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Plasma Soluble CD14 Subtype Levels Are Associated With Clinical Outcomes in Critically Ill Subjects With Coronavirus Disease 2019

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IMPORTANCE: In bacterial sepsis, CD14 and its N-terminal fragment (soluble CD14 subtype, "Presepsin") have been characterized as markers of innate immune responses and emerging evidence has linked both to coronavirus disease 2019 pathophysiology.

OBJECTIVES: Our aim was to determine the relationship between the soluble form of CD14 and soluble CD14 subtype plasma levels, coronavirus disease 2019 status, and coronavirus disease 2019-related outcomes.

DESIGN: A prospective cohort study.

SETTING: ICUs in three tertiary hospitals in Seattle, WA.

PARTICIPANTS: Two-hundred four critically ill patients under investigation for coronavirus disease 2019.

MAIN OUTCOMES AND MEASURES: We measured plasma soluble CD14 and soluble CD14 subtype levels in samples collected upon admission. We tested for associations between biomarker levels and coronavirus disease 2019 status. We stratified by coronavirus disease 2019 status and tested for associations between biomarker levels and outcomes.

RESULTS: Among 204 patients, 102 patients had coronavirus disease 2019 and 102 patients did not. In both groups, the most common ICU admission diagnosis was respiratory failure or pneumonia and proportions receiving respiratory support at admission were similar. In regression analyses adjusting for age, sex, race/ethnicity, steroid therapy, comorbidities, and severity of illness, soluble CD14 subtype was 54% lower in coronavirus disease 2019 than noncoronavirus disease 2019 patients (fold difference, 0.46; 95% CI, 0.28–0.77; $p = 0.003$). In contrast to soluble CD14 subtype, soluble CD14 levels did not differ between coronavirus disease 2019 and noncoronavirus disease 2019 patients. In both coronavirus disease 2019 and noncoronavirus disease 2019, in analyses adjusting for age, sex, race/ethnicity, steroid therapy, and comorbidities, higher soluble CD14 subtype levels were associated with death (coronavirus disease 2019: adjusted relative risk, 1.21; 95% CI, 1.06–1.39; $p = 0.006$ and noncoronavirus disease 2019: adjusted relative risk, 1.19; 95% CI, 1.03–1.38; $p = 0.017$), shock, and fewer ventilator-free days. In coronavirus disease 2019 only, an increase in soluble CD14 subtype was associated with severe acute kidney injury (adjusted relative risk, 1.23; 95% CI, 1.05–1.44; $p = 0.013$).

CONCLUSIONS: Higher plasma soluble CD14 subtype is associated with worse clinical outcomes in critically ill patients irrespective of coronavirus disease 2019 status though soluble CD14 subtype levels were lower in coronavirus disease 2019 patients than noncoronavirus disease 2019 patients. Soluble CD14 subtype levels may have prognostic utility in coronavirus disease 2019.

KEY WORDS: CD-14; coronavirus disease 2019; critical illness; innate immunity; soluble CD14 subtype

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Coronavirus disease 2019 (COVID-19) is caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). The COVID-19 pandemic has had an immense global impact with over 166 million cases and 3.5 million deaths to date (2). Severe disease correlates with early cytokine and chemokine-rich inflammation characteristic of exuberant innate immune system activation (3–5). The protein CD14 may play a role in this early innate immune system activation. CD14, in both its soluble (sCD14) and membrane-bound form (mCD14), is crucial to the recognition of microbial pathogens and products of tissue damage by the innate immune system. The N-terminal fragment of CD14, soluble CD14 subtype (sCD14-ST) also known as Presepsin, is generated by proteolytic cleavage in the setting of innate immune system activation and has been shown to have diagnostic utility in detecting bacterial infection in patients with suspected sepsis (6). CD14 inhibition has been identified as a potential target to modulate aberrant innate immune system activation in COVID-19 and clinical trials are underway (7).

Toll-like receptors (TLRs) bind both viral and nonviral pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) released by host injured and dying cells to initiate an innate immune response (8). CD14 is a necessary cofactor in the binding of a diverse array of PAMPs and DAMPs facilitating signal transduction through TLR-associated pathways (9). In viral infection, mCD14 and sCD14 act in concert with TLRs to bind a variety of viral PAMPs and DAMPs initiating inflammatory and anti-viral responses via nuclear factor kappa-light-chain-enhance of activated B cells and toll-interleukin 1 receptor-domain-containing adapter-inducing interferon-beta pathways, respectively (10–13). Proteomic analysis of plasma from patients with COVID-19 has shown that higher levels of plasma sCD14 are associated with increased oxygen support and preliminary studies have shown that higher levels of sCD14 may be associated with mortality in critically ill subjects (14, 15). However, the relationship of sCD14 to multiple clinical parameters in a large, well-phenotyped clinical cohort remains unknown.

sCD14-ST may be a more specific marker of pathogen-derived innate immune system activation than sCD14 and has been well studied as a biomarker in bacterial sepsis. An elevated circulating level of sCD14-ST is specific and sensitive for the diagnosis of bacterial sepsis, predicts mortality in sepsis and discriminates sepsis versus nonsepsis-associated acute respiratory distress syndrome (ARDS) (16–21). Prior work has reported that sCD14-ST levels are lower in patients with viral as opposed to bacterial infection (22). Less is known about the prognostic importance of sCD14-ST in viral illness but, in small case series of COVID-19 patients, sCD14-ST concentrations have been reported to track with severity of respiratory failure and death (23, 24). In this study, we sought to establish the relationship between early measurements of circulating sCD14 and sCD14-ST levels and presence of COVID-19 and related severity in a prospectively enrolled cohort of critically ill patients admitted under suspicion for COVID-19.

MATERIALS AND METHODS

Clinical Data and Ascertainment of Outcomes

We measured plasma sCD14 and sCD14-ST levels in 204 ICU patients prospectively enrolled in the COVID-19 Host Response and Clinical Outcomes (CHROME) study. CHROME enrolled patients admitted to three University of Washington hospitals between March 2020 and November 2020 under suspicion of COVID-19. All patients had symptoms consistent with COVID-19 and were admitted as persons under investigation for COVID-19. SARS-CoV-2 status was determined by nasopharyngeal reverse transcriptase-polymerase chain reaction assay for SARS-CoV-2. Patients who were less than or equal to 18 years old, pregnant, or incarcerated were excluded. Biomarkers were measured in samples collected within 24 hours of ICU admission.

Clinical data for demographics, comorbidities, events, and outcomes were abstracted from the electronic medical record into standardized case report forms. Inhospital death was defined as death prior to discharge from the index hospitalization. Ventilator-free days (VFDs) were defined as the number of days a patient was alive and not supported by invasive mechanical ventilation in the 28 days following enrollment (25). VFDs were not normally distributed and subjects were grouped into the following VFD tertiles: 0 VFDs, 1–27 VFDs, and 28 VFDs. ARDS was adjudicated based on the Berlin criteria (26) with all chest imaging adjudicated by a thoracic radiologist blinded to clinical data. Severe acute kidney injury (AKI) was

defined as a doubling of serum creatinine compared with baseline value (defined as creatinine value within 12 hr of enrollment sample collection) or need for new renal replacement therapy during the index hospitalization. Culture-verified bacterial or fungal infection at time of ICU admission was defined as growth of a pathogenic organism from a clinical culture and initiation of antimicrobials by the clinical team at the time of enrollment through hospital day 1. All studies were approved by the University of Washington Institutional Review Board (UW IRB: 9763 and 6878).

Biomarker Measurements

We measured plasma sCD14 and sCD14-ST concentrations using immunoassays per the manufacturer's guidelines (sCD14 [R&D Systems, Minneapolis, MN] and sCD14-ST [MyBioSource, San Diego, CA]). Biomarker levels below the lower limit of detection were assigned a value equal to the lowest detected value. Values above and below the standard curve range were extrapolated from the four-parameter equation generated using SoftMax Pro 7.1 (Molecular Devices). Information on quality control including inter-plate and intra-plate covariance can be found in the supplement (**Table S1**, http://links.lww.com/CCX/A864).

Statistical Analysis

Patient characteristics were summarized using standard descriptive statistics. Plasma biomarker concentrations were log-transformed to facilitate parametric statistical analyses. For regression analyses, we defined the covariate race/ethnicity as non-Hispanic White versus other. We used multivariable linear regression adjusting for age, sex, race/ethnicity, steroid therapy, Charlson Comorbidity Index (CCI) without age (27), and Sequential Organ Failure Assessment (SOFA) score (28) to test for associations between COVID-19 status and fold difference in sCD14 and sCD14-ST levels. To evaluate relationships with severity, we stratified our cohort by COVID-19 status and tested for associations between sCD14 and sCD14-ST concentrations and clinical outcomes. We performed multivariable linear regressions adjusting for age, sex, race/ethnicity, steroid therapy, and CCI to test for associations between biomarker concentrations and tertiles of VFDs. We used multivariable relative risk (RR) regression adjusting for age, sex, race/ethnicity, steroid therapy, and CCI to test for associations between biomarker concentrations and hospital mortality, severe AKI, and shock. We created additional models adjusting for SOFA score. We performed sensitivity analyses limiting to patients with COVID-19 testing for associations between sCD14 and sCD14-ST and clinical outcomes with additional adjustment for culture-verified infection. All beta estimates and RR estimates are expressed as either a beta estimate for change in VFD tertile or a RR estimate for each binary outcome per doubling (one $\log_2$ unit) of the specific biomarker concentration.

RESULTS

Study Population

The indication for ICU admission and illness severity were well balanced between the COVID-19 and non-COVID-19 groups. Approximately 75% of patients in both groups were admitted with pneumonia and/or respiratory failure (**Table 1**). A similar proportion of patients received invasive mechanical ventilation on study enrollment and the median National Institute of Health ordinal scale score was 6 in both groups. Baseline severity of illness was lower in COVID-19 than non-COVID-19 patients (Table 1). COVID-19 patients had a higher prevalence of ARDS (Table 1). More patients in the non-COVID-19 group had sepsis or septic shock upon admission (Table 1). Numerically, more patients in the non-COVID-19 group had a culture-verified bacterial or fungal infection (35%) compared with the COVID-19 group (23%), although this difference was not significant ($p = 0.087$) (Table 1). There were two culture-verified fungal infections in the non-COVID-19 group and zero in the COVID-19 group (**Table S4**, http://links.lww.com/CCX/A864). In the non-COVID-19 group, there were only two patients diagnosed with non-SARS-CoV-2 respiratory viral infection. More patients in the COVID-19 group were treated with steroids (Table 1). COVID-19 patients had fewer VFDs, but inhospital mortality was similar between groups (Table 1).

Higher SCD14-ST Levels Are Associated With Non-COVID-19 Critical Illness

At the time of ICU admission, we found that COVID-19 patients had significantly lower sCD14-ST levels than non-COVID-19 patients (COVID-19 patients:

TABLE 1.
Cohort Characteristics and Clinical Outcomes

Parameter	COVID-19 Negative (*n* = 102)	COVID-19 Positive (*n* = 102)	*p*
Cohort characteristics			
Age	55 (17)	55 (16)	0.99
Male sex	63 (62%)	71 (70%)	0.30
Race and ethnicity			
White	64 (63%)	69 (72%)	0.56
Hispanic/Latino	7 (7%)	43 (43%)	< 0.001
Non-White	38 (37%)	33 (28%)	0.56
Admission diagnosis			
Chronic obstructive pulmonary disease or asthma exacerbation	6 (6%)	4 (4%)	0.75
Respiratory failure or pneumonia	80 (78%)	78 (76%)	0.87
Myocardial infarction, cardiac arrest, congestive heart failure, arrhythmia	10 (10%)	6 (6%)	0.44
Sepsis and septic shock	38 (38%)	17 (17%)	0.002
Admission acute respiratory distress syndrome	25 (25%)	40 (40%)	0.035
Culture-verified bacterial or fungal infection	35 (35%)	23 (23%)	0.087
Outside hospital transfer	31 (30%)	45 (44%)	0.059
Admission comorbidities			
Charlson Comorbidity Index[a]	3.42 (± 2.47)	2.25 (± 1.91)	< 0.001
Admission disease severity			
National Institute of Health ordinal scale day 0	6 (5–7)	6 (5–7)	0.75
Acute Physiology and Chronic Health Evaluation III	80.31 (± 29.47)	71.73 (± 28.67)	0.036
Sequential Organ Failure Assessment	6.89 (± 4.42)	4.99 (± 4.42)	0.002
Treated with steroids	34 (33%)	50 (49%)	0.033
Clinical outcomes			
Ventilator-free days	26 (11–28)	13.50 (0–28)	0.012
In hospital death	22 (22%)	30 (30%)	0.28
Acute kidney injury (stage 2+)	10 (10%)	21 (21%)	0.050
Shock	47 (47%)	56 (56%)	0.26

COVID-19 = coronavirus disease 2019.
[a]Charlson Comorbidity Index without age.
Categorical variables: *n* (%).
Continuous variables: mean (± SD) or median (interquartile range) if highly skewed distribution.

median 4.9 ng/mL [interquartile range [IQR], 1.3–14.5 ng/mL vs non-COVID-19 patients: median 12.5 ng/mL [IQR, 3.6–49.7 ng/mL]) (**Table 2**). In regression analyses adjusting for age, sex, race/ethnicity, steroid therapy, comorbidities, and severity of illness, sCD14-ST was 54% lower in COVID-19 than non-COVID-19 patients (fold difference, 0.46; 95% CI, 0.28–0.77; $p = 0.003$) (Table 2). In contrast, sCD14 was not significantly different between COVID-19 and non-COVID-19 groups (Table 2). Notably, sCD14-ST and sCD14 levels were not correlated (**Fig. S1**, http://links.lww.com/CCX/A864). In a sensitivity analysis limited to patients with an admission diagnosis of pneumonia or respiratory failure, we observed similar findings (**Table S2**, http://links.lww.com/CCX/A864).

TABLE 2.
Fold Difference of Soluble CD14 Subtype and Soluble CD14 Levels Between Coronavirus Disease 2019 Negative and Coronavirus Disease 2019 Positive Patients

Plasma Biomarker	COVID-19 Negative (n = 102) Median (IQR)	COVID-19 Positive (n = 102) Median (IQR)	Unadjusted		Adjusted	
			Fold Difference (95% CI)	p	Fold Difference (95% CI)	p
Soluble CD14 subtype	12.5 (ng/mL) (3.6–49.7)	4.9 (ng/mL) (1.3–14.5)	0.34 (0.20–0.56)	< 0.0001	0.46 (0.28–0.77)	0.003
Soluble CD14	3.16 (µg/mL) (2.00–5.47)	3.02 (µg/mL) (1.99, 3.89)	0.92 (0.75–1.15)	0.47	0.94 (0.75–1.18)	0.59

COVID-19 = coronavirus disease 2019, IQR = interquartile range.
Adjusted model: age, gender, race/ethnicity, steroids, Charlson Comorbidity Index without age, and Sequential Organ Failure Assessment.
The non-COVID-19 group is the reference for the fold difference calculation.

Higher sCD14-ST Levels Are Associated With Worse Clinical Outcomes in Patients With COVID-19

Higher sCD14-ST levels were associated with worse clinical outcomes in COVID-19 and non-COVID-19 critically ill patients after adjusting for age, sex, race/ethnicity, steroid therapy, and CCI. Higher plasma sCD14-ST concentrations were associated with inhospital death in COVID-19 patients (adjusted relative risk [aRR], 1.21; 95% CI, 1.06–1.39; p = 0.006) and non-COVID-19 patients (aRR, 1.19; 95% CI, 1.03–1.38; p = 0.017) (**Table 3**). Higher sCD14-ST levels were associated with fewer VFDs in both COVID-19 (p < 0.001) and non-COVID-19 groups (p = 0.022) (**Fig. 1** and Table 3). In both COVID-19 and non-COVID-19 patients, higher sCD14-ST levels were associated with shock (COVID-19 [aRR, 1.15; 95% CI, 1.07–1.23; p < 0.001] and non-COVID-19 [aRR, 1.16; 95% CI, 1.08–1.22; p < 0.001]) (Table 3). In COVID-19 only, higher plasma sCD14-ST was associated with increased risk of severe AKI (aRR, 1.23; 95% CI, 1.05–1.44; p = 0.013) (Table 3).

In COVID-19, with additional adjustment for SOFA observed associations between sCD14-ST and clinical outcomes persisted but were attenuated (Table 3). In the non-COVID-19 group, after adjustment for SOFA associations between sCD14-ST and outcomes were no longer significant (Table 3).

Because sCD14-ST is a well-described prognostic and diagnostic biomarker in bacterial sepsis (16–21, 23, 24), we performed a sensitivity analysis in the COVID-19 group to assess if the presence of concurrent culture-verified bacterial infection confounds the observed associations between our exposure biomarkers and clinical outcomes. After adjustment for bacterial infection, associations between sCD14-ST and outcomes remained significant (**Table S3**, http://links.lww.com/CCX/A864).

sCD14 levels demonstrated a different pattern of risk for clinical outcomes than sCD14-ST. In analyses adjusting for age, sex, race/ethnicity, steroids, and CCI, higher sCD14 levels were associated with less severe organ dysfunction in COVID-19 patients and a reduced risk of death in non-COVID-19 patients (Table 3). With additional adjustment for severity of illness, sCD14 was associated with death in the non-COVID-19 group only (Table 3).

sCD14-ST Is Positively Associated With Inflammatory Biomarkers Levels in COVID-19

In patients with COVID-19, we examined the relationship between circulating sCD14-ST or sCD14 and a panel of inflammatory biomarkers known to be elevated in COVID-19 and to track with severity of illness (29–33). We identified strong direct relationships between sCD14-ST levels and soluble tumor necrosis factor receptor-1 (sTNFR-1) (r^2 = 0.40; p < 0.00001), soluble triggering receptor expressed on myeloid cell (sTREM) (r^2 = 0.30; p < 0.0001), interleukin-6 (IL-6) (r^2 = 0.20; p < 0.0001), and, to a lesser extent, C-reactive protein (CRP) (r^2 = 0.12; p = 0.002) (**Fig. 2**). We fou

TABLE 3.

Association of Soluble CD14 and Soluble CD14 Subtype With Clinical Outcomes Among ICU Patients, Stratified by Coronavirus Disease 2019 Status

Outcome	Plasma Biomarker	Coronavirus Disease 2019 Status	Unadjusted	*p*	Adjusted No. 1	*p*	Adjusted No. 2 (Model No. 1 + Sequential Organ Failure Assessment)	*p*
Hospital mortality (RR, 95% CI)	sCD14-ST	Positive (*n* = 100)	1.18 (1.06–1.32)	0.003	1.21 (1.06–1.39)	0.006	1.18 (1.02–1.37)	0.027
		Negative (*n* = 101)	1.16 (1.03–1.30)	0.016	1.19 (1.03–1.38	0.017	1.12 (0.97–1.29)	0.12
	sCD14	Positive (*n* = 102)	0.91 (0.72–1.16)	0.46	0.92 (0.72–1.17)	0.50	0.91 (0.69–1.21)	0.53
		Negative (*n* = 101)	0.80 (0.71–0.90)	< 0.001	0.84 (0.74–0.95)	0.007	0.85 (0.75–0.96)	0.010
Ventilator-free days (beta, 95% CI)	sCD14-ST	Positive (*n* = 100)	−0.11 (−0.17 to −0.063)	< 0.0001	−0.10 (−0.16 to −0.046)	< 0.001	−0.064 (−0.13 to −0.0024)	0.042
		Negative (*n* = 101)	−0.067 (−0.12 to −0.018)	0.008	−0.063 (−0.12 to −0.0095)	0.022	−0.017 (−0.068 to 0.033)	0.49
	sCD14	Positive (*n* = 102)	0.037 (−0.13 to 0.20)	0.65	0.071 (−0.087 to 0.23)	0.38	0.053 (−0.12 to 0.22)	0.54
		Negative (*n* = 101)	0.052 (−0.082 to 0.19)	0.44	0.037 (−0.092 to 0.017	0.57	0.048 (−0.071 to 0.17)	0.42
Shock (RR, 95% CI)	sCD14-ST	Positive (*n* = 100)	1.14 (1.07–1.22)	< 0.0001	1.15 (1.07–1.23)	< 0.001	1.11 (1.03–1.19)	0.007
		Negative (*n* = 101)	1.16 (1.08–1.25)	< 0.0001	1.16 (1.076–1.25)	< 0.001	1.12 (1.03–1.21)	0.009
	sCD14	Positive (*n* = 102)	0.92 (0.82–1.04)	0.18	0.89 (0.80–0.99)	0.040	0.91 (0.81–1.02)	0.11
		Negative (*n* = 101)	0.98 (0.83–1.16)	0.83	1.01 (0.87–1.19)	0.84	1.02 (0.89–1.16)	0.79
Severe acute kidney injury[a] (RR, 95% CI)	sCD14-ST	Positive (*n* = 96)	1.20 (1.05–1.36)	0.006	1.23 (1.05–1.44)	0.013	1.27 (1.06–1.53)	0.010
		Negative (*n* = 91)	1.21 (0.98–1.50)	0.082	1.23 (0.95–1.59)	0.11	1.16 (0.89–1.52)	0.27
	sCD14	Positive (*n* = 96)	0.83 (0.68–1.01)	0.066	0.80 (0.64–1.00)	0.055	0.80 (0.64–0.99)	0.042
		Negative (*n* = 91)	0.87 (0.62–1.24)	0.45	0.94 (0.66–1.34)	0.75	0.92 (0.67–1.27)	0.61

RR = relative risk, sCD14 = soluble CD14, sCD14-ST = soluble CD14 subtype.

[a]Patients on hemodialysis prior to admission were excluded from severe acute kidney injury analysis.

RR estimates are for a doubling of biomarker concentrations.

Beta estimates are for change between ventilator-free day tertile per doubling of biomarker concentration.

Adjusted model number 1: age, gender, race/ ethnicity, steroids, and Charlson Comorbidity Index without age.

Adjusted model number 2: age gender, race/ ethnicity, steroids, Charlson Comorbidity Index without age, and Sequential Organ Failure Assessment.

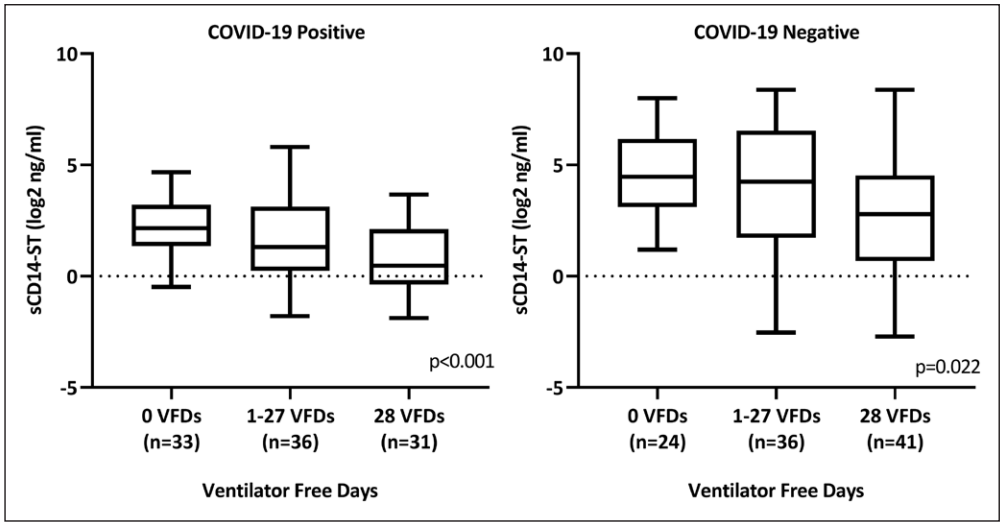

Figure 1. In coronavirus disease 2019 (COVID-19) and non-COVID-19 groups, a rise in soluble CD14 subtype (sCD14-ST) is associated with fewer ventilator-free days (VFDs). *Boxes* and *horizontal bars* denote interquartile range (IQR) and median of the log2 transformed sCD14-ST levels, respectively. *Whisker* endpoints are equal to the maximum and minimum values below or above the median ± 1.5 times the IQR. The *x*-axis denotes the following VFD bins: 0 VFDs, 1–27 VFDs, and 28 VFDs. The *p* value denotes significance of association between a doubling of sCD14-ST levels and change between VFD bin using linear regression with robust standard corrections adjusting for age, sex, race/ethnicity, steroid therapy, and Charlson Comorbidity Index.

nd only weak relationships between sCD14 and CRP (r^2 = 0.070; p = 0.015) and sTNFR-1 (r^2 = 0.11; p = 0.018) but not sTREM or IL-6 (**Fig. S2**, http://links.lww.com/CCX/A864). We tested for associations between sCD14-ST and VFDs with added adjustments for IL-6 and CRP and minimal attenuation was observed (**Table S5**, http://links.lww.com/CCX/A864). Of note, sCD14-ST concentrations were not significantly correlated with any WBC subpopulations at admission, while sCD14 levels were weakly correlated with admission lymphocyte count only (**Fig. S3**, http://links.lww.com/CCX/A864).

DISCUSSION

Our study found that early plasma sCD14-ST levels were significantly lower in critically ill patients with COVID-19 than those without COVID-19. This is consistent with prior work that has established sCD14-ST as a prognostic and diagnostic biomarker in bacterial sepsis that is lower in viral infection than bacterial infection (16–24). However, irrespective of COVID-19 status, CD14-ST is strongly associated with clinical outcomes. Of importance, our work validates a prior finding, demonstrating that higher plasma sCD14-ST levels are associated with fewer VFDs and more AKI, shock, and death in patients with COVID-19. Taken together, our findings support the utility of sCD14-ST in distinguishing bacterial infection from other causes of infection but also support a novel role for sCD14-ST in predicting organ dysfunction and death in subjects with COVID-19.

Our findings expand upon early work investigating sCD14-ST in COVID-19 in several important ways. A descriptive case series of patients hospitalized with COVID-19 suggested that sCD14-ST may predict severity of lung injury (n = 6), and a retrospective cohort study reported that sCD14-ST was higher in those who died or required ICU admission (total n = 75; ICU n = 21); the timing of sample collection in this study varied during the hospitalization period (23, 24). Here, we expanded on this work by investigating the relationship between plasma sCD14-ST levels and COVID-19 status and related ICU outcomes in a much larger, well phenotyped, and prospectively enrolled ICU cohort. Additionally, in our study, we used standard timing of sample collection for plasma measurements, and we adjusted for potentially important covariates. In COVID-19, adjustment for illness severity attenuates but does not eliminate associations between sCD14-ST and clinical outcomes. However, the elements included in the SOFA score (e.g., shock) may sit within the causal pathway for clinical outcomes, and, therefore, may result in over-adjustment. From a prognostic standpoint, sCD14-ST, a single biomarker, may be easier to apply clinically than a multivariable illness severity score. Additionally, we believe our findings may be biologically informative. As is described in bacterial infection, we predict that in COVID-19, CD14 cooperates with TLRs to recognize and bind viral PAMPs, potentially nonviral PAMPs from gut translocation, and DAMPs to initiate downstream signaling

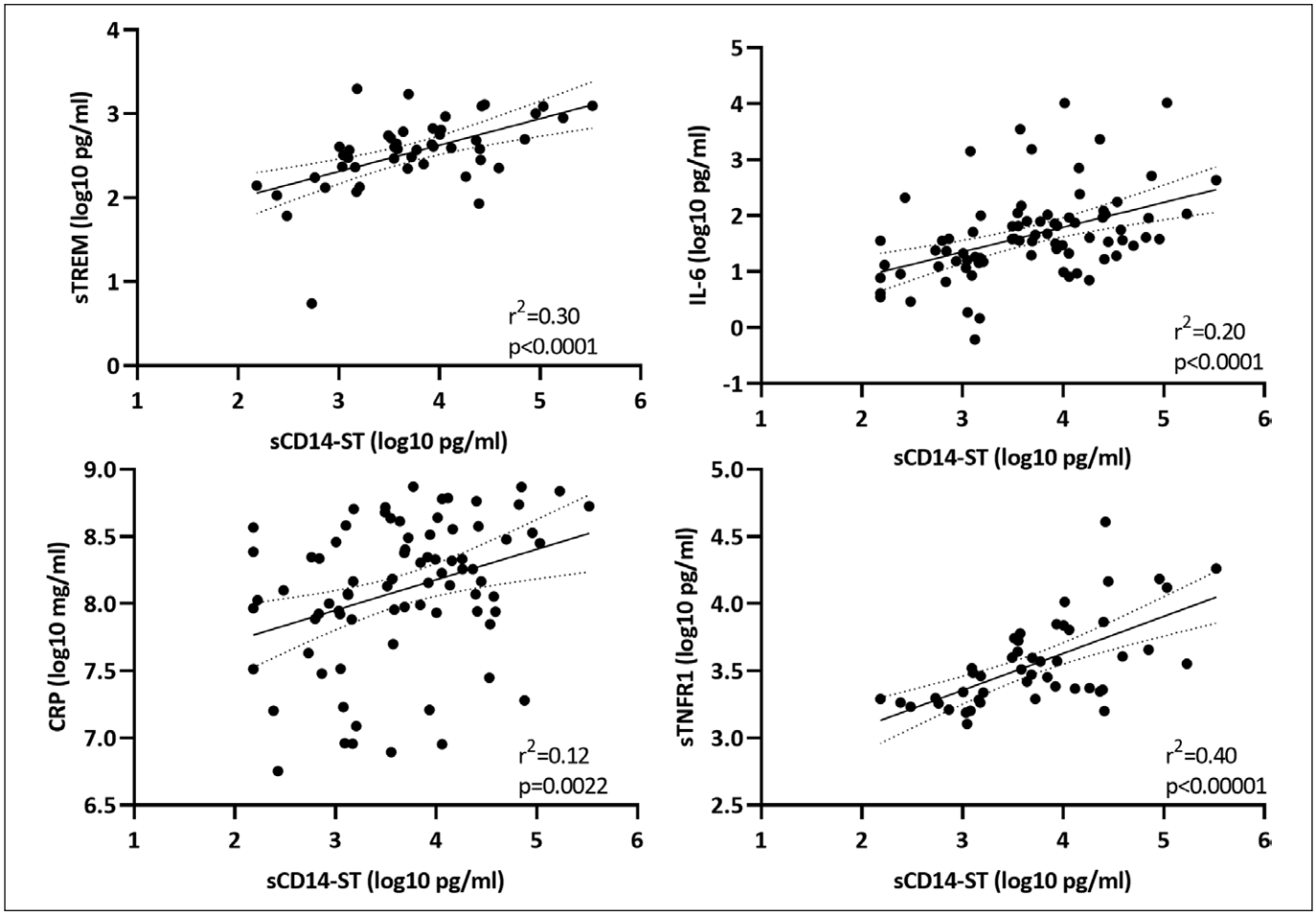

Figure 2. In patients with coronavirus disease 2019 (COVID-19), *dot plots* describing the relationship observed between soluble CD14 subtype (sCD14-ST) and other inflammatory biomarkers of interest in COVID-19. The *x*-axis denotes log10 transformed sCD14-ST or soluble CD14 levels in pg/mL. *Y*-axis display log10 transformed biomarkers (interleukin-6 [IL-6] pg/mL, soluble triggering receptor expressed on myeloid cell [sTREM] pg/mL, soluble tumor necrosis factor receptor-1 [sTNFR-1] pg/mL, C-reactive protein [CRP] mg/mL). Unadjusted linear regression was used to fit a line with 95% CIs, and the *p* values and r^2 values are reported.

through specific innate immune pathways (34, 35). In other work, bacterial PAMPs, lipopolysaccharide, and bacterial RNA, were increased in severe COVID-19, and this correlated with other inflammatory biomarkers including IL-6 and tumor necrosis factor (36). This suggests bacterial PAMPs related to gut translocation or overt bacterial infection may play a role in innate immune activation in COVID-19. However, in our study, the associations between sCD14-ST and clinical outcomes in COVID-19 were independent of concurrent, overt bacterial infections. sCD14-ST, the 13 kDa N-terminal fragment of CD14, may be a marker CD14-related innate immune activation as it is generated via elastase-dependent proteolysis following CD14-TLR-PAMP endocytosis (37). Given its mechanism of production and its strong association with outcomes, we speculate that sCD14-ST may actually be useful as a predictive marker of treatment response to anti-CD14 antibody therapy in COVID-19; as noted clinical trials testing this therapeutic are underway (7). Future work will be aimed at testing this hypothesis.

In contrast to sCD14-ST, in our study, sCD14 levels were associated with less severe organ dysfunction in COVID-19. We also found that sCD14 was inversely associated with death in non-COVID-19 patients, and this is congruent with what has been described in bacterial sepsis studies (38–40). Prior work has shown that higher levels of plasma sCD14 were associated with SARS-CoV-2 positivity (compared with healthy controls). Further, in COVID-19, sCD14 was associated with increased oxygen support, and in critically ill patients (n = 15), sCD14 was higher in those who died (14, 15). In contrast, we show that sCD14 does not differ between COVID-19 and non-COVID-19 critically ill patients. Our findings contradict prior work assessing the relationship between sCD14 and

clinical outcomes in COVID-19 and should be validated. Potential explanations for the contradictory findings include differences in severity of illness, timing of sample collection within the clinical course, and treatments received (i.e., corticosteroids). However, of note, adjustment for steroid administration in our risk models did not fully attenuate associations between sCD14 and outcomes.

While our findings show that sCD14 and sCD14-ST levels are not correlated early in COVID-19, the associations we identified between biomarkers and clinical outcomes suggest an inverse relationship between sCD14 and sCD14-ST and clinical outcomes. Of note, circulating sCD14 concentrations (μg/mL) are orders of magnitude higher than sCD14-ST concentrations (ng/mL), which may limit our ability to detect a weak correlation. An increase in sCD14 levels are likely to be dependent on either shedding of mCD14 through cleavage from myeloid cells or, possibly, from secretion of sCD14 (9, 39, 41, 42); whereas sCD14-ST levels are likely to require mCD14 endocytosis and proteolysis (37). Therefore, sCD14-ST production following infection may be a marker of innate immune activation and proteolytic cleavage of sCD14. The fact that we found sCD14-ST, but not sCD14 levels, to be strongly associated with inflammatory biomarkers that have been implicated as markers of disease severity in COVID-19 (3, 5, 29–33, 43) suggests that sCD14-ST levels may be a good indicator of the pathophysiologic processes leading to these outcomes. Future work should be aimed at clarifying whether sCD14-ST indicates a causal relationship between CD14 and these inflammatory pathways. Enhanced understanding of pathogenic immune pathways has the potential to inform an improved approach to therapy. We found that sCD14-ST is independently associated with VFDs when adjusting for IL-6 and CRP, commonly measured inflammatory biomarkers (44). We hypothesize that the use of a therapeutic targeting the CD14 pathway may have benefit in addition currently used immunomodulatory medications. There are two clinical trials testing the efficacy of an anti-CD14 monoclonal in severe COVID that will help inform this question.

There are several limitations to our study. We do not know precisely when we are capturing patients in their clinical course; however, limiting our analysis to biomarkers drawn at the time of ICU admission should narrow the range of sampling time to be around the time of or just before peak disease severity. Although our cohort was large compared with other published data on sCD14-ST in COVID-19, it was not powered to detect minor differences between groups.

CONCLUSIONS

Plasma levels of sCD14-ST are significantly lower in critically ill patients infected with SARS-CoV-2 compared with subjects who are not infected with SARS-CoV-2. However, in COVID-19 critical illness, higher sCD14-ST levels, were associated with worse COVID-19–related outcomes. Early measurement of sCD14-ST in critically ill subjects with COVID-19 may have prognostic utility.

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