Elevated plasma sTIM-3 levels in patients with severe COVID-19

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Background: The pathogenesis of coronavirus disease 2019 (COVID-19) is still incompletely understood, but it seems to involve immune activation and immune dysregulation.
Objective: We examined the parameters of activation of different leukocyte subsets in COVID-19 – infected patients in relation to disease severity.
Methods: We analyzed plasma levels of myeloperoxidase (a marker of neutrophil activation), soluble (s) CD25 (sCD25) and soluble T-cell immunoglobulin mucin domain-3 (sTIM-3) (markers of T-cell activation and exhaustion), and sCD14 and sCD163 (markers of monocyte/macrophage activation) in 39 COVID-19 – infected patients at hospital admission and 2 additional times during the first 10 days in relation to their need for intensive care unit (ICU) treatment.
Results: Our major findings were as follows: (1) severe clinical outcome (ICU treatment) was associated with high plasma levels of sTIM-3 and myeloperoxidase, suggesting activated and potentially exhausted T cells and activated neutrophils, respectively; (2) in contrast, sCD14 and sCD163 showed no association with need for ICU treatment; and (3) levels of sCD25, sTIM-3, and myeloperoxidase were inversely correlated with degree of respiratory failure, as assessed by the ratio of PaO_2 to fraction of inspired oxygen, and were positively correlated with the cardiac marker N-terminal pro-B – type natriuretic peptide.
Conclusion: Our findings suggest that neutrophil activation and, in particular, activated T cells may play an important role in the pathogenesis of COVID-19 infection, suggesting that T-cell – targeted treatment options and downregulation of neutrophil activation could be of importance in this disorder. (J Allergy Clin Immunol 2020;*nnn*:*nnn-nnn*.)

Key words: COVID-19, outcome, T cell, TIM-3, neutrophil

The present coronavirus disease 2019 (COVID-19) pandemic has spread to most countries in the world and is defined by the World Health Organization as a public health emergency of international concern.[1] This pandemic has already resulted in a high number of critically ill patients in need of hospitalization and respiratory support, in some countries overwhelming the capacity of the health facilities.[2]

Whereas COVID-19 is caused by the coronavirus SARS-CoV-2, the disease's severity is, at least only partly, driven by the virus itself.[3,4] Thus, some patients with COVID-19 infection are characterized by persistent inflammation and immune dysregulation, with downregulation of angiotensin-converting enzyme ACE2, the putative SARS-CoV-2 receptor, as an important molecular mechanism, resulting in impaired signaling through the antiproliferative and anti-inflammatory angiotensin (1-7) pathway.[5-7] Several reports have demonstrated elevated levels of a wide range of inflammatory cytokines, such as IL-6, TNF, and various inflammatory chemokines such as CXCL10 in patients with COVID-19 with severe disease, compared with the levels in patients with mild-to-moderate disease.[8-10] There are also reports of lymphopenia (in particular, T-cell lymphopenia) in those with severe disease.[10,11] So far, however, the role of the different leukocyte subsets in the pathogenesis of COVID-19 infection has not been fully elucidated.

In the present study we examined plasma parameters of activation of different leukocyte subsets, including myeloperoxidase as a marker of neutrophil activation, soluble (s) CD25 and soluble T-cell immunoglobulin mucin domain-3 (sTIM-3) as markers of T-cell activation/exhaustion, and sCD14 and sCD163 as markers of monocyte/macrophage activation in relation

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Abbreviations used

COVID-19: Coronavirus disease 2019
eGFR: Estimated glomerular filtration rate
Fio_2: Fraction of inspired oxygen
hsCRP: High-sensitivity C-reactive protein
ICU: Intensive care unit
NT-proBNP: N-terminal pro-B – type natriuretic peptide
OUH: Oslo University Hospital
P/F ratio: Ratio of Pao_2 to Fio_2
s: Soluble
sTIM-3: Soluble T-cell immunoglobulin mucin-3

to disease severity, as reflected by intensive care unit (ICU) admission in COVID-19 – infected patients. In addition, we analyzed correlations between these leukocyte activation markers and the degree of respiratory failure as assessed by the ratio of PaO_2 to the fraction of inspired oxygen (Fio_2) (P/F ratio), and as cardiac involvement is relatively frequent in hospitalized patients with COVID-19 (reference), we also analyzed the correlation of these markers with the cardiac marker N-terminal pro-B – type natriuretic peptide (NT-proBNP).

METHODS

Study population

Between March 6 and April 14, 2020, a total of 39 adult patients (≥18 years old) with confirmed COVID-19, as assessed by a positive SARS-CoV-2 PCR test targeting the E-gene on oropharyngeal and nasopharyngeal specimens, were consecutively recruited from Oslo University Hospital (OUH) Ullevål and Drammen Hospital, Vestre Viken Hospital Trust, to a clinical cohort study (Norwegian SARS-CoV-2 study [ClinicalTrials.gov identifier NCT04381819]). Clinical information and laboratory samples were collected at the earliest time point after hospitalization. Peripheral blood was collected within 48 hours of inclusion and up to 10 days after hospitalization with 1 to 3 samples per patient. Through use of a modified version of the International Severe Acute Respiratory and Emerging Infection Consortium/World Health Organization clinical characterization protocol, clinical and routine data were abstracted from electronic medical records into the International Severe Acute Respiratory and Emerging Infection Consortium (isaric.tghn.org) Research Electronic Data Capture web application (Vanderbilt University, hosted by University of Oxford, United Kingdom). Sequential (Sepsis-Related) Organ Failure Assessment score was calculated at each time point, whereas Quick Sequential (Sepsis-Related) Organ Failure Assessment score and National Early Warning Score were calculated at admission.

For reference, leukocyte markers were also analyzed in plasma from 16 healthy controls who were recruited at the Research Institute of Internal Medicine, OUH, on the basis of disease history and normal laboratory test results (Table I).

Ethical considerations

Informed consent was obtained from all patients or from next of kin if patients were incapacitated and therefore unable to give consent. The study was approved by the South-Eastern Norway Regional Health Authority (reference no. 106624).

Study outcome definition

Outcome was defined as the need for treatment in an ICU during hospitalization. The indication for admission to ICU was respiratory failure requiring mechanical ventilation support or noninvasive ventilation support that could not be given in the hospital ward.

Sample processing

Peripheral blood was collected with 4-mL Vacutainer tubes (BD Biosciences, San Diego, Calif) with EDTA as an anticoagulant. The samples were immediately stored on ice and processed within 30 minutes; plasma was isolated by centrifugation at 2000 *g* for 20 minutes at 48C to obtain platelet-poor plasma. The plasma samples were immediately stored at − 80^0C in several aliquots until analysis. Samples were thawed only once.

Measurement of leukocyte activation markers

Plasma levels of sCD14, sCD163, sCD25, sTIM-3, and myeloperoxidase were measured in duplicate by enzyme immunoassays using commercially available antibodies (R&D Systems, Minneapolis, Minn) in a 384 format using a combination of a SELMA pipetting robot (Analytik Jena AG, Jena, Germany) and a BioTek dispenser/washer (BioTek Instruments, Winooski, Vt). Absorption was read at 450 nm by using an enzyme immunoassay plate reader (BioTek Instruments) with wavelength correction set to 540 nm. Samples from all patients and controls were run on the same 384-well plate; the intraassay coefficients of variation for sCD14, sCD163, sCD25, sTIM-3, and myeloperoxidase were 5.5%, 5.2%, 5.4%, 3.6%, and 2.3%, respectively.

Measurement of NT-proBNP and hsCRP

Cardiac marker and high-sensitivity C-reactive protein (hsCRP) levels were measured at the medical biochemistry departments at the 2 centers: at OUH, levels of NTproBNP and hsCRP were analyzed on a cobas 8000 analyzer (Roche Diagnostics, Basel, Switzerland), and at Drammen Hospital, hsCRP level was analyzed on a Alinity/Architect ci8200 analyzer (Abbott, Abbott Park, Ill) and NTproBNP level was on a cobas E411 analyzer (Roche). The reference values for NT-proBNP were as follows: for women younger than 50 years, at least 170 ng/L; for women aged 50-69 years, at least 300 ng/L; for women aged 70 years or older, at least 760 ng/L; for men younger than 50 years, at least 85 ng/L; for men aged 50 to 69 years, ≥250 ng/L; and for men aged 70 years or older, 500 ng/L.

Statistical analysis

Patient characteristics were compared by using the Student *t* test or chi-square test for continuous and categoric variables, respectively (Tables I and II). We did not have samples from all patients at all time points or for analyzing the temporal profile of the leukocyte activation markers; we therefore used a univariate general linear model. Markers were categorized as day 1 (ie, within 48 hours of admission [n 5 33]), day 3 to 5 [n 5 23], and day 7-10 [n 5 17], giving 3 time categories (Fig 1), and they were log-transformed. Marker was used as dependent, ICU admission (yes/no) and time as fixed factors, patient number as random factor and estimated glomerulus filtration rate (eGFR) as covariate. The SPSS syntax was as follows: UNIANOVA marker BY time ICU patient WITH eGFR/RANDOM5patient/METHOD5SSTYPE(3)/INTERCEPT5INCLUDE/CRITERIA5ALPHA(0.05)/DESIGN5patient(ICU) time ICU ICU*time eGFR.

Correlation analysis between leukocyte markers and other continuous variables was performed by using partial correlation with adjustment for patient and time point, and in some cases, also for eGFR (Table III). Correlation analysis between selected markers was also performed at individual time points (Pearson correlation [Fig 2]).

To limit multiple comparisons, *post hoc* testing was performed only on variables where outcome or outcome*time interaction was significant with use of multivariate regression at each time point with eGFR and age as covariate (Fig 1). In addition, we multiplied the *P* values with the number of leukocyte markers assessed (ie, with 5 in the overall univariate and partial correlation analysis (Fig 1 and Table III) and with 3 in the *post hoc* and individual correlation analysis (Figs 1 and 2).

P values are 2 sided and considered significant when less than .05. SPSS software (release 26.0.0.1 [IBM, Armonk, NY]) was used for statistical analysis.

TABLE I. Characterization of the study group

Charact eristic	Controls (n 5 16)	Patients (n 5 39)
Women, no. (%)	7 (44)	10 (26)
Age (y), mean 6 SD	66 6 7	61 6 15
Time from symptoms (d)	—	9.6 6 3.7
White, no. (%)	16 (100)	27 (69)
Current smoker, n (%)	3 (19)	8 (21)
P/F ratio (kPa)	—	41 6 15
Need for oxygen therapy, n (%)	—	28 (72)
Comorbidities, no (%)		
Cardiovascular	0 (0)	9 (23)
Pulmonary	0 (0)	9 (23)
Renal	0 (0)	4 (10)
Liver	0 (0)	0 (0)
Neurologic	0 (0)	1 (3)
Cancer	0 (0)	1 (3)
Hematologic	(0)	1 (3)
Obesity	0 (0)	5 (13)
Diabetes	0 (0)	3 (8)
Rheumatic	0 (0)	4 (10)
Biochemistry		
Hemoglobin level (g/dL), mean 6 SD	14.4 6 0.9	13.3 6 1.7*
Leukocyte count (3 10^9/L), mean 6 SD	5.6 6 1.9	6.6 6 3.2
Lymphocyte count (3 10^9/L), mean 6 SD	1.68 6 0.66	1.07 6 0.45*
Monocyte count (3 10^9/L), mean 6 SD	0.54 6 0.18	0.44 6 0.2
Neutrophil count (3 10^9/L), mean 6 SD	3.24 6 0.71	5.09 6 3.2†
Platelet count (3 10^9/L), mean 6 SD	254 6 70	202 6 59*
ALT level (U/L), mean 6 SD	29 6 13	43 6 40
AST level U/L), mean 6 SD	32 6 9	49638
eGFR level (mL/min/1.73 m^2), mean 6 SD	82 6 12	82630
hsCRP level (mg/L), median (25th, 75th percentile)	1.6 (0.8, 3.9)	53 (31, 153)%
NT-proBNP level > cutoff, no. (%)	0 (0)	18 (50)
Risk scores, no. (%)	—	37 (95)
qSOFA score ≥ 1	—	23 (62)
NEWS ≥ 5	—	17 (46)
Radiologic findings, no. (%)		
Radiography performed	—	35 (90)
Infiltrates present	—	28 (80)

ALT, Alanine transaminase; ***AST***, aspartate transaminase; ***NEWS***, National Early Warning Score; ***qSOFA***, quick Sequential (Sepsis-Related) Organ Failure Assessment.
Respiratory failure defined as a P/F ratio of 26.6 kPa or lower.
Cutoffs for NT-proBNP level are given in the Methods section.
*$P < .01$ versus patients with no ICU treatment or death.
†$P < .05$ versus patients with no ICU treatment or death.
%$P < .001$ versus patients with no ICU treatment or death.

Role of the funding source

The sponsor of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and takes final responsibility for the decision to submit for publication.

RESULTS

Table I shows demographic and clinical characteristics of the patients with COVID-19 (n 5 39) and healthy controls (n 5 16). Patients were an average of 60 years old (range 45-91 years) and mostly men (75%), with an average 9.6 days from first symptoms to hospital admission. Chronic cardiovascular disease was the major comorbidity (23%). Biochemically, patients had lower hemoglobin, lymphocyte, and platelet counts and higher neutrophil counts, as well as higher NTproBNP and hsCRP levels than the healthy controls did.

TABLE II. Characterization of the study group at baseline according to outcome (ICU versus non-ICU)

Characteristic	ICU	
	No (n 5 30)	Yes (n 5 9)
Women, no. (%)	9 (31)	1 (10)
Age (y), mean 6 SD	60 6 16	63 6 14
Time from symptoms (d), mean 6 SD	9.7 6 3.8	9.3 6 3.0
P/F ratio (kPa), mean 6 SD	44 6 14	27 6 10*
Need for oxygen therapy, no. (%)	20 (69)	8 (80)
Current smoker, no. (%)	6 (21)	2 (20)
Pulmonary disease, no. (%)	7 (24)	2 (20)
Diabetes, no. (%)	2 (7)	1 (10)
Obesity, no. (%)	4 (14)	1 (10)
Leukocyte count (3 10^9/L), mean 6 SD	5.8 6 2.3	8.8 6 4.4†
Lymphocyte count (3 10^9/L), mean 6 SD	1.13 6 0.47	0.91 6 0.33†
Monocyte count (3 10^9/L), mean 6 SD	0.47 6 0.21	0.38 6 0.17
Neutrophils count (3 10^9/L), mean 6 SD	4.2 6 2.4	7.5 6 4.2†
eGFR level (mL/min/1.73 m^2), mean 6 SD	81 6 27	67 6 32
hsCRP level (mg/L), median [25th, 75th percentile]	51 [31,100]	140 [117, 259]*
NT-proBNP level > cutoff, no. (%)	10 (37)	8 (89)*
qSOFA score ≥ 1, no. (%)	16 (57)	7 (78)
NEWS ≥ 5, no. (%)	11 (39)	6 (67)
Radiography performed, no. (%)	23 (89)	7 (88)
Infiltrates present, no. (%)	16 (70)	7 (100)

NEWS, National Early Warning Score; ***qSOFA***, quick Sequential (Sepsis-Related) Organ Failure Assessment.
Cutoffs for NT-proBNP level are given in the Methods section. Respiratory failure defined as a P/F ratio less than 26.6 kPa.
*$P < .01$ versus non-ICU patients.
†$P < .05$ versus non-ICU patients.

Characteristics within outcome groups

The demographic and clinical characteristics of the patients with COVID-19 according to ICU admission are presented in Table II. Of the 39 patients with the COVID-19 virus, 9 were admitted to ICU (of the 3 patient deaths, 2 were due to respiratory failure and 1 was due to multiorgan failure related to COVID-19). The ICU patients were characterized by low P/F ratio and lymphocyte counts and high NT-proBNP and hsCRP levels. No significant differences in age, time from symptoms to admission, major comorbidities, or eGFR level were detected in relation to ICU (Table II).

Leukocyte markers in relation to outcomes

The temporal course of leukocyte markers in relation to ICU admission (ICU patients, n 5 9; non-ICU patients, n 5 30) is shown in Fig 1. Patients admitted to ICU were characterized by high levels of the T-cell activation marker sCD25 and sTIM-3 in *a priori* analysis, and for sTIM-3, also in *post hoc* testing. Moreover, whereas sCD25 displayed stable levels between samples obtained at days 3 to 5 and days 7 to 10, sTIM-3 levels increased until day 7 to 10 in the ICU group. Finally, sCD25 and sTIM-3 levels were markedly higher than the control levels at all time points.

The level of the neutrophil marker myeloperoxidase was significantly higher in the ICU group than in the non-ICU group

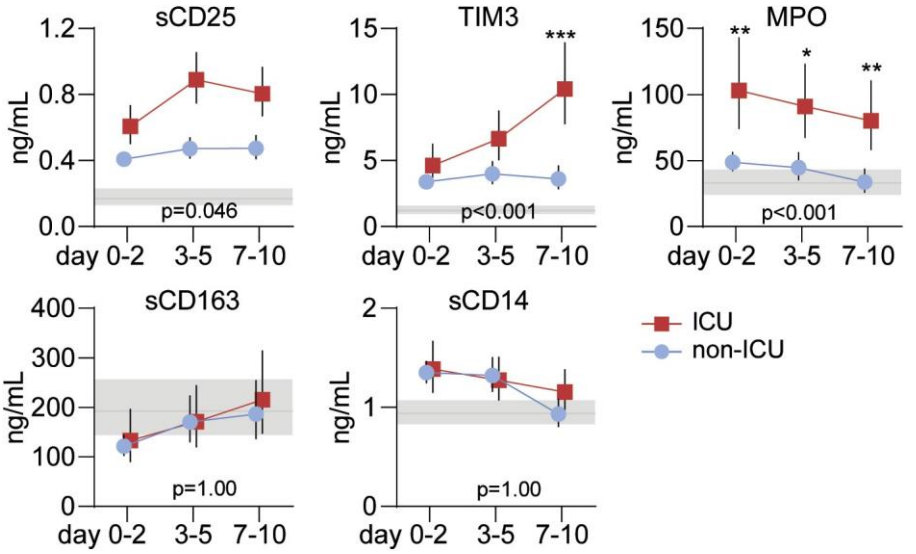

FIG 1. Temporal course of leukocyte activation markers during COVID-19 infection according to ICU admission (ICU patients, n 5 9; non-ICU patients, n 5 30). Data are presented as back-transformed estimated marginal means with 95% CIs from the general linear model procedure (see the Statistical Analysis section). The *P* value given in the graph is the overall effect of ICU admission. *P* values versus non-ICU patients at the same time point are as follows: **P* < .05; ***P* < .01; ****P* < .001. Statistical differences were analyzed with multivariate regression with adjustment for age and eGFR level. The gray area represents the estimated marginal mean (*line*) and 95% CI (*gray area*) in 16 healthy controls analyzed at the same time. Sample numbers in non-ICU patients at days 0 to 2, 3 to 5, and 7 to 10: 27, 16, and 12; sample numbers in ICU patients at the same days: 7, 7, and 6.

TABLE III. Associations of leukocyte activation markers with P/F ratio as a marker of respiratory function, routine biochemical markers, and troponins and NT-proBNP as markers of cardiac involvement

Variable	sCD25	sTIM-3	MPO	sCD163	sCD14
P/F ratio (n 5 72)	− 0.35*	− 0.43†	− 0.39*	− 0.14	− 0.01
Leukocyte count (n 5 73)	0.23	0.36*	0.46†	0.33%	− 0.02
Lymphocyte count (n 5 69)	− 0.01	− 0.07	− 0.04	0.30%	− 0.09
Neutrophil count (n 5 69)	0.15	0.32%	0.42†	0.09	− 0.01
Monocyte count (n 5 68)	− 0.05	0.02	− 0.05	0.40†	− 0.33%
eGFR level (n 5 70)	− 0.45†	− 0.61†	− 0.20	− 0.30%	− 0.27%
hsCRP level (n 5 73)§	0.64†	0.70†	0.51†	0.20	0.27%
NT-proBNP level (n 5 55)§	0.51†	0.58†	0.47†	0.18	0.17

MPO, Myeloperoxidase.

Analysis performed by using partial correlation with adjustment for time point (ie, day 1, days 3-5, and days 7-10) and for some analysis (§) eGFR. The numbers of available data are listed for each variable.

**P* < .01.

†*P* < .001.

%*P* < .05.

at all time points (Fig 1). Myeloperoxidase levels in the non-ICU patients were comparable to the control levels. In contrast to the level of the T-cell markers, however, myeloperoxidase level in the ICU patients decreased during follow-up.

In contrast to the T-cell markers and myeloperoxidase, plasma levels of sCD14 and sCD163, reflecting activation of monocytes/macrophages, were similar in ICU and non-ICU patients (Fig 1).

For the aforementioned analysis of outcomes, age and eGFR were included as covariates when comparing time points; however, excluding these covariates gave similar results.

Association between leukocyte activation markers and markers of respiratory failure and cardiac involvement

Table III shows correlations between levels of leukocyte activation markers and P/F ratio as a marker of respiratory function and NT-proBNP as a marker of cardiac involvement during the course of the study. sCD25, sTIM-3, and myeloperoxidase levels were negatively correlated with the P/F ratio and positively correlated with NT-proBNP level. Notably, these correlations were consistent with a similar pattern at all time points, in particular, for sTIM-3 (Fig 2). Finally, patients with pulmonary infiltrates (regardless of ICU) on their radiographs had higher levels of myeloperoxidase (an estimated mean of 33 ng/mL without infiltrate [95% CI 5 20-54 ng/mL] vs an estimated mean of 66 ng/mL with infiltrate [95% CI 5 51-85 ng/mL]; *P* 5 .015) and sTIM-3 (an estimated mean of 2.3 ng/mL without infiltrate [95% CI 5 1.6-3.4 ng/mL] vs an estimated mean of 4.3 ng/mL with infiltrate [95% CI 5 3.6-5.3 ng/mL]; *P* 5 .006); however, their levels of CD25, CD14, and CD163 were not higher than those without pulmonary infiltrates.

Association between leukocyte activation markers and biochemical variables

Whereas myeloperoxidase levels were positively correlated with neutrophil counts (Table III), none of the T-cell marker levels correlated with lymphocyte counts, of which T cells were the dominating cell subset, suggesting that the increased levels of sCD25 and sTIM-3 do not merely reflect altered numbers of T cells. All markers were correlated with kidney function, but importantly, the associations of sCD25, sTIM-3, and myeloperoxidase levels with ICU admission were also seen after adjustment

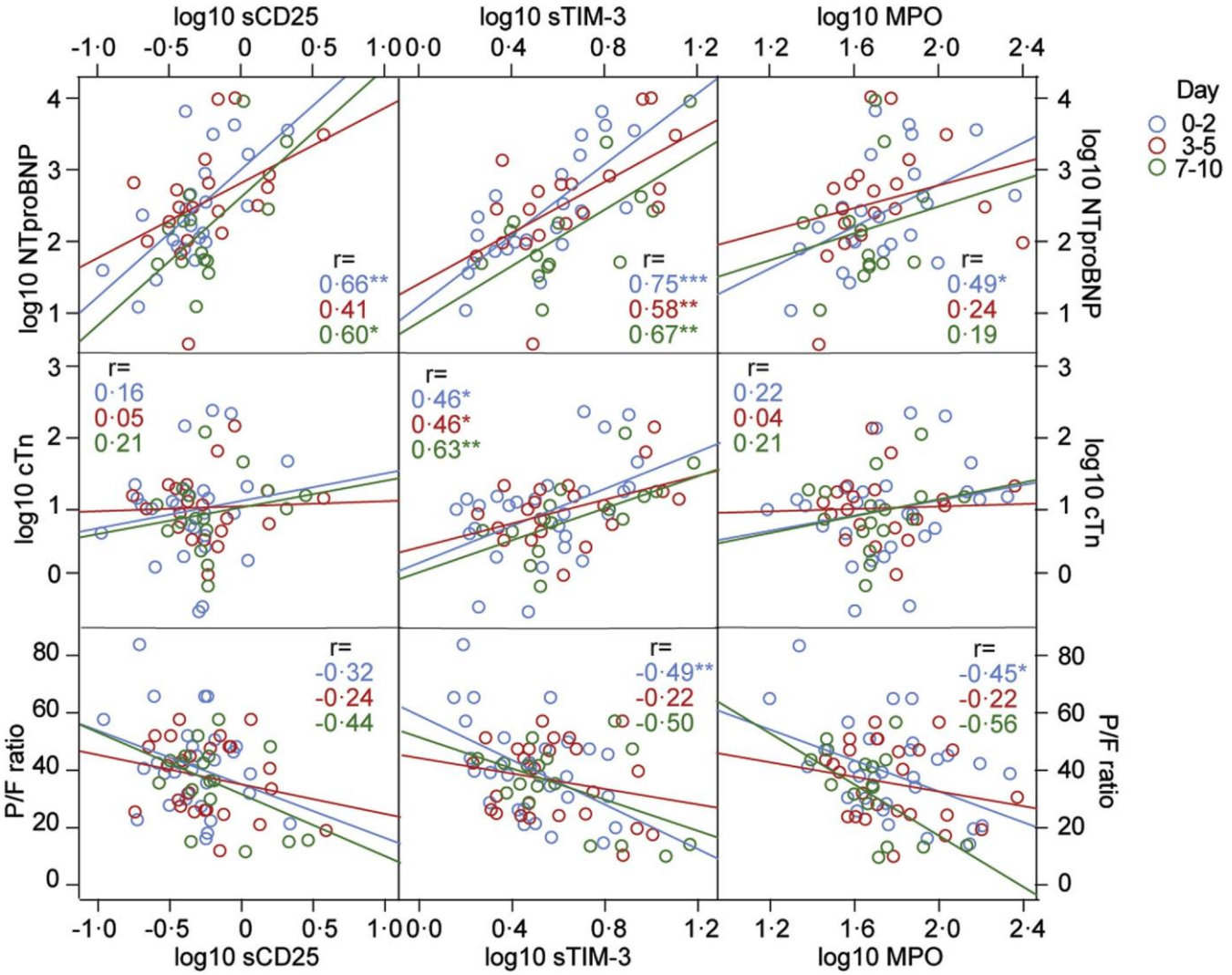

FIG 2. Correlation between selected leukocyte activation markers in 39 hospitalized patients with COVID-19 and NT-proBNP as a marker of cardiac involvement and P/F ratio as a marker of respiratory failure at different time points (day 1 [*blue*], n 5 25-33; day 3-5 [*red*], n 5 19-23; and day 7-10 [*green*], n 5 11-17) during the course of the study. Pearson *r* value is given. *$P < .05$; **$P < .01$; ***$P < .001$.

for eGFR. Finally, the 2 markers of T-cell activation, sCD25 and sTIM-3, were strongly correlated (r 5 0.71; $P < .001$).

DISCUSSION

Rapidly mounting evidence implicates dysregulated immune responses in patients with COVID-19 with severe clinical outcome, characterized by high levels of inflammatory cytokines and low lymphocyte counts.[10-12] Several studies have demonstrated that lymphopenia (in particular, a low number of T cells), is an important feature of COVID-19 infection.[8,10,11,13,14] We have shown that despite signs of T-cell depletion, severe COVID-19 infection as reflected by ICU admission is also characterized by raised levels of sTIM-3, suggesting activated and potentially exhausted T cells.

There are a few reports of elevated sTIM-3 levels in infectious disorders such as HIV,[15] hepatitis B,[16] hepatitis C,[17] malaria falciparum,[18] and pulmonary tuberculosis.[19] To the best of our knowledge, however, this is the first report on sTIM-3 in COVID-19, and to this end, there are no data on sTIM-3 in acute respiratory distress syndrome or influenza infection. Notably, a recent study reported raised serum levels of sTIM-3 in patients with adult Still disease,[20] a condition characterized by hyperinflammation with elevated serum levels of ferritin and C-reactive protein (ie, a state of hyperinflammation that can also exist in severe COVID-19 infection). Although the function of sTIM-3 is not clarified, it seems to reflect the degree of membrane expression of this checkpoint inhibitor.[15] Because we examined a larger number of patients as well as an association with admission to the ICU, our finding extends findings of the study by Diao et al, who showed that T cells in patients with COVID-19 appear functionally exhausted, as reflected by higher expression of TIM-3 on T cells.[21] However, the term ***T-cell exhaustion*** is mostly used in relation to chronic infections.[22] During acute COVID-19 infection, enhanced sTIM-3 level could be regarded as a marker of T-cell activation, as underscored by the strong correlation between sTIM-3 and sCD25 in our study. Moreover, whereas long-term T-cell exhaustion could contribute to immune

dysregulation, T-cell exhaustion in the short-term could be beneficial by dampening inflammation. We speculate that the increase in sTIM-3 level toward the end of the observation period in the ICU patients in the present study could reflect a mechanism to prevent persistent and overshooting T-cell activation, which could harm the host.[23] However, further studies are needed to evaluate the pathogenic significance of T-cell activation and exhaustion during COVID-19 infection.

In contrast to the association of T-cell markers with outcome, we observed no association for the monocyte/macrophage markers sCD14 and sCD163. However, myeloperoxidase being released from activated neutrophils,[24] as well as neutrophil counts, were positively associated with ICU admission. Neutrophils are important cellular sources of matrix-degrading enzymes, and Zuo et al recently showed increased levels of myeloperoxidase DNA and other indices of neutrophil extracellular trap formation in COVID-19 – infected patients compared with those in controls.[25] Moreover, myeloperoxidase and TIM-3 levels were both correlated with respiratory function as reflected by the P/F ratio, and higher levels were observed in those with pulmonary infiltrates than in those with no pulmonary infiltrates. Thus, whereas much focus has been placed on the role of monocytes/macrophages in the development of COVID-19 – related respiratory failure, our finding may suggest that excessive T-cell and neutrophil activation could also be involved.

Increasing evidence indicates that COVID-19 – infected patients are at higher risk of developing cardiac involvement or cardiovascular-related death,[3,26] and accordingly, a majority of ICU patients in our study had elevated NT-proBNP levels. The spectrum of cardiac involvement in COVID-19 infection ranges from type 1 and type 2 myocardial infarction to myocarditis, and in more general terms, T cells are known to participate in these conditions.[27,28] The consistent correlation between the T-cell markers with NTproBNP at all time points supports a pathogenic role for T cells in the cardiac involvement during COVID-19 infection. In the present study, however, the cardiac involvement as reflected by raised levels of NT-proBNP was most probably secondary to disease severity, and larger studies are need to clarify a role for T-cell activation in COVID-19 – related cardiac disease.

Our study has some limitations. First, this study included only a small number of patients with ICU admission. Thus, the results should be interpreted with caution. Second, we did not have blood samples at all time points in all patients. Third, although we found no association with outcome for the monocyte/macrophage markers, this does not exclude a vital role for these cells. Local activation in the lungs, heart, or other tissues may not necessarily be reflected by systemic levels of monocyte/macrophage activation. Finally, we lack data on functional impairment of cardiac function by, for example, echocardiographic examination.

In conclusion, we found that patients admitted to the ICU during COVID-19 hospitalization were associated with high plasma levels sTIM-3 and myeloperoxidase. Our findings may suggest that T-cell activation and exhaustion and neutrophil activation play an important role in the pathogenesis of COVID-19 infection. Thus, T-cell – targeted treatment options, as well as modalities targeting neutrophil activation, could be of interest in this disorder. However, as most patients with severe COVID-19 infection present with T-cell lymphopenia, additional experimental data are needed to support the rationale of T-cell – targeted therapy for the exhausted T cells.

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Key messages

- Patients with COVID-19 with severe outcome were characterized by high plasma levels of markers of T-cell and neutrophil activation, but not by markers of monocyte activation.
- Despite lymphopenia, T cells in patients with COVID-19 were activated and potentially exhausted, and this process was associated with severe outcome.
- Therapy targeting T-cell activation and exhaustion as well as neutrophil activation could be of particular interest in hospitalized patients with COVID-19.

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