Levels of Soluble CD14 and Tumor Necrosis Factor Receptors 1 and 2 may be predictive of death in Severe Coronavirus Disease 2019 (COVID-19)

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Summary

Individuals infected with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) display increased inflammation and monocyte activation, regardless of disease severity. Higher levels of TNFR1 and 2 were associated with death in patients with severe Coronavirus Disease-19 (COVID-19).

Abstract

People infected with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) display a wide range of illness, from asymptomatic infection to severe respiratory distress resulting in death. We measured serum biomarkers in uninfected individuals and in individuals with mild, moderate, or critical COVID-19 disease. Levels of monocyte activation (sCD14 and FABP4) and inflammation (TNFR1 and 2) were increased in COVID-19 individuals, regardless of disease severity. Among patients with critical disease, individuals who recovered from COVID-19 had lower levels of TNFR1 and TNFR2 at hospital admission compared to these levels in patients with critical disease that ultimately died.

Keywords: SARS-CoV-2; monocytes; tumor necrosis factor; COVID-19

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a viral infection that causes Coronavirus Disease 2019 (COVID-19) and has resulted in over 52 million confirmed cases and over 1.28 million deaths worldwide as of November 11, 2020. The disease course for individuals infected with SARS-CoV-2 is varied, with some experiencing no symptoms, whereas others require hospitalization. Several factors may play a role in the severity of COVID-19; underlying medical conditions including cardiovascular disease (CVD), obesity, and diabetes have been identified as risk factors for a severe disease course[1]. A significant proportion of individuals with COVID-19 experience damage to their lungs, heart, or kidneys[2], even if their disease course is not severe. There is a growing body of literature describing profiles of immune responses to SARS-CoV-2 that may relate to disease severity, but our understanding of how the immune activation may result in clearance of the virus or induce tissue damage and death is incomplete. Severe COVID-19 appears to be associated with increased inflammatory cytokines and markers of endothelial cell activation and coagulation[3, 4]. Recently, several immune phenotypes were identified in COVID-19; a subset of infected individuals with severe disease showed increased T and B cell activation, while others with severe disease had minimal lymphocyte activation[5].

We have reported on the immune response to severe acute respiratory syndrome (SARS)[6] and here, we explored immune activation in COVID-19. In this cross sectional and longitudinal study, we examined serum levels of immune activation and inflammation, including markers associated with monocyte/macrophage activation (soluble CD14 (sCD14), soluble CD163 (sCD163), fatty acid binding protein 4 (FABP4)). COVID-19 patients were stratified into mild, moderate, or critical disease, based on oxygen requirements and the need for mechanical ventilation. Approximately half of the patients with critical disease died from the infection. We have identified markers of immune activation that were increased in patients who died of COVID-19 compared to levels in patients with critical disease who recovered. As patients recovered from COVID-19, we observed decreases in immune

activation over time, while patients who did not recover tended to maintain elevated levels of monocyte activation and inflammation. Interestingly, biomarkers associated with CVD and obesity (lipoprotein-associated phospholipase A2 (Lp-Pla2), oxidized low-density lipoprotein (OxLDL), adiponectin) were not associated with severity of disease or death.

Materials and Methods

Study participants

COVID-19 participants were enrolled at MetroHealth Medical Center in Cleveland, OH, and provided informed consent (IRB 20-00198). Disease severity was classified based on oxygen requirements and the need for mechanical ventilation. Patients who did not need oxygen supplementation were categorized as 'mild', patients who required oxygen were categorized as 'moderate', and those who were intubated were categorized as 'critical'.

Control participants were recruited from the general population of the Ohio State University (OSU); experiments were performed in compliance with OSU IRBs 2014H0467 and 2020H0339.

Sample collection

Blood samples were collected in serum separating tubes (SST; BD Biosciences) and centrifuged for 10 minutes at 1000 x g. Serum was collected and frozen at -80°C until thawed once and analyzed in batch. Samples from COVID-19 patients were collected at hospital admission (approximately), prior to treatment, and longitudinally.

Soluble markers

Serum markers of immune activation were measured by enzyme linked immunosorbent assays (ELISA, R&D Systems, unless otherwise noted): sCD14, sCD163, tumor necrosis factor receptor (TNFR) 1, TNFR2, Lp-Pla_2, adiponectin, OxLDL (Mercodia) and LPS-binding protein (LBP) (Hycult Biotech).

Statistical Analyses

Differences in biomarker levels between groups were determined using nonparametric t tests. Changes in longitudinal timepoints from COVID-19 patients were analyzed by paired t tests (GraphPad Prism 6).

Results

Demographic information for control (N=14) and COVID-19 (N=44) study participants is provided in **Supplemental Table 1**. We measured levels of OxLDL, adiponectin, and Lp-PLA_2, as these markers are associated with CVD, obesity, and diabetes, and these comorbid conditions predict more severe COVID-19[1]. Levels of these markers were not different between the COVID-19 patients and controls (**Figure 1**) and these markers were not associated with disease course (**Supplemental Figure 1, 2**). Levels of lipopolysaccharide binding protein (LBP) were increased in COVID-19 patients (33379 ng/mL v 19889 ng/mL, p<0.0001; **Figure 1, 2**), and may reflect increased translocation of microbial products from the gastrointestinal tracts of these patients. Patients with moderate disease displayed a significant decrease in LBP levels over time, whereas critical patients did not (**Supplemental Figure 3**).

Morbidity and mortality in COVID-19 is also associated with hyperinflammation and a better understanding of how inflammatory biomarker levels relate to differential clinical outcomes in COVID-19 patients is important. Serum levels of monocyte and macrophage activation, sCD14 (2964 ng/mL v 1495 ng/mL, p<0.0001), sCD163 (1072 ng/mL v 644 ng/mL, p=0.01), and FABP4 (52,969 pg/mL v 18,733 pg/mL, p=0.0001), were increased in COVID-19 patients compared to levels in controls (**Figure 1**). COVID-19 patients with critical disease who ultimately died displayed higher levels of sCD14 and FABP4 than did critical disease patients who recovered and patients with more mild disease (**Figure 2**). Additionally, we observed a significant decrease in sCD14 levels over time in COVID-19

patients with moderate disease, whereas critical patients who died did not display similar reductions in sCD14 (**Supplemental Figure 3**). Although sCD163 levels at initial time points did not differ among disease categorizations, we observed a significant increase in sCD163 serum concentrations over time only in patients who ultimately died (**Supplemental Figure 3**).

Markers of systemic inflammation (TNFR1; 2980 pg/mL v 1063 pg/mL, p=0.003) and TNFR2 (7797 pg/mL v 1694 pg/mL, p<0.0001) were increased in COVID-19 patients, and were associated with disease severity (**Figure 1, 2**). Upon initial evaluation following hospital admission, serum concentrations of TNFR1 and TNFR2 were higher in critical patients who ultimately died compared to concentrations in critical patients who recovered. Levels of TNFR1 and 2 were also increased in patients with mild to moderate disease compared to levels in controls (**Figure 2**). Furthermore, COVID-19 patients with mild to moderate disease displayed reduced levels of TNFR1 and TNFR2 over time; we did not observe similar decreases in concentrations of these markers in critical patients.

Discussion

Critically ill patients with COVID-19 display a broad spectrum of T and B cell activation [5], suggesting that lymphocyte activation may not accurately predict disease course in COVID-19. Here, we report that elevated biomarkers of monocyte activation and inflammation may be associated with severe disease outcomes in COVID-19 patients. Compared to levels measured in controls, levels of the monocyte/macrophage activation markers, sCD14, sCD163, and FABP4, were increased in patients with SARS-CoV-2. Levels of TNFR1 and TNFR2 were also increased in COVID-19 patients, and at the time of hospital admission, prior to treatment, levels of these markers were elevated in patients with critical disease who ultimately died compared to levels in critical patients who recovered. Furthermore, sCD14 levels were directly correlated with TNFR1 (p=0.005; r=0.433) and TNFR2 (p=0.0004; r=0.600). These findings suggest that markers of monocyte activation

and levels of inflammatory biomarkers TNFR1 and TNFR2 are associated with disease severity and may be predictive of mortality in critically ill patients.

Soluble CD14 levels were also associated with LBP (p=0.018; r=0.373). Gastrointestinal (GI) epithelial cells express ACE-2 receptors through which SARS-CoV-2 can invade the intestinal tract, causing GI dysfunction[7]. Increased inflammatory responses also disrupt intestinal barriers leading to translocation of microbial products, which can amplify systemic inflammation[8]. Our observation that circulating LBP levels are increased in COVID-19 patients suggests GI involvement in COVID-19 disease progression. GI complications (e.g. diarrhea) were also reported at higher frequency in patients with critical disease (mild: 27%; moderate: 29%; critical recovered/deceased: 53%). Although LBP levels were not significantly different among critical patients with or without reported diarrhea, several inflammatory markers were increased in critical patients with diarrhea (sCD14: 3499 ng/mL; TNFR1: 4920 pg/mL; TNFR2: 8173 pg/mL) compared to critical patients without reported diarrhea (sCD14: 2785 ng/mL, p=0.04; TNFR1: 2660 pg/mL, p=0.06; TNFR2: 4376 pg/mL, p=0.03). Studies exploring associations among microbial translocation, the composition of the gut microbiome, and excessive inflammatory responses in COVID-19 should be considered.

Our data implicate monocyte/macrophage activation as a potential contributor to deaths related to critical COVID-19 infection. Monocyte activation is associated with mortality and cardiovascular risk in the general population and in people with HIV (PWH) [9, 10]. We have reported associations between sCD14 and HIV viremia, microbial translocation, and proinflammatory lipid profiles [11]; the mediators of increased levels of sCD14 levels in COVID-19 should be identified. Monocytes are made up of distinct subgroups based on CD14 and CD16 expression[12]. Proportions of “inflammatory” $CD14^+$ $CD16^+$ monocytes are increased in PWH, sepsis, diabetes, and CVD [11, 13]. We have also reported that $CD16^+$ monocytes in PWH and in people without HIV who have recently experienced an acute coronary event express high levels of tissue factor (TF), a

procoagulant molecule that correlates with plasma D-dimer levels. Patrolling monocytes ($CD14^{Dim}CD16^{+}$) recognize viral products[12, 13] and express high levels of the fractalkine receptor (CX3CR1) which may cause these cells to home to the vascular endothelium. COVID-19 is associated with vascular inflammation and coagulopathy[1, 2], including increased levels of D dimer and fractalkine [3]. Induction of TF expression following recognition of viral products, and the ability of these cells to migrate to the endothelium, likely make $CD14^{Dim}CD16^{+}$ cells potential mediators of vascular inflammation and coagulation in viral infections, including SARS-CoV-2. Further characterization of these cells in COVID-19 patients and their association with coagulation and microthrombi formation is warranted.

Increased levels of TNFR1 and/or TNFR2 are associated with mortality in PWH, renal disease, obesity, diabetes, and acute lung and kidney injuries. Several inhibitors of TNF are already used clinically (e.g. Etanercept and Adalimumab) for inflammatory diseases. Exposure of monocytes and macrophages to microbial products may result in increased production of TNF, enhancing the activation status of these cells, promoting adhesion molecule expression on endothelial cells, and increasing expression of procoagulant molecules including TF. Here, increased levels of TNFR1 and TNFR2 were related to mortality in COVID-19 patients. At the time of hospital admission and prior to treatment, patients with critical COVID-19 who eventually died, had significantly higher levels of both TNFR1 and TNFR2, compared to levels in critical COVID patients who recovered. Levels of TNFR1 and 2 often decreased overtime in critical/recovered patients; levels in critical/deceased patients remained elevated. Therefore, the TNF/TNFR signaling cascade may be an effective target to reduce mortality in patients with critical COVID-19. Our findings are in line with results from a study that demonstrated dexamethasone treatment reduced mortality in COVID-19 patients receiving ventilation or oxygen support, compared to the rates of mortality in patients of similar disease severity who received the standard of care[14]. Dexamethasone may inhibit the upstream signaling that leads to TNF production

and may also inhibit the downstream effects of TNF: TNF receptor engagement. Further study on treatments aimed at inhibition of TNF in COVID-19 are needed.

Our study has limitations. The control group and COVID-19 patients were not ideally matched based on age and ethnicity. COVID-19 patients also had increased incidence of diabetes and hypertension relative to the controls, and patients with critical disease tended to be male (**Supplemental Table 2**). This relatively small cohort may not reflect the global population of COVID-19 patients. While we report several important clinical cofactors that may be related to disease severity (e.g. age, smoking status, BMI), samples from the COVID-19 population may be collected at different time points post-infection. This limitation is made less important due to the longitudinal results we present, that demonstrate predictable changes in inflammatory markers in mild, moderate, and critical populations. Although we did not observe alterations in markers associated with obesity (adiponectin), oxidative stress (OxLDL), and vascular inflammation (Lp-PLA_2) in the severity of COVID-19 participants, more expansive future studies might demonstrate associations with these indices and morbidity/mortality. We report the incidence of several other underlying conditions in this cohort, but none were especially related to disease severity (**Supplemental Table 2**). Interpretation of these findings in a larger study are needed, yet, our data suggest that activation of monocytes/macrophages and TNF-associated signaling cascades may be prognostic of disease course in COVID-19. Exploring relationships among specific immune activation profiles, COVID-19 symptoms, and end organ involvement, may enhance clinical care.

Conflict of Interest

Dr. Funderburg has served as a consultant for Gilead.

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Figure legends

Figure 1. Biomarker levels in serum samples from healthy (n=14, blue) and COVID-19 patients (n=44, red) were analyzed by ELISA. Bars indicate mean values with standard deviation. Unpaired t tests were used for statistical comparison among groups **p<0.01; ***p<0.001; ****p<0.0001

Figure 2. COVID-19 patients were categorized by disease state (mild, n=10; moderate, n=14; critical recovered, n=7; critical deceased, n=8), and serum biomarker levels from patients in each clinical classification were compared to levels measured in healthy controls. Bars indicate mean values with standard deviation. Unpaired t tests were used for statistical comparison among groups *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

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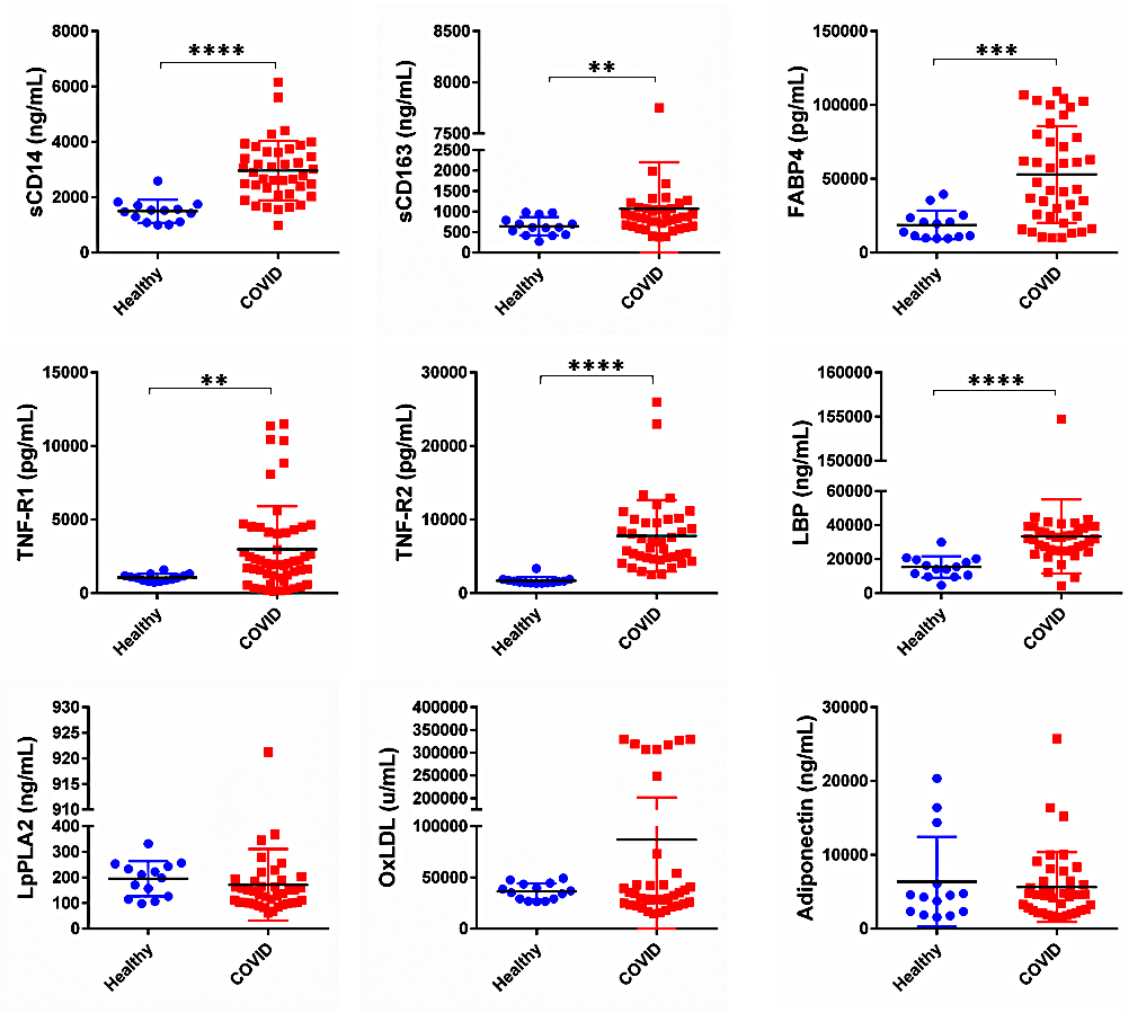
Figure 1


sCD14 (ng/mL)
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Healthy
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sCD163 (ng/mL)
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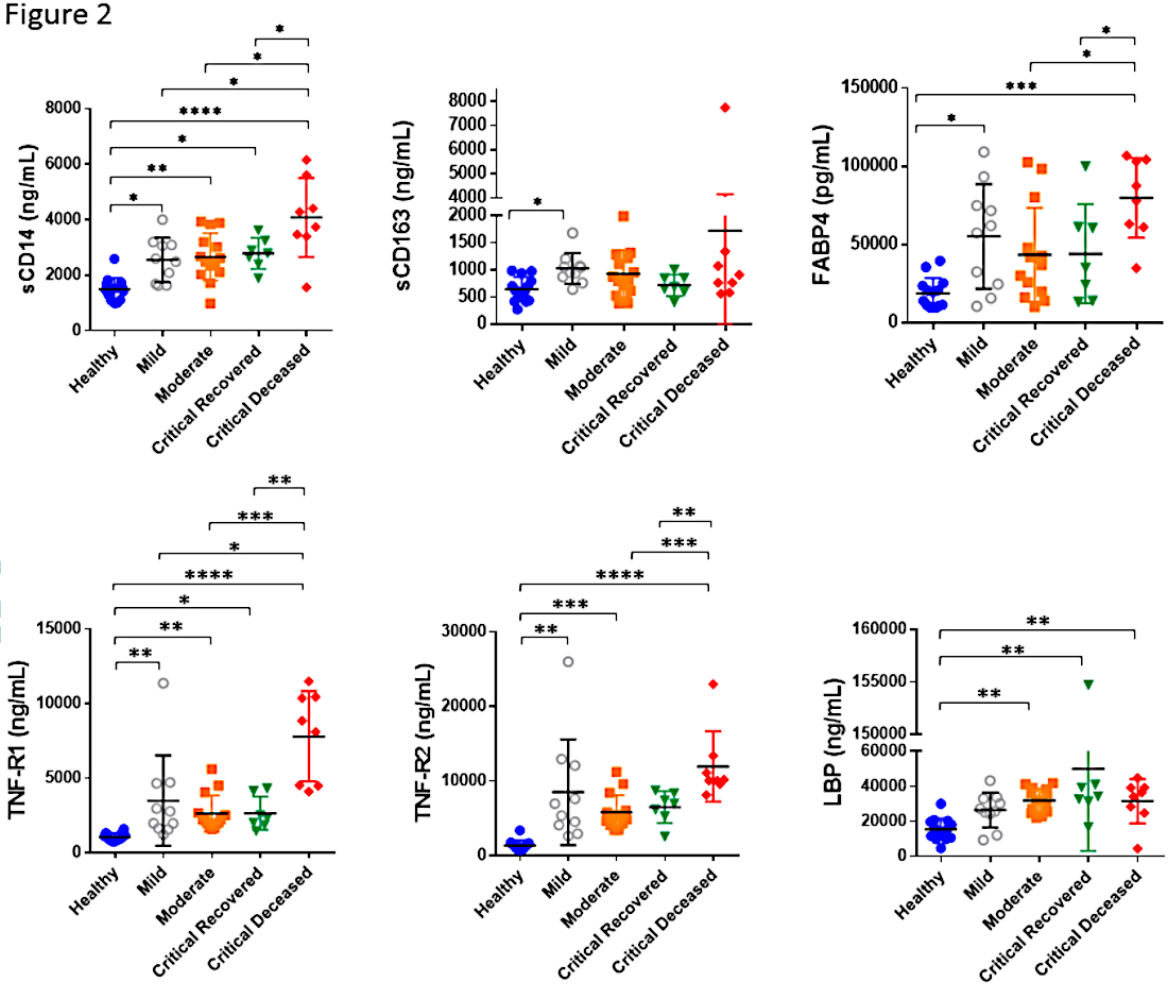
Figure 2


sCD14 (ng/mL)
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Healthy
Mild
Moderate
Critical Recovered
Critical Deceased