysfunction were evident in either group, the group of patients who would later develop ICU-acquired AKI had higher plasma concentrations of IL-6 and renin compared with patients who would not develop AKI ($p = 0.011$ and $p = 0.036$, respectively; **Fig. 4*A***). Also at baseline, concentrations of IL-10, von Willebrand factor domain A2 (VWF-A2), and a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 were significantly lower in the ICU-AKI group ($p = 0.037$, $p = 0.041$, and $p = 0.020$, respectively). Interaction analyses, examining the relative change in biomarkers between the two groups from baseline until day 12, revealed that eleven biomarkers were increased in ICU-AKI patients compared with the no AKI group. These biomarkers were related to the inflammatory response pathways (IL-1 receptor antagonist, TREM-1, IL-18, TNF-R1), lung dysfunction (receptor for advanced glycation endproducts, CC16), and coagulation activation (tissue factor pathway inhibitor [TFPI], D-dimer, von Willebrand Factor A2 domain [VWF-A2], urokinase-type plasminogen activator; **Fig. 4**).

DISCUSSION

The purpose of this study was to provide insight into mechanisms implicated in COVID-AKI pathogenesis using sequential plasma protein biomarker analyses. Persistent AKI was associated with more severe kidney dysfunction and tubular damage at ICU admission, as reflected by plasma creatinine, NGAL, and cystatin C. Persistent AKI was also associated with stronger host-response anomalies in the inflammatory and pulmonary damage domains but not with endothelial dysfunction and coagulation activation. This suggests that inflammation is the most important mediator in the pathogenesis of COVID-AKI on the ICU. In the subgroup analysis of patients without AKI in the first 48 hours of admission, a stronger shift toward the pro-inflammatory IL-6 and away from the anti-inflammatory IL-10 cytokines was observed in patients that would later develop ICU-acquired AKI compared with those who would remain free of AKI. This shift supports the suggested importance of the role of the inflammatory domain in the pathogenesis of COVID-AKI and indicates insufficient counter-regulation of inflammation. For a more thorough appreciation of the relevance of host-response anomalies in COVID-AKI, we will discuss the results of the present study in order of each biomarker's potential place in the development of AKI.

Patients with persistent AKI exhibited more pronounced inflammatory host-response anomalies. In the persistent AKI group, levels of TNF-RI, TREM-1, procalcitonin, and MASP-2 were higher than in no AKI. MASP-2 was also higher in persistent AKI compared with transient AKI. Levels of TNF-R1, TREM-1, and procalcitonin were also relatively increased in the patients who developed ICU-acquired AKI compared with patients who never had AKI. Prior studies have linked TNF-receptor levels to AKI severity in sepsis (25) and COVID-19 (26, 27). Of interest, observations of a positive impact on COVID-19 mortality with TNF inhibition using infliximab have prompted some guidelines to suggest its consideration as an alternative immunomodulatory agent to baricitinib (Janus kinase 1/2 inhibitor) or tocilizumab (IL-6 inhibitor) (28, 29). TREM-1 receptors are present on immune and endothelial cells (30). Increased soluble TREM-1 (which also reflects the activation of cell-bound TREM-1 receptors) is associated with disease severity in viral pneumonia and with AKI development in sepsis and cardiothoracic surgery (31–34). Inhibition of endothelium-bound TREM-1 has been linked to improved clinical status and reduced mortality in COVID-19 (35). Murine studies suggest that TREM-1 pathway inhibition could protect against ischemia-reperfusion injury induced AKI (36), although this effect may be compound specific (37). Elevated procalcitonin might not only suggest bacterial co-infection in viral pneumonia (including COVID-19) but can also reflect disease severity (38, 39). In the present study, we also found that AKI duration corresponded with overall disease severity. These latter two findings fit in the hypothesis that kidney damage is aggravated by remote organ damage and are also in line with pathology findings, which show that the extent of lung injury corresponds to the extent of kidney injury in COVID-19 patients (40, 41). Elevated MASP-2 suggests a role for lectin pathway complement activation in COVID-AKI pathophysiology, which has also been suggested by earlier pathology findings (40).

In contrast with what is known of host-response profiles in SA-AKI (12), the data presented here do not show an association of anomalies in the coagulation and endothelial activation domains with COVID-AKI

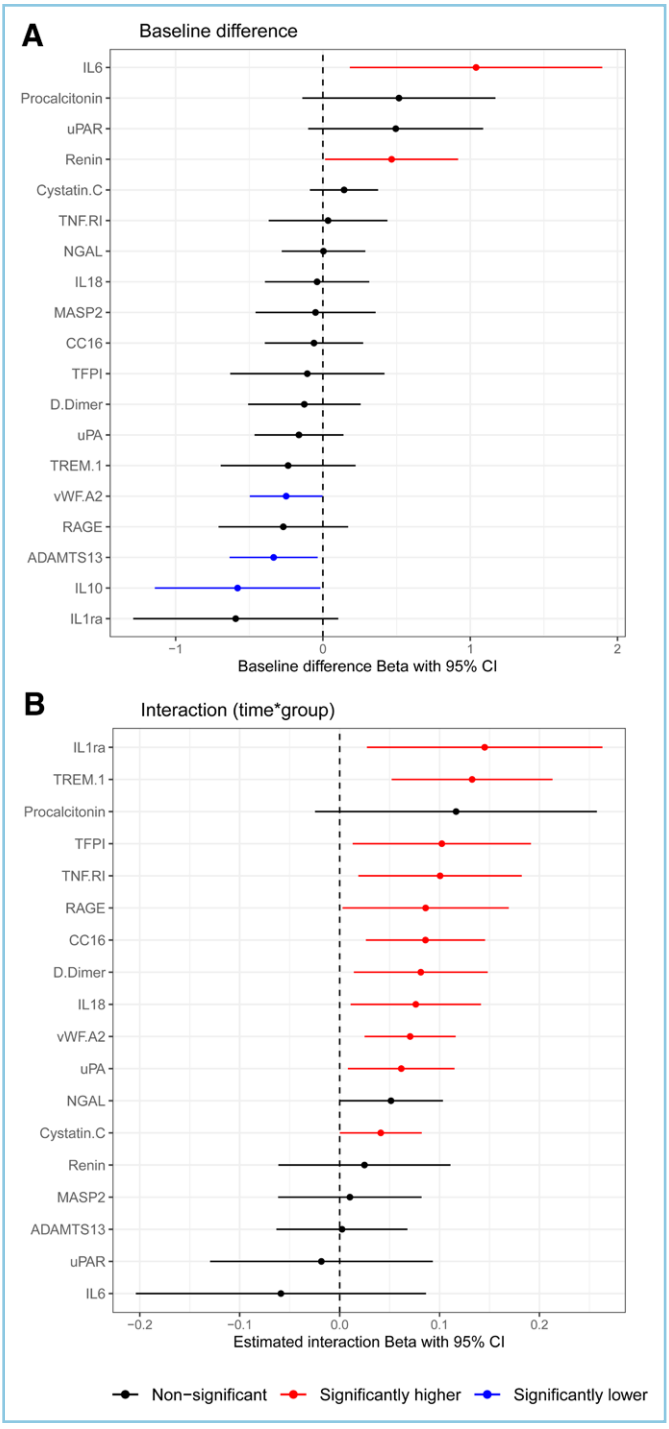

Figure 4. Mixed model analyses of sequential plasma concentrations of biomarkers in patients who did not have acute kidney injury (AKI) in the first 48 hr and were at risk of developing AKI after 48 hr. In this analysis, patients who developed ICU-acquired AKI ($n = 19$) were compared with patients who did not acquire AKI ($n = 12$). **A**, Baseline differences between patients who develop ICU-acquired AKI vs. who did not, while at this stage both groups had no sign of AKI. **B**, Interaction analyses between the two groups and time showing biomarkers increased relatively over time in patients who developed ICU-acquired AKI compared with patients who did not acquire AKI. Mixed model included time

duration. Despite evidence for the involvement of increased coagulation and endothelial dysfunction in COVID-19 associated lung injury (42) and the frequent suggestion of their involvement in the pathogenesis of COVID-19-associated AKI (6), findings of (micro-)thrombi in histopathologic evaluation of kidney tissue are rare rather than pathognomonic for AKI in COVID-19 (6, 8, 43). The marked increase of coagulation and endothelial cell activation markers in the ICU-acquired AKI group—which was not observed in the AKI at admission group—might suggest that ICU-acquired COVID-AKI shows stronger resemblance to SA-AKI than community and ward-acquired COVID-AKI (12). However, in the first 48 hours after ICU admission, absolute levels of endothelial and coagulation markers were lower in this group compared with the no-ICU-acquired group providing additional evidence for the hypothesis that COVID-AKI differs from SA-AKI.

Interestingly, the lung epithelial injury marker CC16 was highly associated with AKI severity, a persistent AKI course of admission AKI, and with the development of ICU-acquired AKI. CC16 is mainly produced and secreted by the club cells in the distal respiratory tract or terminal bronchioles (44). It is commonly used as a marker for acute respiratory distress syndrome (ARDS) (45, 46). However, its predictive capacity for ARDS is decreased in patients with impaired renal function (44). The latter emphasizes the association between impaired renal function and plasma CC16 clearance (47). In light of these previous data, we argue that the present findings of elevated levels of CC16 should be interpreted primarily as a reflection of diminished renal function rather than lung injury.

Figure 4. (*Continued*). (per day), group, and their interaction (group × time) as fixed effects, and patients specific intercept and linear slope of time as random effects. Models were not adjusted for other variables due to low sample size. Only significant estimates of biomarkers, either at baseline or interaction, are shown with $p < 0.05$. ADAMTS13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, CC16 = Clara cell secretory protein 16, IL = interleukin, MASP-2 = mannose-binding protein-associated serine protease 2, NGAL = neutrophil gelatinase-associated lipocalin, RAGE = receptor for advanced glycation endproducts, TFPI = tissue factor pathway inhibitor, TNF.R1 = tumor necrosis factor receptor 1, TREM.1 = triggering receptor expressed on myeloid cells 1, uPA = urokinase-type plasminogen activator, uPAR = urokinase-type plasminogen activator receptor, VWF.A2 = von Willebrand factor A2 domain.

This study was conducted in three large medical centers with complete registration of ICU stay characteristics. The registration procedure made it feasible to use both creatinine and urine output for the recognition and classification of AKI. These urine output criteria are used in only few studies, but are a critical addition to ensure accurate AKI recognition (48). As is often the case in the clinic, baseline creatinine values were mostly unavailable and needed to be estimated. The assumption of normal preadmission kidney function may have led to over-estimation of AKI occurrence rate. The study was carried out in the first wave of SARS-CoV-2 infections in the Netherlands. This timing is both a strength and a limitation to the study. The exceptionally long average admission duration and homogenous patient population on the ICU resulted in an unusually low number of dropout during the first week of follow-up and sample collection showed only limited between-patient variation. These factors might reduce the chance of type II error and selection bias often associated with small to moderate-size cohort studies. However, it should also be noted that there were differences between the biomarker cohort and the complete cohort, which may influence the missing at random assumption. The timing also provides unique insight into COVID-AKI unclouded by the use of medication that could alter the host-response (such as hydroxychloroquine, remdesivir, neutralizing SARS-CoV-2 monoclonal antibodies, corticosteroids, and IL-6 antagonists). The timing might, however, limit the translatability of our results because the extensive use of diuretics was common, which may have exacerbated the persistence of AKI. The use of immunosuppressive medication was also only adapted as a treatment strategy for COVID-19 after this study was completed (13, 49). Also, the dominant strain of the SARS-CoV-2 virus at the time of data collection differs from the most prevalent mutations at the time of writing (50). Additionally, the cohort of patients presenting with sequential biomarkers may be relatively small when compared with other large cohorts possessing renal data. Nevertheless, the completeness and low variability of the included dataset with sequential measurements enabled prospective investigation of patients who developed ICU-acquired AKI. This provides further understanding of COVID-AKI at various stages, despite the fact that these analyses may lack sufficient statistical power.

CONCLUSIONS

In this multicenter retrospective cohort study, COVID-AKI persistence was associated with induced inflammatory host-response parameters but not with host-response anomalies in coagulation and endothelium activation pathways. These findings could suggest that the COVID-AKI pathophysiologic process is distinct from that of SA-AKI. Additionally, future research should be carried out to establish CC16 as a suitable marker for the identification and follow-up on kidney dysfunction for COVID-AKI. Last, we underscore to potential of TREM-1 as a therapeutic target in this setting. This investigation offers deeper insights into the various stages of COVID-AKI, which may inform strategies aimed at mitigating its persistence.

ACKNOWLEDGMENTS

COVID-19 Biobank Study Group: Diederik van de Beek and Sanne de Bruin.

CovidPredict Study Group: Daisy Rusch, Suat Simsek, Kees Brinkman, Niels Gritters van den Oever, Joop van den Bergh, Hamza Moeniralam, N. Bokhizzou, Martijn de Kruif, Helen Leavis.

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Please see COVID-19 Biobank Study Group and COVID-19 Biobank Study Group in the Acknowledgements.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccejournal).

This study was funded by the Amsterdam University Medical Center (UMC), Amsterdam UMC Corona Research Fund, and personal grants from the Dutch Kidney Foundation (to Dr. Peters-Sengers [KOLFF grant number 19OK009] and Dr. Kers [KOLFF grant number 17OKG23]).

The authors have disclosed that they do not have any potential conflicts of interest.

Drs. Steenvoorden, de Kruijf, Vogt, and Peters-Sengers contributed equally to this study.

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The data underlying this article will not be made publicly available because of the privacy of the study participants. These data will be shared on reasonable request to the corresponding author.

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