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Evaluation of Plasma Biomarkers to Predict Major Adverse Kidney Events in Hospitalized Patients With COVID-19

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Rationale & Objective: Patients hospitalized with COVID-19 are at increased risk for major adverse kidney events (MAKE). We sought to identify plasma biomarkers predictive of MAKE in patients hospitalized with COVID-19.

Study Design: Prospective cohort study.

Setting & Participants: A total of 576 patients hospitalized with COVID-19 between March 2020 and January 2021 across 3 academic medical centers.

Exposure: Twenty-six plasma biomarkers of injury, inflammation, and repair from first available blood samples collected during hospitalization.

Outcome: MAKE, defined as KDIGO stage 3 acute kidney injury (AKI), dialysis-requiring AKI, or mortality up to 60 days.

Analytical Approach: Cox proportional hazards regression to associate biomarker level with MAKE. We additionally applied the least absolute shrinkage and selection operator (LASSO) and random forest regression for prediction modeling and estimated model discrimination with time-varying C index.

Results: The median length of stay for COVID-19 hospitalization was 9 (IQR, 5-16) days. In total, 95 patients (16%) experienced MAKE. Each 1 SD increase in soluble tumor necrosis factor receptor 1 (sTNFR1) and sTNFR2 was significantly associated with an increased risk of MAKE (adjusted HR [AHR], 2.30 [95% CI, 1.86-2.85], and AHR, 2.26 [95% CI, 1.73-2.95], respectively). The C index of sTNFR1 alone was 0.80 (95% CI, 0.78-0.84), and the C index of sTNFR2 was 0.81 (95% CI, 0.77-0.84). LASSO and random forest regression modeling using all biomarkers yielded C indexes of 0.86 (95% CI, 0.83-0.89) and 0.84 (95% CI, 0.78-0.91), respectively.

Limitations: No control group of hospitalized patients without COVID-19.

Conclusions: We found that sTNFR1 and sTNFR2 are independently associated with MAKE in patients hospitalized with COVID-19 and can both also serve as predictors for adverse kidney outcomes.

Visual Abstract online

Complete author and article information (including a list of the members of the TRIKIC Consortium) provided before references.

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The spread of severe acute respiratory SARS-CoV-2 infections has led to a global pandemic since the end of 2019.[1,2] Acute kidney injury (AKI), defined using Kidney Disease: Improving Global Outcomes (KDIGO) serum creatinine criteria, occurs in 30%-50% of hospitalized patients with COVID-19. Among patients with AKI, nearly 20% require dialysis.[3-6] Furthermore, clinical AKI during hospital admission has been associated with a greater need for intensive care unit admission (ICU),[7] worse short-term mortality, and adverse long-term outcomes.[8] Moreover, compared with AKI due to other causes, COVID-19–associated AKI may be associated with a greater long-term decline in kidney function.[9]

The pathogenesis of COVID-19–associated AKI is likely multifactorial, with contributions by endothelial dysfunction, coagulopathy, complement activation, systemic inflammation, and immune cell activation affecting the kidney.[10] Older age, male sex, diabetes mellitus, hypertension, obesity, and heart failure have all been associated with increased risk of AKI in the setting of COVID-19.[7,10] We previously showed that even after adjusting for such risk factors, patients with COVID-19 have a more than 40% higher risk of AKI compared with patients without COVID-19.[5] Further, the long-term impact of COVID-19 remains concerning, with numerous physiological and psychological effects reported.[11] Thus, there remains a need to identify patients at the greatest risk of developing adverse kidney outcomes after COVID-19 infection.

Biomarkers of kidney injury, inflammation, and repair may offer further insight beyond the current standard methods of characterizing COVID-19–associated AKI.[12-16] Plasma and urinary biomarkers have also been associated with COVID-19 disease severity.[17,18] We recently showed that biomarkers of injury and inflammation measured in the urine are significantly associated with stage 3 AKI, dialysis, or death up to 60 days from hospital admission for COVID-19.[19] Model discrimination using single biomarker values was moderate, with area under the receiver operating characteristic curve (AUC) levels approaching 0.80 at 60 days. A model combining the urine biomarkers epidermal growth factor (EGF) and neutrophil gelatinase-associated lipocalin (NGAL) yielded an AUC of 0.85.

PLAIN-LANGUAGE SUMMARY

Patients hospitalized with COVID-19 are at increased risk for long-term adverse health outcomes, but not all patients suffer long-term kidney dysfunction. Identification of patients with COVID-19 who are at high risk for adverse kidney events may have important implications in terms of nephrology follow-up and patient counseling. In this study, we found that the plasma biomarkers soluble tumor necrosis factor receptor 1 (sTNFR1) and sTNFR2 measured in hospitalized patients with COVID-19 were associated with a greater risk of adverse kidney outcomes. Along with clinical variables previously shown to predict adverse kidney events in patients with COVID-19, both sTNFR1 and sTNFR2 are also strong predictors of adverse kidney outcomes.

Based on our previous work in other clinical settings of AKI, we identified 26 candidate plasma biomarkers representing different biological pathways of injury, inflammation, and repair.[20-24] In this study, therefore, we evaluate the association of plasma biomarkers with major adverse kidney events (MAKE) and assess the predictive capability of top biomarkers. We hypothesized that plasma biomarkers would be strongly associated with MAKE in the setting of COVID-19 and have clinically significant predictive potential.

Methods

Study Design, Population, and Data Sources

We obtained patient data and biosamples for this study from 3 academic medical centers as part of the Translational Investigation of Kidney Disease in COVID-19 (TRIKIC) Consortium between March 2020 and January 2021: the Johns Hopkins Hospital in Baltimore, Maryland; Yale-New Haven Hospital in New Haven, Connecticut; and Mount Sinai Hospital in New York, New York. All 3 health care systems used similar institutional protocols to prospectively collect data and biosamples in hospitalized adult inpatients with COVID-19. At all 3 sites, adult patients with suspected COVID-19 were screened by polymerase chain reaction (PCR) testing; patients with confirmed COVID-19 were approached for enrollment in institutional protocols to prospectively collect samples during hospitalization, linked with outpatient follow-up data. Institutional review boards at each participating site of the TRIKIC Consortium approved the study, and written informed consent was obtained from all participants.

The baseline values for laboratory and clinical variables were defined as the values present before hospital admission. Inpatients with baseline serum creatinine ≥ 4 mg/dL were excluded. We additionally limited our cohort to patients who were hospitalized for at least 2 days and did not have prior end-stage kidney disease (ESKD) defined by *International Classification of Diseases* (ICD) diagnosis codes for ESKD and kidney transplant and an admission serum creatinine of ≥4 mg/dL. Baseline comorbidities were defined by mapping ICD-10 codes to the Elixhauser comorbidity index.[25] Laboratory values and vital signs on admission were defined as the first available values upon hospital presentation.

Sample Collection and Biomarker Measurement

Plasma samples were collected after a patient's admission to the hospital and with a confirmed COVID-19 test. Samples were collected following each institution's informed consent protocol. After collection, all samples were centrifuged promptly at 3,000*g* at 4°C and stored in multiple aliquots at −80°C until biomarker measurement. For each participant, we used the first available plasma sample before the onset of stage 3 AKI if more than 1 plasma sample was collected. Patients who had stage 3 AKI present before the time of plasma sample collection, such as patients with AKI on presentation to the emergency department or admission to the hospital, were excluded from our analysis.

We selected 26 plasma biomarkers as the primary exposure variables based on assay validity; previous work by our group and others have demonstrated significant associations between plasma biomarkers and AKI in various clinical settings.[26-30] These 26 plasma biomarkers include the following: tumor necrosis factor-α (TNF-α); soluble TNF receptor 1 and 2 (sTNFR1 and sTNFR2); angiopoietin 1 and 2 (ANG1 and ANG2); chitinase-like protein-1 (YKL-40); monocyte chemoattractant protein-1 (MCP-1); kidney injury molecule-1 (KIM-1); neutrophil gelatinase-associated lipocalin (NGAL); interleukin 1β (IL-1β), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and IL-18; soluble forms-like tyrosine kinase 1 (sFlt-1); basic fibroblast growth factor (bFGF); placental growth factor (PIGF); vascular endothelial growth factor A (VEGFA), VEGFC, and VEGFD; angiopoietin-1 receptor (TIE2); and interferon-γ (IFN-γ). All biomarkers were measured blinded, using the Meso Scale Discovery (MSD) platform after a single freeze-thaw cycle (Table S1).

Kidney Function Evaluation and Outcome Definitions

Estimated glomerular filtration rates (eGFRs) were calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[31] Baseline kidney function was defined using the median of all outpatient serum creatinine measurements 7 to 365 days before hospitalization. In patients without any available outpatient serum creatinine measurements, the minimum serum creatinine value obtained during the COVID-19 hospitalization was used as the baseline creatinine (n = 369; 64%), as described elsewhere.[32] Clinical AKI was defined as an increase of ≥50% in serum creatinine from baseline or dialysis at any time during the index hospitalization. AKI severity was classified

by modified KDIGO staging criteria on the basis of the peak serum creatinine level during the index hospitalization. The 60-day time period for primary outcome determination was chosen as a relevant window of time to assess intermediate outcomes, aligning with our previous work.[19]

The primary outcome of the study was a composite of KDIGO stage 3 AKI, dialysis, or death within 60 days of hospital admission, which together defined MAKE. Additional details are outlined in Item S1.

Statistical Analysis

We limited the analytic cohort to the subset of participants who had all 26 biomarkers measured at least once during hospitalization. We summarized descriptive characteristics using the mean ± standard deviation or median and interquartile range for continuous variables and frequency and percentage for categorical variables. All biomarkers were modeled continuously after $\log_2$ transformation. To explore the changes in biomarker levels over time, we used linear regression to calculate the slope of key biomarkers for each participant in the subset of participants with more than 1 plasma sample. World Health Organization (WHO) disease severity scale, as a marker of COVID-19 severity, was determined at the time of sample collection.[33] Patients who did not experience the primary outcome were censored at 60 days.

Cox proportional hazards regression was used to examine the association between plasma biomarker level and MAKE using the first available biomarker measurement for each participant. Model 1 included each individual $\log_2$-transformed biomarker alone. Model 2 was adjusted for demographic variables (age, sex, race), diabetes mellitus, obesity, hypertension, and baseline serum creatinine. Model 3 was further adjusted for WHO disease severity scale. Kolmogorov-type supremum tests were used to evaluate proportional hazards assumptions for all models. Hazard ratios (HRs) are reported per 1-SD increase for each $\log_2$-transformed biomarker.

To identify biomarker combinations, we used the end point of time to the development of MAKE within 60 days. We applied the least absolute shrinkage and selection operator (LASSO) to model the risk of MAKE by biomarker level for the 26 candidate plasma biomarkers. We used 5-fold cross-validation for optimal shrinkage parameter selection. We performed random forest analysis as an alternative approach for dimensionality reduction in evaluating key biomarkers associated with MAKE to allow for more flexibility in accounting for interaction, with the data randomly partitioned: 80% (n = 461) into a training dataset and 20% (n = 115) into a test dataset.

We evaluated model discrimination by calculating the time-varying C indexes.[34] We examined C indexes for each individual biomarker as well as several models including the LASSO and random forest model (on the test dataset). To identify the 2 highest performing biomarkers, we examined biomarkers with the highest individual C indexes, with non-0 coefficients in LASSO models and the top biomarkers in variable importance plots from the random forest model. For the 2 highest-performing biomarkers, we created models with key clinical demographics to assess for any additional improvement in model performance. These clinical variables included age, sex, and race/ethnicity; baseline diabetes, hypertension, and obesity; baseline serum creatinine; and WHO disease severity scale at the time of sample collection. We additionally added in C-reactive protein (CRP) to each model as a clinically available and widely measured inflammatory biomarker in patients with COVID-19. We evaluated receiver operating characteristic (ROC) curves at 60 days, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Based on these curves we determined optimal cutoff points for these biomarkers. Using the coefficients from the Cox model, we back-calculated the biomarker value for the optimal threshold.

In addition to analyses in the overall study population, we performed Cox proportional hazards regression and calculated C indexes in key subgroups, including in the subset of 499 of 576 patients (87%) with biosample collection occurring within the first week of admission, and in the 436 of 576 patients (76%) who did not receive steroids before biosample collection given the previously described association between steroid use and reduction in sTNFR1 and sTNFR2 levels.[35] Furthermore, we developed Cox models and calculated model discrimination for the outcome of stage 3 AKI or dialysis. The 38 patients who died without experiencing either kidney outcome were censored at the time of death.

To determine whether the association between biomarkers and outcomes was modified by patient comorbidities, we examined models with an interaction term between the biomarker and covariate of interest. P of the interaction term < 0.1 were considered statistically significant. Hazard ratios at each covariate value were calculated.

Because the timing of the plasma sample collection was not consistent across patients, we completed a supplementary analysis where time 0 was defined as the time of sample collection. The imputation strategy for patients without a baseline outpatient serum creatinine measurement was modified to use the lowest serum creatinine before the time of sample collection as the baseline measure. Hazard ratios for individual biomarkers were calculated using the Cox models as defined for the primary analysis.

All analyses were performed in SAS (version 9.4; SAS Institute) and R (version 3.1.2; R Foundation for Statistical Computing). All tests of statistical significance were 2-sided, with $P < 0.05$ considered significant.

Table 1. Inpatient Characteristics of Patients With COVID-19

Variable	Overall (N = 576)	Hopkins (N = 182)	Mt Sinai (N = 291)	Yale (N = 103)
Age, y	60.34 ± 16.2	55.25 ±15.82	62.88 ±15.9	62.14 ±15.72
Female	243 (42%)	82 (45%)	116 (40%)	45 (44%)
Black race	171 (30%)	90 (49%)	48 (16%)	33 (32%)
Diabetes	203 (35%)	90 (49%)	70 (24%)	41 (40%)
Obesity	168 (29%)	104 (57%)	32 (11%)	32 (31%)
Hypertension	293 (51%)	121 (66%)	106 (36%)	66 (64%)
Length of hospital stay (d)	9 [5-16]	9 [5-17]	8 [5-15]	11 [7-19]
WHO disease severity scale–maximum				
3	84 (16%)	31 (17%)	52 (18%)	1 (2%)
4	264 (49%)	88 (48%)	135 (46%)	41 (64%)
5	64 (12%)	22 (12%)	34 (12%)	8 (13%)
6	3 (1%)	—	3 (1%)	—
7	75 (14%)	26 (14%)	39 (13%)	10 (16%)
8	47 (9%)	15 (8%)	28 (10%)	4 (6%)
Admission CRP (mg/dL)	83.9 [35.9-163.6]	60.7 [25.3-111.3]	101.7 [46.3-189.6]	85 [39.8-146]
Serum creatinine				
No. of values measured	9 [5-16]	8 [4-14]	9 [5-15]	10 [7-22]
Baseline, mg/dL	0.87 ± 0.45	0.83 ± 0.38	0.88 ± 0.49	0.90 ± 0.46
Admission, mg/dL	1.23 ± 1.11	1.09 ± 0.53	1.34 ± 1.4	1.15 ± 0.85
Discharge, mg/dL	1.14 ± 1.08	0.96 ± 0.51	1.28 ± 1.31	1.07 ± 1.02
Baseline eGFR (mL/min/1.73 m^2)[a,31]	77 (30%)	83 (31%)	78 (30%)	65 (25%)
Baseline CKD[a]	62 (30%)	11 (21%)	30 (26%)	21 (54%)
AKI stage 3, any dialysis, death within 60 d	95 (16%)	15 (8%)	66 (23%)	14 (14%)
No. of days to MAKE	13 [9-24]			
Dialysis requirement	31 (5%)	4 (2%)	22 (8%)	5 (5%)
Time to dialysis (d)	14 [9-22]	11 [8-24.5]	15 [12-22]	14 [13-14]
Death within 60 d	69 (12%)	13 (7%)	46 (16%)	10 (10%)
Time to death (d)	18 [10-27]	26 [15-32]	13 [9-25]	22.5 [15-35]
KDIGO AKI stage				
1	102 (18%)	45 (25%)	46 (16%)	11 (11%)
2	65 (11%)	18 (10%)	38 (13%)	9 (9%)
3	57 (10%)	7 (4%)	41 (14%)	9 (9%)
Days to COVID-19–associated AKI	5 [0-12]	3 [1-10]	5 [0-11]	7 [1-15]
Biosample collection from time of admission				
Within 72 hours (3 d)	385 (67%)	99 (17%)	235 (41%)	51 (13%)
4-7 d	114 (20%)	40 (7%)	38 (7%)	36 (6%)
After first week	77 (13%)	43 (7%)	18 (3%)	16 (3%)
Medication use before biomarker measurement				
Tocilizumab	61 (11%)	7 (4%)	3 (1%)	51 (50%)
Remdesivir	125 (22%)	40 (22%)	50 (17%)	35 (34%)
Hydroxychloroquine	196 (34%)	25 (14%)	111 (38%)	60 (58%)
Steroid[b]	140 (24%)	45 (25%)	65 (22%)	30 (29%)

Values are reported as mean ± SD, n (%), or median [IQR]. Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; KDIGO, Kidney Disease: Improving Global Outcomes; MAKE, major adverse kidney events; WHO, World Health Organization.
[a]Baseline eGFR and CKD (defined as eGFR ≤ 60) is reported for the 206 patients with baseline serum creatinine available before the time of admission.
[b]Steroid is any administration of dexamethasone, methylprednisolone, or hydrocortisone.

Results

Study Population

We included samples from a total of 576 patients during hospitalization for COVID-19 after applying inclusion and exclusion criteria (Fig S1). The mean patient age on admission was 60.3 ± 16.2 years, and 243 (42%) were women. The mean baseline serum creatinine was 0.87 ± 0.5 mg/dL, and the mean admission serum creatinine was 1.23 ± 1.1 mg/dL (Table 1). Of the 576 patients, 203 (35%) had diabetes mellitus, 293 (51%) had hypertension, and 168 (29%) had obesity, with a BMI ≥ 30 kg/m^2. A total of 62 patients (30%) had baseline chronic kidney disease (CKD), defined by an estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2. The median length of a COVID-19 hospitalization in this study was 9

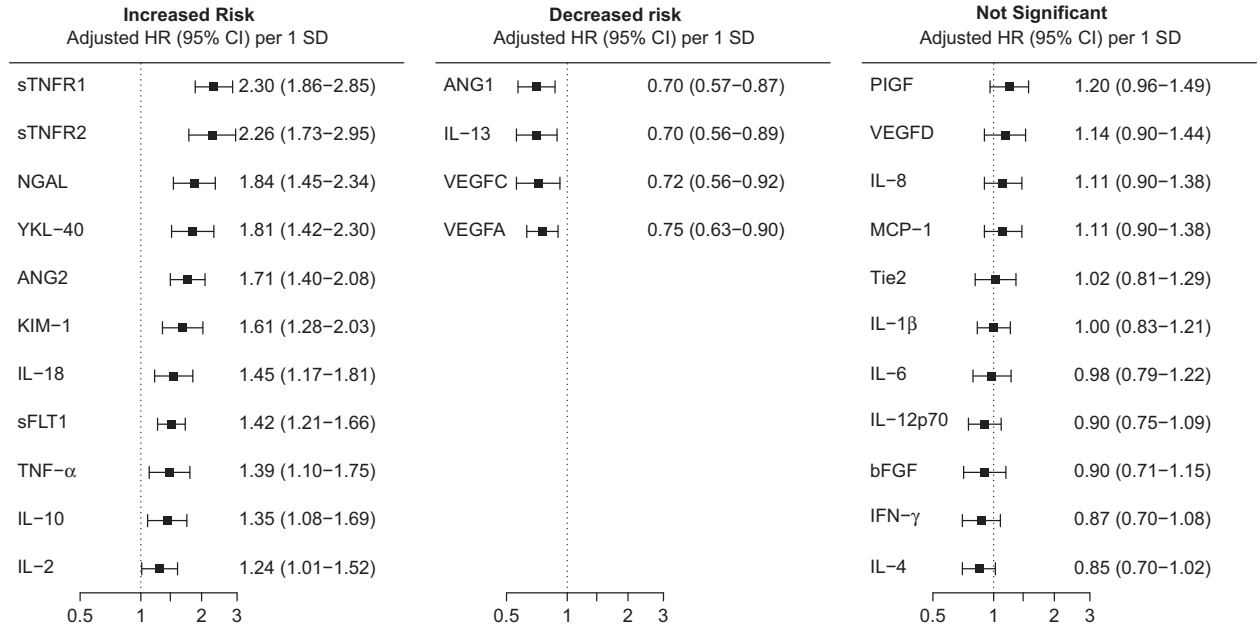

Figure 1. Risk of MAKE by 1-SD increase in plasma biomarker level in the fully adjusted analysis. MAKE is defined as having at least stage 3 AKI, dialysis, or death within 60 days of hospital admission with a confirmed COVID-19 test. Four biomarkers (ANG1, VEGFA, VEGFC, and IL-13) were associated with lower risk of MAKE, and 11 biomarkers were associated with higher risk of MAKE. The remaining 11 were not significantly associated with risk of MAKE. *Fully adjusted for the following: demographics (age, sex, race), diabetes, obesity, hypertension, baseline serum creatinine, and World Health Organization (WHO) disease severity scale (reported as Model 3 in Table S2). Abbreviations: ANG, angiopoietin; bFGF, basic fibroblast growth factor; IFN-γ, interferon γ; IL, interleukin; KIM-1, kidney injury molecule-1; MAKE, major adverse kidney events; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; sTNFR, soluble tumor necrosis factor receptor; TIE2, angiopoietin-1 receptor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; YKL-40, chitinase-like protein-1.

(IQR, 5-16) days. In total, 95 (16%) patients experienced the primary outcome of MAKE. Of these 95 patients, 57 (60%) developed stage 3 AKI, 31 (33%) required dialysis, and 69 (73%) died during the study period. A total of 125 (22%) patients received remdesivir, and 140 (24%) received steroids before biomarker measurement. The majority (67%) of biosamples were collected within 72 hours of hospital admission, with a smaller percentage collected between 4 and 7 days and later in admission.

Associations of Biomarkers With the Primary Outcome

Figure 1 (Table S2) highlights the risk of MAKE by individual biomarker level, using biomarkers measured from the first available plasma sample during admission. In the fully adjusted analysis, 15 of 26 candidate biomarkers were significantly associated with MAKE, 11 with increased risk, and 4 with decreased risk. Each 1-SD increase in $\log_2$-transformed ANG1, IL-13, VEGFA, and VEGFC was significantly associated with a lower risk of MAKE after adjustment for clinical covariates. Of the 11 biomarkers associated with increased risk of MAKE, each 1-SD increase in either sTNFR1 (adjusted HR [AHR], 2.30 [95% CI, 1.86-2.85]) or sTNFR2 (AHR, 2.26 [95% CI, 1.73-2.95]) was associated with a greater than 2-fold higher risk of 60-day MAKE.

In the subgroup analyses, we saw largely similar results in the subset of 499 patients whose biosample collection occurred within the first week of admission, with 73 events observed (Table S3). In the subset of 436 patients who did not receive steroids before biosample collection, we observed 73 events, with sTNFR1 and sTNFR2 still most strongly associated with risk of MAKE (Table S4).

In an additional secondary analysis limiting the outcome to stage 3 AKI or dialysis, sTNFR1 (AHR, 2.98 [95% CI, 2.31-3.84]), sTNFR2 (AHR, 3.35 [95% CI, 2.35-4.77]), and NGAL (AHR, 2.34 [95% CI, 1.70-3.24]) were still most strongly associated with greatest risk (Table S5). Figure S2 highlights sTNFR1 and sTNFR2 levels relative to the number of days from sample collection to the event for those that experienced MAKE. Similar trends were noted for other biomarkers based on time to MAKE (Fig S3). In a supplementary analysis on the subset of 70 participants with more than 1 plasma biomarker measurement before the onset of stage 3 AKI, both sTNFR1 and sTNFR2 increased in patients who went on to develop MAKE compared with those who did not (Table S6). Redefining time 0 to the time of plasma sample collection resulted in a

Table 2. Individual Biomarker Model Discrimination

Direction of Association[a]	Biomarker	C Index (95% CI)
Increased risk	sTNFR2	0.81 (0.77-0.84)
	sTNFR1	0.80 (0.78-0.84)
	YKL-40	0.78 (0.74-0.82)
	NGAL	0.77 (0.72-0.81)
	ANG2	0.73 (0.69-0.79)
	KIM-1	0.73 (0.69-0.77)
	sFLT-1	0.69 (0.65-0.74)
	IL-18	0.68 (0.63-0.73)
	TNF-α	0.67 (0.63-0.71)
	IL-10	0.63 (0.58-0.68)
	IL-2	0.63 (0.57-0.68)
Decreased risk	ANG1	0.61 (0.56-0.66)
	VEGFC	0.56 (0.51-0.62)
	VEGFA	0.55 (0.50-0.62)
	IL-13	0.52 (0.50-0.58)
Not statistically significant	PIGF	0.66 (0.60-0.72)
	IL-6	0.64 (0.60-0.69)
	IL-8	0.63 (0.58-0.67)
	MCP-1	0.63 (0.59-0.68)
	VEGFD	0.62 (0.56-0.68)
	IL-1β	0.59 (0.53-0.64)
	IL-12p70	0.56 (0.51-0.61)
	IFN-γ	0.55 (0.50-0.62)
	IL-4	0.55 (0.51-0.59)
	Tie2	0.51 (0.50-0.59)
	bFGF	0.51 (0.50-0.57)

Taking into account time to event among 576 patients. Abbreviations: ANG, angiopoietin; bFGF, basic fibroblast growth factor; IFN-γ, interferon γ; IL, interleukin; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; sTNFR, soluble tumor necrosis factor receptor; TIE2, angiopoietin-1 receptor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; YKL-40, chitinase-like protein-1.
[a]Direction of association is based on the hazard ratio of the biomarker adjusted for demographics (age, sex, race, diabetes, obesity, hypertension), baseline serum creatinine, and World Health Organization disease severity scale, as presented in Figure 1.

slightly lower rate of 91 MAKE events out of 575 patients included in this sensitivity analysis. Biomarker associations with MAKE remained largely unchanged (Table S7).

Discrimination of Biomarkers With Outcomes

Table 2 displays the results of discrimination for individual biomarkers using the first available measurement for each. Notably, sTNFR1 and sTNFR2 had the 2 highest C index values of 0.80 (95% CI, 0.78-0.84) and 0.81 (95% CI, 0.77-0.84), respectively. Table S8 demonstrated individual biomarker discrimination for our key secondary analyses including the subgroups of patients with biosample collection with 7 days of hospital admission, patients without steroid exposure before biosample collection, and in the overall patient population limiting the outcome to stage 3 AKI and dialysis.

Using LASSO, 20 of 26 biomarkers were found to have non-0 coefficients and were included in the final model: IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12p70, IL-13, IL-18, TNF-α, TIE2, PIGF, bFGF, sFLT1, NGAL, MCP-1, YKL-40, ANG1, ANG2, and sTNFR1. This combined model yielded a C index of 0.86 (95% CI, 0.83-0.89) (Table 3). As an alternative, complementary approach, we performed random forest regression using the first biomarker measurements of all 26 plasma biomarkers, which yielded a similar C index of 0.84 (95% CI, 0.78-0.91) in the validation dataset. Several biomarkers were found to be significant predictors for MAKE, as determined through both LASSO and random forest analysis, with sTNFR1 and sTNFR2 being among the top predictors (Fig 2).

A clinical prediction model that contained 8 variables based on known risk factors for adverse outcomes yielded a C index of 0.82 (95% CI, 0.76-0.88), similar to the C indexes of sTNFR1 and sTNFR2 alone (Table 3).[36,37] The addition of sTNFR1 to this 8-variable clinical model improved the C index to 0.86 (95% CI, 0.81-0.92), similar to the LASSO and random forest regression models that utilized all 26 plasma biomarkers. Inclusion of CRP, as a marker of general inflammation and widely measured in patients with COVID-19, did not appreciably improve model discrimination of either the clinical model but did yield a higher C index to the biomarker-enriched models, particularly to a model containing clinical variables, sTNFR1, and CRP (C index, 0.87 [95% CI, 0.81-0.92]).

A concentration of sTNFR1 at the optimal cutoff of 2,130 pg/mL, based on the ROC curve at 60 days, had a specificity of 65%, sensitivity of 70%, PPV of 30%, and most notably a NPV of 92% for the time to MAKE (Table S9). A concentration of sTNFR2 at the optimal cutoff of 14,670 pg/mL had a specificity of 74%, sensitivity of 70%, PPV of 35%, and NPV of 94%. There was no significant interaction on the association between sTNFR1 or sTNFR2 and MAKE by age, sex, race, diabetes, CKD (eGFR $<$ 60 vs $\geq$ 60), or obesity status (Fig S4).

Discussion

In this study, we demonstrated that 15 of 26 candidate plasma biomarkers were associated with MAKE up to 60 days from hospital admission, with each SD elevation in levels of sTNFR1 and sTNFR2 associated with an over 2-fold higher risk of MAKE. Through the complementary LASSO and random forest regression techniques using all 26 plasma biomarkers, we found that combination models yielded C indexes of 0.86 and 0.84. The biomarkers sTNFR1 or sTNFR2 were found to be the strongest individual biomarkers predictive of MAKE and in combination with clinical variables yielded C indexes of 0.85 and 0.86.

TNFR1 and TNFR2 are cell-surface receptors that bind TNF-α and are important in mediating inflammatory responses. Downstream signaling through the TNF-α pathway is mediated through TNFR1 and TNFR2, and includes activation of the transcription factors nuclear factor κB (NF-κB) and Jun N-terminal kinase (JNK).[38] Of note, the measured levels of sTNFR1 and sTNFR2 reflect the soluble forms of these receptors, in contrast to being

Table 3. Combination Biomarker-Clinical Model Discrimination

Model	C Index (95% CI)
LASSO (20 biomarkers)[a]	0.86 (0.83-0.89)
Random forest (26 biomarkers)[b]	0.84 (0.78-0.91)
Clinical model[c]	0.82 (0.76-0.88)
Clinical model + sTNFR1[c]	0.86 (0.81-0.92)
Clinical model + sTNFR2[c]	0.85 (0.79-0.91)
Clinical model + admission CRP[c]	0.82 (0.77-0.88)
Clinical model + sTNFR1 + admission CRP[c]	0.87 (0.81-0.92)
Clinical model + sTNFR2 + admission CRP[c]	0.85 (0.79-0.91)

Abbreviations: CRP, C-reactive protein; LASSO, least absolute shrinkage and selection operator; sTNFR, soluble tumor necrosis factor receptor.
[a]LASSO model had 20 biomarkers with nonzero coefficients.
[b]Random forest includes all 26 biomarkers.
[c]Clinical model comprises 8 variables: age; sex; race; baseline serum creatinine; history of diabetes, hypertension, or obesity; and World Health Organization disease severity score at time of sample collection.

bound to cell membrane.[39] These soluble forms may result from either cleavage of the cell surface receptor or from the release of full-length sTNFR1 via exosome-like vesicles.[40,41] However, the mechanistic relationship between elevated plasma sTNFR1 and adverse outcomes remains unclear. Xu et al[42] demonstrated that sTNFR1 plays a key role in TNF-mediated damage to glomerular epithelium in a mouse model of sepsis-associated AKI. Specifically, they showed that knockout mice deficient in sTNFR1 were resistant to lipopolysaccharide-induced AKI, whereas wild-type mice suffered significant kidney injury.

Elevations in sTNFR1 and sTNFR2 have been associated with progression of kidney disease in a number of clinical settings.[40,43] In 2012 White et al[44] demonstrated that sTNFR1 is implicated in cellular apoptosis in the pulmonary microvasculature, noted on bronchoalveolar lavage analysis, in the setting of ischemic AKI. Both sTNFR1 and sTNFR2 predict CKD and ESKD in the setting of types 1 and 2 diabetes mellitus.[45,46] Furthermore, sTNFR1 was shown to be a strong prognostic factor for all-cause mortality in patients with CKD and type 2 diabetes mellitus.[39]

Our study adds to the growing body of literature evaluating the role of sTNFR1 and sTNFR2 as prognostic biomarkers in patients with COVID-19. Sancho Ferrando et al[47] demonstrated that in the acute setting, sTNFR1 and sTNFR2 are associated with AKI, with levels trending significantly higher by AKI stage. Additionally these authors showed that sTNFR1 was associated with 30-day mortality in critically ill hospitalized patients with COVID-19.[47] Our study expands on these findings, evaluating the need for dialysis in our MAKE outcome with outcome ascertainment over a longer time period of 60 days. Notably, discrimination in a model containing CRP along with the 8 clinical variables was not as strong as a clinical model containing either sTNFR1 or sTNFR2. While CRP is a marker of general inflammation, sTNFR1 and sTNFR2 have been more strongly associated with inflammation in the kidney and may help explain this difference.[48]

Although the focus of this investigation centered on hospitalized patients with COVID-19, our study adds to the existing literature of sTNFR1 as a marker of MAKE after hospitalized AKI in the general setting. A recent investigation using data from the AKI Risk in Derby Study has shown that sTNFR1 and sTNFR2 measured after discharge are strong predictors of CKD progression up to 3 years after hospitalization for AKI.[49] Similarly, sTNFR1 and sTNFR2 were found to be significantly associated with long-term CKD and mortality in the Assessment, Serial Evaluation, and Subsequent Sequelae in Acute Kidney Injury Study.[50] Furthermore, among patients with existing CKD, hospitalized AKI has been associated with increases in sTNFR1 and sTNFR2 even several months after hospital discharge.[51] A number of experimental models have demonstrated that antibodies against sTNFR1 may in fact reduce chronic inflammation and tissue injury.[52,53] Given their role in the body's response to inflammation, elevations in sTNFR1 and sTNFR2 in the setting of COVID-19 may serve as markers of disease severity, with prolonged inflammation leading to worse kidney outcomes.

Our results have implications both for future research and clinical application. The ultimate goal of biomarker-based research is to improve the timely diagnosis and management of patients. Although a model containing 8 clinical variables did perform well in prediction of MAKE in our analysis, at the individual level, this may be difficult to implement given the lack of availability of all necessary variables, including baseline kidney function. On the other hand, the robust performance of sTNFR1 can be leveraged for early clinical prediction and timely management of patients who are likely to have poor outcomes. Our proposed cutoffs for sTNFR1 and sTNFR2 of 2,130 pg/mL and 14,670 pg/mL had high NPVs of 92% and 94% for MAKE over 60 days respectively, largely excluding the outcome in our cohort at concentrations below the cutoff.

Future studies can investigate sTNFR1 as a "rule-out" test with potential early discharge from ICU and hospital if the patient is improving clinically. After identification of high-risk patients—that is, patients above the cutoff of 2,130 pg/mL—the goal of management would be to slow or prevent progression of CKD through meaningful post-discharge nephrology care. There has been increasing interest in evaluating the AKI-to-CKD transition, with interventions to help slow or prevent this process. The use of an sTNFR1 cutoff may offer significant contribution in such risk stratification. Although our current study was conducted exclusively in patients with COVID-19–associated AKI and in other clinical settings, an sTNFR1 cutoff for risk stratification should be explored more broadly.[54,55]

The strengths of our study include the use of a large and diverse patient population from 3 academic medical centers, allowing for improved generalizability of our study findings. We also validated our discriminatory findings with high C indexes for a combined biomarker model generated through LASSO regression using the

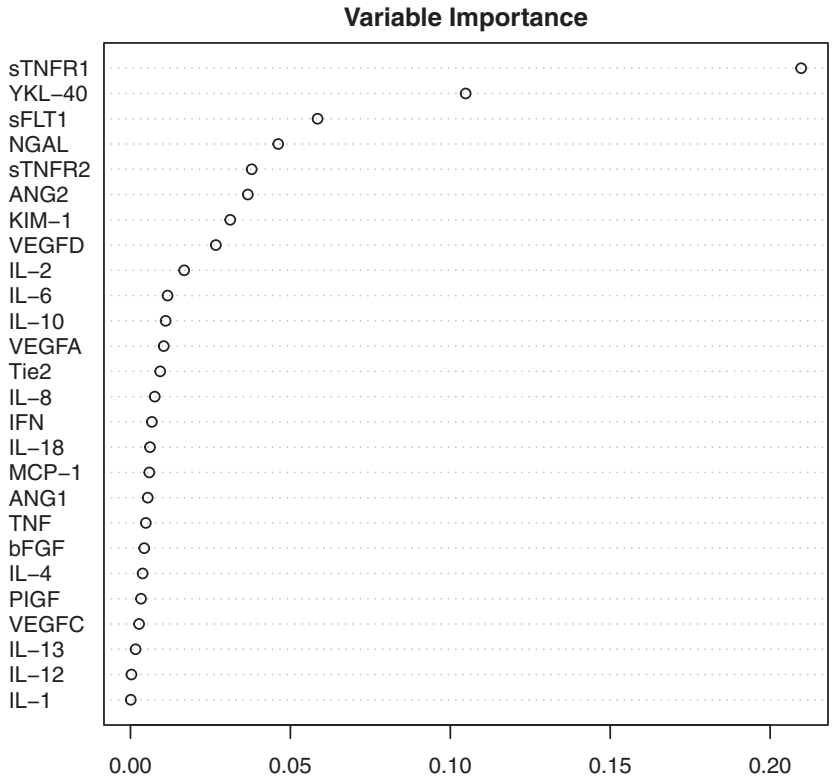

Figure 2. Variable importance in risk prediction using random forest regression, yielding an overall C index of 0.84 (95% CI, 0.78-0.91). Abbreviations: ANG, angiopoietin; bFGF, basic fibroblast growth factor; IFN-γ, interferon γ; IL, interleukin; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; NGAL, neutrophil gelatinase-associated lipocalin; PIGF, placental growth factor; sFlt-1, soluble fms-like tyrosine kinase 1; sTNFR, soluble tumor necrosis factor receptor; TIE2, angiopoietin-1 receptor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; YKL-40, chitinase-like protein-1.

complementary approach of random forest. Given the predictive power of sTNFR1 alone, taking the first biomarker measurement available after admission may be a feasible approach to improve risk stratification of patients for future risk of adverse kidney outcomes. We have also identified a number of other plasma biomarkers including sTNFR2 that are strongly associated with MAKE and require further investigation. Our use of the agnostic LASSO approach for variable selection did not in fact identify sTNFR2 as having a non-0 coefficient, though this is likely due to its high collinearity with sTNFR1.

This study has several limitations. We previously showed that urinary biomarker measurements are robust to variations in sample collection, processing, and storage, though this is as yet unproven for plasma biomarkers.[56] Despite standardized protocols, it is possible that the samples had differential handling across sites during the COVID-19 epidemic. Other biomarkers such as soluble urokinase plasminogen activator receptor (suPAR) and cystatin-C have been associated with COVID-19–associated AKI but were not clinically available in this cohort; such associations were not known at the time of our biomarker measurement, and we did not have additional samples available at all sites to perform these additional biomarker measurements. Additionally, although this study includes data on over 500 patients with nearly 100 MAKE events, it is not sufficiently powered to separately evaluate the individual outcomes of KDIGO stage 3 AKI, dialysis, or death. Although we accounted for COVID-19 disease severity by adjusting for WHO disease severity score, this may not account for other unmeasured potentially confounding comorbidities. Similarly, we explored patient cohorts across 3 academic medical centers, but we did not externally validate our findings in a separate, larger patient cohort. The development of new therapies against SARS-CoV-2 infection may modify these associations and overall generalizability over time. Similarly, the disease severity of COVID-19 has varied over time, with the emergence of new variants that may differentially impact kidney outcomes, which we were not able to assess in this study.

In summary, increased plasma concentrations of sTNFR1 and sTNFR2 are each independently and strongly associated with MAKE in patients hospitalized with COVID-19. In particular, combining clinical variables with either sTNFR1 or sTNFR2 has very strong discrimination for predicting MAKE, and further studies should confirm these findings in COVID-19 and other hospitalized clinical settings to identify high-risk patients. These results support that those with severe disease need postdischarge care and longer follow-up studies in a larger population are

necessary to understand the full spectrum of health consequences from COVID-19.

Supplementary Material

Supplementary File (PDF)

Figure S1: CONSORT flow diagram.

Figure S2: Measurement of sTNFR1 (A) and sTNFR2 (B) in relationship to MAKE.

Figure S3: Measurement of select biomarkers relative to time of MAKE.

Figure S4: Adjusted HRs stratified by covariates for sTNFR1 and sTNFR2.

Item S1: Supplemental methods.

Table S1: Biomarker measurement details, quality control.

Table S2: Risk of major adverse kidney events by 1-SD increase in plasma biomarker level.

Table S3: Risk of major adverse kidney events by 1-SD increase in plasma biomarker level, among patients with biosample collection occurring within the first week of hospital admission.

Table S4: Risk of major adverse kidney events by 1-SD increase in plasma biomarker level, among patients receiving steroids before biosample collection.

Table S5: Risk of stage 3 AKI or dialysis by 1-SD increase in plasma biomarker level.

Table S6: Change in biomarker level per day among 70 patients with more than 1 biomarker measurement available by MAKE status.

Table S7: Risk of MAKE when time 0 is set to the time of plasma sample collection and imputation of baseline creatinine is before the time of sample collection.

Table S8: Individual biomarker model discrimination, using time-varying C index.

Table S9: Receiver operating characteristic curve values at 60 days for top individual biomarkers.

Article Information

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Evaluation of Plasma Biomarkers to Predict Major Adverse Kidney Events in Hospitalized Patients With COVID-19

Setting and Participants

Multicenter prospective cohort study

N = 576 patients admitted with COVID-19

March 2020-January 2021

Analysis

Exposures
26 plasma biomarkers

Outcomes
Major adverse kidney events (MAKE) up to 60 days from admission:

- KDIGO stage 3 AKI
- Dialysis-requiring AKI
- Mortality

Analytic Approach

- Cox proportional hazards regression
- LASSO and random forest regression
- Discrimination with time-varying C-indices

Results

Model Discrimination	C-index (95% CI)
sTNFR1	0.80 (0.78-0.84)
sTNFR2	0.81 (0.77-0.84)
LASSO (20 biomarkers)	0.86 (0.83-0.89)
Random forest (26 biomarkers)	0.84 (0.78-0.91)
Clinical model*	0.82 (0.76-0.88)
Clinical model* + sTNFR1	0.86 (0.81-0.92)
Clinical model* + sTNFR2	0.85 (0.79-0.91)

*Clinical model comprises 8 variables: age, sex, race, baseline serum creatinine, history of diabetes, hypertension or obesity, and WHO disease severity score at time of sample collection

CONCLUSION: sTNFR1 and sTNFR2 are independently associated with MAKE in patients with COVID-19, and can both also serve as predictors for adverse kidney outcomes.

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