OPEN

The Association of Inflammatory Cytokines in the Pulmonary Pathophysiology of Respiratory Failure in Critically Ill Patients With Coronavirus Disease 2019

Sophie Stukas, PhD[1,2]; Ryan L. Hoiland, PhD[3,4]; Jennifer Cooper, BMLSc[1,2]; Sonny Thiara, MD[5]; Donald E. Griesdale, MD, MPH[3,5]; Adam D. Thomas, MD[5]; Matthew M. Orde, MBChB[6]; John C. English, MD[6]; Luke Y. C. Chen, MD, MMEd[7]; Denise Foster, RN[5]; Anish R. Mitra, MD, MPH[5]; Kali Romano, MD[5]; David D. Sweet, MD[5,8]; Juan J. Ronco, MD[5]; Hussein D. Kanji, MD, MSc[5,8]; Yu-Wei R. Chen, MSc[6]; Sophia L. Wong, MD MBA[1,6]; Cheryl L. Wellington, PhD[1,2,9]; Mypinder S. Sekhon, MD[5]

Objectives: The majority of coronavirus disease 2019 mortality and morbidity is attributable to respiratory failure from severe acute respiratory syndrome coronavirus 2 infection. The pathogenesis underpinning coronavirus disease 2019-induced respiratory failure may be attributable to a dysregulated host immune response. Our objective was to investigate the pathophysiological relationship between pro-inflammatory cytokines and respiratory failure in severe coronavirus disease 2019.

Design: Multicenter prospective observational study.

Setting: ICU.

Patients: Critically ill patients with coronavirus disease 2019 and noncoronavirus disease 2019 critically ill patients with respiratory failure (ICU control group).

Interventions: Daily measurement of serum inflammatory cytokines.

Measurements and Main Results: Demographics, comorbidities, clinical, physiologic, and laboratory data were collected daily. Daily serum samples were drawn for measurements of interleukin-1β, interleukin-6, interleukin-10, and tumor necrosis factor-α. Pulmonary outcomes were the ratio of Pao_2/Fio_2 and static lung compliance. Twenty-six patients with coronavirus disease 2019 and 22 ICU controls were enrolled. Of the patients with coronavirus disease 2019, 58% developed acute respiratory distress syndrome, 62% required mechanical ventilation, 12% underwent extracorporeal membrane oxygenation, and 23% died. A negative correlation between interleukin-6 and Pao_2/Fio_2 (rho, −0.531; $p = 0.0052$) and static lung compliance (rho, −0.579; $p = 0.033$) was found selectively in the coronavirus disease 2019 group. Diagnosis of acute respiratory distress syndrome was associated with significantly elevated serum interleukin-6 and interleukin-1β on the day of diagnosis.

Conclusions: The inverse relationship between serum interleukin-6 and Pao_2/Fio_2 and static lung compliance is specific to severe acute respiratory syndrome coronavirus 2 infection in critically ill patients with respiratory failure. Similar observations were not found with interleukin-β or tumor necrosis factor-α.

[1]Department of Pathology and Laboratory Medicine, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada.

[2]Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada.

[3]Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, BC, Canada.

[4]Centre for Heart, Lung, & Vascular Health, School of Health and Exercise Sciences, University of British Columbia Okanagan, Kelowna, BC, Canada.

[5]Division of Critical Care Medicine, Department of Medicine, University of British Columbia, Vancouver, BC, Canada.

[6]Department of Pathology and Laboratory Medicine, Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada.

[7]Division of Hematology, Department of Medicine, University of British Columbia, Vancouver, BC, Canada.

[8]Department of Emergency Medicine, University of British Columbia, Vancouver, BC, Canada.

[9]International Collaboration on Repair Discoveries, School of Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada.

Crit Care Expl 2020; 2:e0203

DOI: 10.1097/CCE.0000000000000203

Key Words: acute respiratory distress syndrome; coronavirus disease 2019; interleukin-6; respiratory failure; severe acute respiratory syndrome coronavirus 2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and resultant coronavirus disease 2019 (COVID-19) has caused greater than 12.9 million confirmed cases and greater than 570,000 deaths globally as of July 14, 2020 (1). COVID-19 may progress to severe respiratory dysfunction, manifesting as acute respiratory distress syndrome (ARDS), and multiple organ failure (2–4).

The symptomology and morbidity/mortality of COVID-19 stem from respiratory pathophysiologic sequelae, but the precise mechanisms are uncertain, with an immunopathological response or direct viral replication-induced lung injury suggested as culprits (5). The onset of severe respiratory failure (3) and mortality (6) are both linked to an exaggerated host immune response (7). This has increased interest in immunomodulatory therapy for COVID-19 (5, 8). However, it remains unclear whether it is appropriate to ascribe the severity of COVID-19-related respiratory failure to cytokine storm syndrome (9), a debate driven by lack of prospective data in critically ill patients with COVID-19.

Although studies to date have included severe COVID-19 patients, the proportion of critically ill COVID-19 patients is minimal and existing studies have assessed a single time point with cross-sectional designs rather than prospective repeated longitudinal measures (6, 10–12). This highlights a major gap in COVID-19 pathophysiology as the most significant deterioration in respiratory function and elevations of inflammatory cytokines would reasonably be expected to occur in critically ill patients, a population in which the majority of mortality occurs, and that poses a sizeable resource burden for healthcare systems (13). Interleukin (IL)–6, IL-1β, and tumor necrosis factor-α (TNFα) have emerged as potential targets given their central role in inflammatory signaling cascades and readily available immunomodulatory therapies that target their specific actions (5).

We conducted a prospective observational study to investigate the relationships between IL-6, IL-1β, and TNFα and measures of clinical and physiologic respiratory function, namely the ratio of Pao_2/Fio_2 and static lung compliance, in critically ill patients with severe COVID-19. We hypothesized that elevated IL-6, IL-1β, and TNFα would be associated with worse Pao_2/Fio_2 and static lung compliance.

MATERIALS AND METHODS

The study was approved by the University of British Columbia Clinical Research Ethics Board (H20-00971) and registered on ClinicalTrials.gov (NCT04363008). All research was conducted in accordance with the principles of the Helsinki declaration and Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

Patient Management and Study Site

The study was conducted in the ICUs of Vancouver General Hospital and Surrey Memorial Hospital, mixed medical and surgical academic closed quaternary ICUs. Clinical management was in accordance with the Surviving Sepsis Campaign (14). Intubated patients with COVID-19 were ventilated with a lung protective strategy (~6 mL/kg tidal volume), maintenance of plateau pressure less than 30 cm H_2O and titration of positive end-expiratory pressure (PEEP) to optimize systemic oxygenation and maintain recruited lung. Prone positioning, inhaled nitric oxide, and venovenous extracorporeal membrane oxygenation (ECMO) were used for refractory hypoxemia.

Patients and Controls

COVID-19 patients admitted to ICU with a diagnosis of pneumonia secondary to SARS-CoV-2 infection, confirmed by reverse transcription polymerase chain reaction (RT-PCR) positive nasopharyngeal or tracheal swabs were enrolled. Consecutive patients admitted with a primary diagnosis of hypoxemic respiratory failure ($Pao_2/Fio_2 \leq 300$) secondary to community- or hospital-acquired pneumonia and two successive (nasopharyngeal and tracheal) negative SARS-CoV-2 RT-PCR tests were designated as ICU controls. Serum samples were collected from nine healthy community controls ($n = 2$ males [22%]; median age 27 [interquartile range (IQR), 22–37]) collected prior to the SARS-CoV-2 pandemic to compare against COVID-19 patients and ICU controls.

Data Collection and Inflammatory Biomarkers

ICU admission demographics pertaining to age, sex, medical comorbidities, smoking (current status) and date of: symptom onset, initiation of mechanical ventilation, and hospital and ICU admission were collected. Median daily measurements of Pao_2/Fio_2, static lung compliance (tidal volume [mL]/[plateau pressure–total PEEP (cm H_2O)]), and dead space fraction ([$Paco_2$ (mm Hg)–end-tidal carbon dioxide tension (mm Hg)]/$Paco_2$) were recorded. A daily inspiratory and expiratory hold was conducted to measure static lung compliance. Daily medians from hourly recordings of heart rate (beats/min), mean arterial pressure (mm Hg), IV norepinephrine dose (μg/min), temperature (°C), PEEP (cm H_2O), tidal volume (mL), and minute ventilation (L/min) were collected. A diagnosis of ARDS was denoted by fellowship-trained intensivists in accordance with the Berlin criteria (S.T., M.S.S.) (15). Daily laboratory values included as follows: C-reactive protein (CRP), D-dimer, complete blood count (WBC count and differential, hemoglobin concentration, and platelet count), ferritin, creatinine, liver enzymes, and bilirubin. In a subcohort of 15 patients, viral load (log_{10} copies) was quantified using nasopharyngeal ($n = 13$) or tracheal ($n = 2$) swabs.

Daily arterial blood specimens were obtained from each SARS-CoV-2 positive patient for days 1–7, 10, 14, and 21 following ICU admission. For ICU controls, arterial blood specimens and data collection for all variables were collected on day 1 of ICU admission. Serum cytokines were quantified using the Simoa HD-1 platform from Quanterix (Billerica, MA) following the manufacturer's protocol, using a 128-fold in lieu of four-fold dilution for the 3-plex assay to accommodate the IL-6 response. IL-6, IL-10, and TNFα were measured using a cytokine 3-plex A advantage assay (101160), while IL-1β was measured using the human IL-1β advantage assay (101605). The lower limit of detection, lower limit

of quantification, and upper limit of quantification as measured in the assay well are: 0.006, 0.011, and 20 pg/mL (IL-6); 0.0022, 0.0073, and 6 pg/mL (IL-10); 0.011, 0.051, and 28 pg/mL (TNFα); and 0.016, 0.083, and 120 pg/mL (IL-1β).

Cross-Platform Analytical Validation

A subset of 39 serum specimens spanning the analytical range of IL-6 (0.597–5,029 pg/mL) were selected for secondary quantification using the commercially available Siemens ADVIA Centaur XP analyzer (Munich, Germany) for cross-platform comparison with the Quanterix Simoa HD-1 platform. The dynamic range of the ADVIA Centaur IL-6 assay (10995080) is 2.7–5,500 pg/mL. Study members analyzing serum specimens were blinded to all clinical data and to whether specimens were from COVID-19 patients or ICU controls.

Outcome Measures

Primary, secondary, and tertiary outcomes were selected a priori. The primary outcome was the relationship between serum inflammatory cytokines and study enrollment Pao_2/Fio_2 (before use of rescue maneuvers for refractory hypoxemia) in patients with COVID-19 compared with ICU controls. The secondary outcome was the relationship between inflammatory cytokines and static lung compliance. The tertiary outcome was the quantifiable difference in serum inflammatory cytokines between patients with COVID-19 with and without ARDS (15). Relationships between clinical inflammatory variables (CRP, ferritin) and the aforementioned pulmonary outcomes were also analyzed.

Statistical Analysis

Descriptive statistics, including median, IQRs, and frequency, were used to describe continuous and categorical variables, respectively. Group differences were tested using a Mann-Whitney *U* test (two groups) or Kruskal-Wallis test (three or more groups) for continuous variables, or Fisher exact test for categorical variables. Associations between serum cytokines and Pao_2/Fio_2 and static lung compliance were assessed using the Spearman rank correlation test. All statistical tests were two-sided and a *p* value of less than 0.05 was considered significant. *p* values from analyses of secondary or subgroups comparisons are reported after correcting for multiple comparisons to limit type I errors. For comparisons of three or more groups of non-normally distributed data, we corrected for multiple comparisons using Dunn test, following the groupwise Kruskal-Wallis test. All statistical analyses were completed using Graphpad Prism (Version 7.03; San Diego, CA).

RESULTS

Between March 30, 2020, and May 17, 2020, 48 patients were enrolled; 26 were in the COVID-19 group and 22 in the ICU control group. The etiology of respiratory failure in the ICU control cohort was community-acquired pneumonia (n = 16, 73%) and hospital-acquired pneumonia (n = 6, 27%).

Demographics, clinical characteristics, and laboratory findings are shown in **Table 1** and **sTable 1** (http://links.lww.com/CCX/A347). Compared with ICU controls, COVID-19 patients had a lower lymphocyte count (0.6 vs 0.9×10^9/L; $p = 0.0016$) and higher alanine aminotransferase (83 vs 32 U/L; $p = 0.033$), lactate dehydrogenase (407 vs 262 U/L; $p < 0.0001$), and ferritin (1,257 vs 349 μg/L; $p < 0.0001$). Both groups had elevated D-dimer (1,422 vs 2,298 mg/L) and CRP (112 vs 140 mg/L) compared with established reference ranges. Within the COVID-19 group, 16 patients (62%) required mechanical ventilation, with a median duration of ventilation of 8 days (IQR, 5–12 d); five patients underwent prone ventilation (31%), one received inhaled nitric oxide (6%), three patients (12%) underwent venovenous ECMO, and six patients (23%) died (**sTable 2**, http://links.lww.com/CCX/A348). Twelve patients (46%) with COVID-19 were administered corticosteroids and six patients (23%) were given tocilizumab. All IL-6 data shown is prior to tocilizumab administration.

The concentrations of serum IL-6, IL-1β, IL-10, and TNFα were significantly higher in both COVID-19 and ICU control patients compared with healthy control values. Of these, the most marked difference was the ~100-fold higher concentration of IL-6 (Table 1 and **sFig. 1**, http://links.lww.com/CCX/A344). A significant negative correlation between serum IL-6 and Pao_2/Fio_2 (rho, –0.531; $p = 0.0052$), and static lung compliance (rho, –0.579; $p = 0.033$), was observed only in patients with COVID-19 (**Fig. 1**). There were no correlations between Pao_2/Fio_2 or static lung compliance and serum IL-1β, TNFα, IL-10, CRP, ferritin, or viral load (log_{10} copies) in patients with COVID-19 or ICU controls (Fig. 1 and **sTables 3**, http://links.lww.com/CCX/A349 and **4**, http://links.lww.com/CCX/A350).

Thirteen out of 26 patients with COVID-19 (50%) developed ARDS over the course of the study. On the day of ARDS diagnosis, serum IL-6 ([median ARDS vs non-ARDS] 167 vs 59.2 pg/mL; $p = 0.0029$) and IL-1β (0.53 vs 0.16 pg/mL; $p = 0.022$) were significantly increased in the ARDS versus non-ARDS group (**Fig. 2** and **sTable 5**, http://links.lww.com/CCX/A351). The requirement for mechanical ventilation was associated with increased inflammatory cytokines; the maximum concentration of IL-6 (1,329 vs 62.2 pg/mL; $p = 0.0005$), IL-1β (0.61 vs 0.16 pg/mL; $p = 0.0021$), TNFα (28.6 vs 7.64 pg/mL; $p = 0.0002$), and CRP (188 vs 118 mg/dL; $p = 0.036$) were increased in COVID-19 patients requiring mechanical ventilation (**sFig. 2**, http://links.lww.com/CCX/A345 and **sTable 6**, http://links.lww.com/CCX/A352).

Longitudinally, we observed three temporal patterns of cytokine responses: 1) decreased IL-6 over time in patients who recovered; 2) elevated IL-6 prior to death from refractory distributive shock; and 3) elevated IL-6 preceding worsening respiratory failure (**sFig. 3**, http://links.lww.com/CCX/A346). **Figure 3** demonstrates data derived from a patient who succumbed to COVID-19, with longitudinal measurements of serum IL-6 and Pao_2/Fio_2. Chest radiography demonstrates progressive bilateral opacification, and chest CT reveals bilateral pulmonary fibrosis with traction bronchiectasis. This patient exhibited a pronounced increase of serum IL-6 (775 pg/mL) on the day following intubation, coinciding with a rapid decline in Pao_2/Fio_2. Postmortem examination demonstrated firm, airless, contracted lungs, with microscopic features of acute and organizing diffuse alveolar damage (DAD). All lobes of both lungs showed similar features, with prominent expansion of alveolar walls by fibroblastic inflammatory matrix, and atelectasis and fusion of large zones of airspace parenchyma. Ongoing acute

TABLE 1. Demographic and Clinical Characteristics for ICU Controls and Patients With Coronavirus Disease 2019 Upon Admission to the ICU

Parameters	*n* Reporting on	ICU Controls (*n* = 22)	Coronavirus Disease 2019 (*n* = 26)	*p*
Demographics				
Male, *n* (%)	22; 26	10 (45)	18 (69)	0.14
Age, yr, median (IQR)	22; 26	65 (44–76)	70 (58–77)	0.28
Hypertension, *n* (%)	19; 26	12 (63)	16 (62)	> 0.99
Diabetes, *n* (%)	18; 26	7 (31)	8 (39)	0.75
Obesity, *n* (%)	18; 26	4 (22)	2 (8)	0.21
Dyslipidemia, *n* (%)	21; 25	8 (48)	12 (38)	0.78
Chronic kidney disease, *n* (%)	22; 26	3 (19)	5 (14)	0.71
Coronary artery disease, *n* (%)	12; 21	1 (8)	5 (24)	0.39
Chronic obstructive pulmonary disease, *n* (%)	19; 26	3 (16)	1 (4)	0.30
Smoking, *n* (%)	20; 23	6 (30)	3 (13)	0.26
Angiotensin-converting enzyme 1 inhibitor, *n* (%)	22; 26	7 (32)	10 (38)	0.76
Angiotensin II receptor blocker, *n* (%)	22; 26	2 (9)	2 (8)	> 0.99
Fever, *n* (%)	22; 26	13 (59)	23 (88)	0.042
Cough, *n* (%)	22; 26	14 (64)	24 (92)	0.029
Headache, *n* (%)	22; 26	2 (9)	7 (27)	0.15
Time, d, median (IQR), symptom onset to ICU admission	19; 25	5 (4–9)	9 (7–13)	0.001
Day of study enrollment, median (IQR), where ICU admission is day 1	22; 26	1	1 (1–4)	N/A
Serum inflammatory markers, median (IQR)				
Ferritin, µg/L	16; 26	349 (100–749)	1,257 (801–2,969)	< 0.0001
C-reactive protein, mg/L	12; 25	140 (33.3–218)	112 (74.4–170)	0.84
IL-1β, pg/mL	22; 26	0.23 (0.082–0.40)	0.19 (0.16–0.59)	0.56
IL-6, pg/mL	22; 26	65.0 (25.2–154)	79.9 (27.7–200)	0.43
IL-10, pg/mL	22; 26	8.44 (3.42–22.7)	15.0 (4.45–30.9)	0.24
Tumor necrosis factor-α, pg/mL	22; 26	6.16 (4.09–10.0)	10.2 (6.05–16.9)	0.035
Pulmonary function				
pH, median (IQR)	22; 25	7.38 (7.34–7.41)	7.39 (7.34–7.46)	0.47
Pao_2, mm Hg, median (IQR)	22; 25	86 (74–110)	81 (70–94)	0.38
$Paco_2$, mm Hg, median (IQR)	22; 25	41 (38–43)	40 (36–44)	0.72
Bicarbonate, meq/L, median (IQR)	22; 26	24 (22–26)	24 (21–26)	0.94
Lactate, mmol/L, median (IQR)	22; 24	1.7 (1.0–3.1)	1.2 (0.93–2.1)	0.23
Fio_2, mm Hg, median (IQR)	22; 26	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.56
Mechanical ventilation, *n* (%)	22; 26	15 (68)	16 (62)	0.76
Pao_2/Fio_2, median IQR)	22; 26	198 (147–251)	162 (136–209)	0.14

IL = interleukin, IQR = interquartile range.

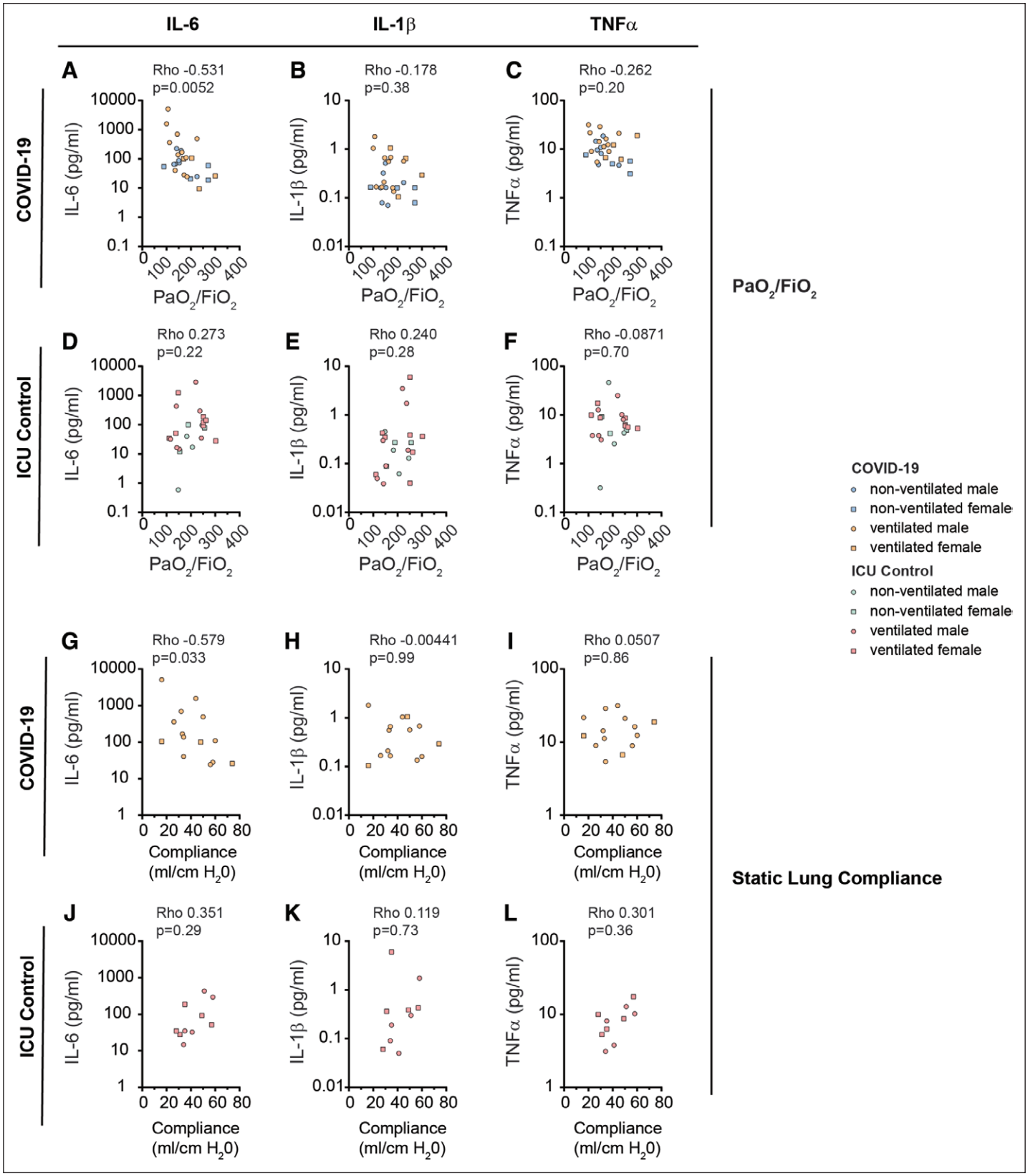

Figure 1. Association of oxygenation, as measured by the ratio of Pao_2/Fio_2, static lung compliance, and serum proinflammatory cytokines in coronavirus disease 2019 (COVID-19) patients and ICU controls. Levels of (**A**, **D**, **G**, **J**) interleukin (IL)–6, (**B**, **E**, **H**, **K**) IL-1β, and (**C**, **F**, **I**, **L**) tumor necrosis factor-α (TNFα) were quantified in serum samples taken upon study enrollment in (**A–C**) 26 COVID-19 patients, and (**D–F**) 22 ICU controls and plotted against their initial ratio of Pao_2/Fio_2. Within the subset of ventilated patients where static lung compliance could be calculated (on assist control or pressure control ventilators), serum cytokines were plotted against initial static lung compliance in (**G–I**) 14 COVID-19 patients and (**J–L**) 11 ICU controls. Data were analyzed using a Spearman correlation. In all graphs, *circles* represent males, whereas *squares* represent females; *blue/teal* represents nonventilated patients, whereas *orange/red* represents mechanically ventilated patients.

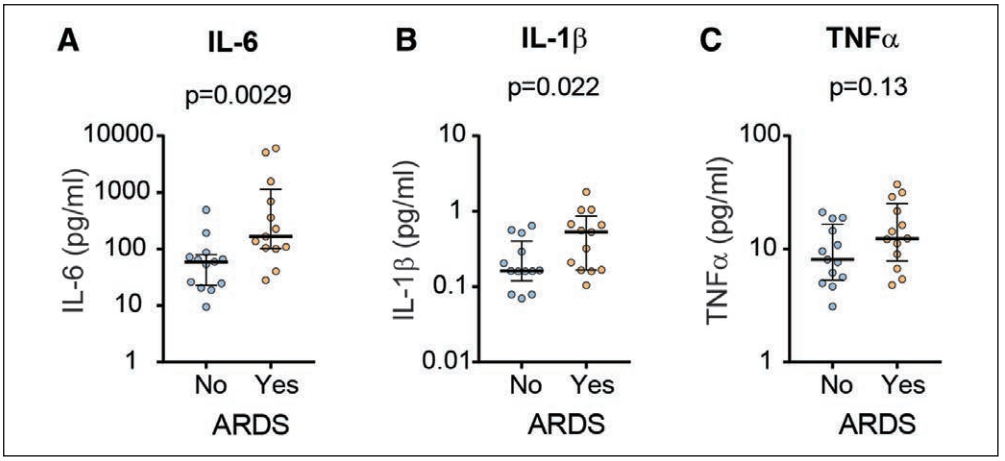

Figure 2. Serum inflammatory markers in coronavirus disease 2019 patients diagnosed with or without acute respiratory distress syndrome (ARDS). Concentration of (**A**) interleukin (IL)–6, (**B**) IL-1β, and (**C**) tumor necrosis factor-α (TNFα) were compared between patients diagnosed without (no) ARDS ($n = 13$) versus those with an ARDS diagnosis (yes; $n = 13$). Graph represents median and interquartile range. Data were analyzed using a Mann-Whitney *U* test.

lung injury was evidenced by residual airspace fibrin aggregates, including rare hyaline membranes, and hyperplastic type II pneumocytes, but the majority of areas conformed to the proliferative/fibrotic stage of DAD with elasto-collagenous deposition. Hyperplastic type II pneumocytes exhibited reactive cytological atypia, but there were no definitive features of viral cytopathic effect.

As the Simoa HD-1 analyzer is not certified for use in a clinical laboratory setting, we performed a cross-platform validation study of IL-6 on the clinically certified Siemens ADVIA Centaur platform. There was a strong correlation between the two

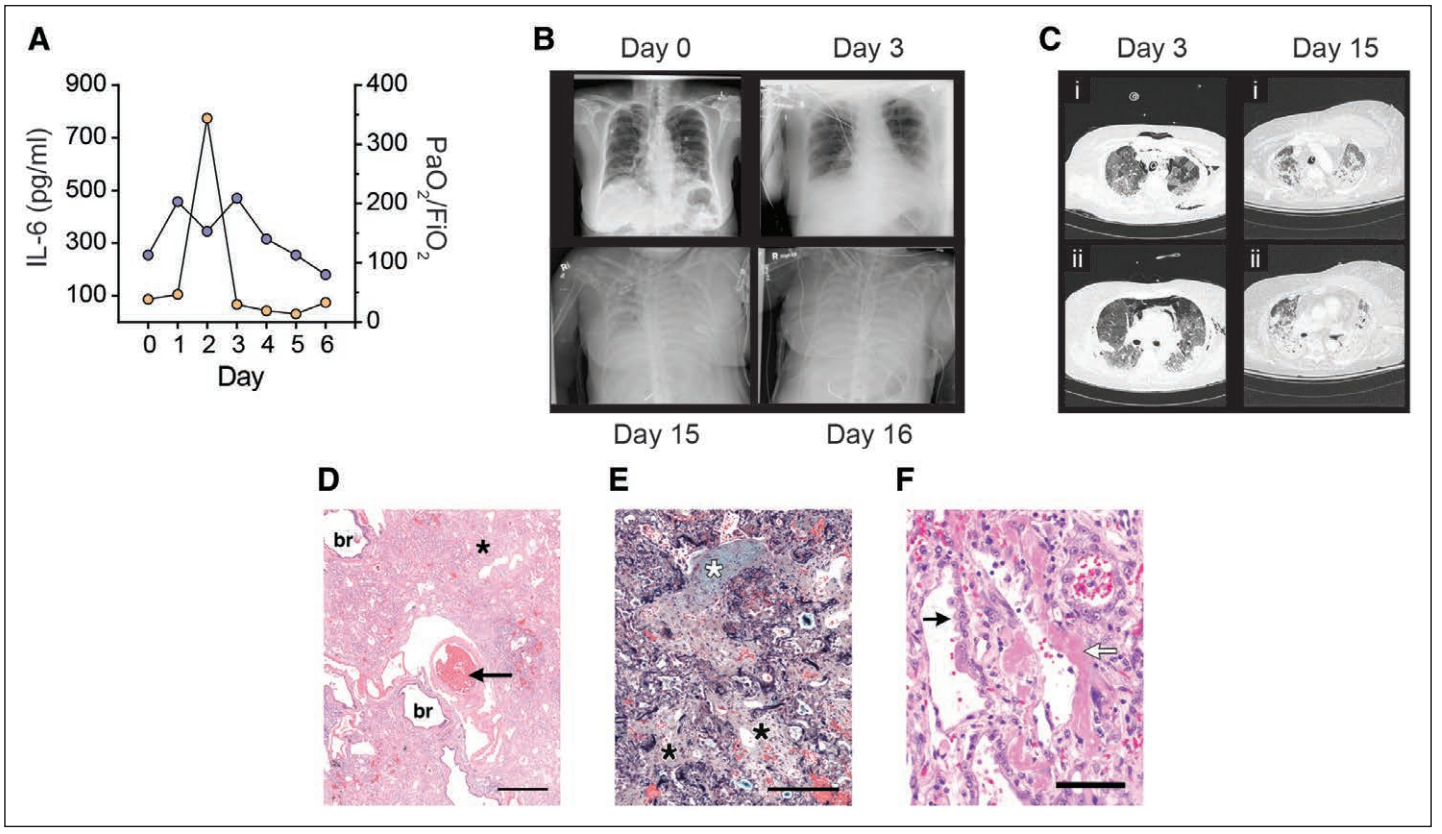

Figure 3. Characterization of pulmonary pathophysiology, serum inflammation, and postmortem histopathology in an individual patient with coronavirus disease 2019 (COVID-19). This patient was intubated and ventilated on day 1 (27 d follow symptom onset), placed onto extracorporeal membrane oxygenation on day 7, and succumb to COVID-19 on day 16. **A**, Graph displaying daily measures of Pao_2/Fio_2 (*blue*, graphed on right-hand *y*-axis) and interleukin-6 (IL-6) (*orange*, graphed on left-hand *y*-axis) during 1 wk of ICU stay. **B**, Chest radiographs (L, left; R, right) of patient 7 taken on days 0, 3, 15, and 16. **C**, Chest CT scans taken on days 3 and 15, with two axial slices shown per day. **D–F**, Postmortem lung histopathology. **D**, Scanning low power micrograph showing large zones of atelectatic and fibrotic parenchyma with compressed and obliterated airspaces. A small organizing pulmonary arterial thrombus is identified (*arrow*) (hematoxylin and eosin stain; bar = 1 mm). **E**, Higher magnification of one of the advanced zones of fibrosis demonstrating collagenized granulation tissue plugs, with a slight arborizing pattern, filling airspaces (*white* and *black asterisks*). Movat pentachrome stain (a connective tissue stain; alveolar and vascular elastica stain black) (bar = 125 μm). **F**, Medium magnification demonstrating a hyaline membrane (*white arrow*) applied to the inner wall of an alveolus. An adjoining alveolus is lined with hyperplastic type II pneumocytes showing reactive cytological atypia (hematoxylin and eosin stain; bar = 250 μm). br = bronchioles.

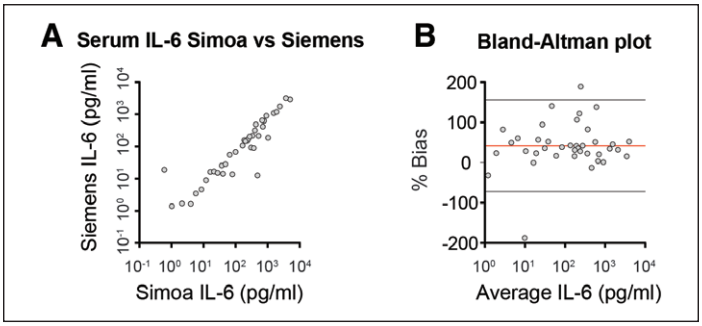

Figure 4. Cross-platform validation of serum interleukin-6 (IL-6) quantification using the Quanterix Simoa HD-1 (Billerica, MA) and Siemens ADVIA Centaur XP platforms (Munich, Germany). IL-6 was quantified in 39 serum samples on both the Quanterix and Siemens platforms. All measures were performed in duplicate. **A**, Scatter-plot of IL-6 quantified using Quanterix (*x*-axis) versus Siemens (*y*-axis). Data were analyzed using a Spearman correlation (Rho, 0.903; 95% CI, 0.819–0.949; $p < 0.0001$). **B**, Bland-Altman plot of average IL-6 versus % bias ([Quanterix–Siemens]/average) × 100%. The mean (SD) bias (41.8% [58.3]) is noted by a *red line*, and 95% limit of agreement (−72%, 156%) is noted by *black horizontal lines*.

platforms (Spearman rho, 0.903; $p < 0.0001$); the mean bias was 41.8 (bias [%] = difference/mean), with the 95% limit of agreement from −72 to 156 (**Fig. 4**).

DISCUSSION

Herein we evaluate the association between serum inflammatory cytokines and pulmonary pathophysiology in critically ill patients with COVID-19 compared with a non-COVID-19 critically ill control group. Our data demonstrate an inverse relationship between serum IL-6 with Pao_2/Fio_2 ratio and static lung compliance selectively in patients with severe COVID-19 but not in ICU controls, a pattern not observed with IL-1β or TNFα. Collectively, these data suggest a specific association between elevated IL-6 and severe COVID-19-associated lung injury.

Increasingly, it is suggested that an unregulated and exaggerated host immune response may contribute to a systemic inflammatory response (7), termed cytokine storm syndrome (5, 16), of which the downstream sequelae are associated with respiratory failure (16), cardiovascular (17), and neurologic dysfunction (18). Specifically, previous studies have demonstrated that higher serum cytokine levels are associated with severe disease in patients with COVID-19 who exhibit an ARDS phenotype as well as increased mortality (3, 11, 19, 20). Studies of cytokine levels in patients with COVID-19 to date have been cross-sectional (11, 20), lack characterization of the longitudinal host inflammatory response, and have not delineated associations between serum cytokines and detailed descriptions of specific patterns of pulmonary physiology associated with severe COVID-19-induced lung injury. Understanding these critical knowledge gaps is imperative in informing clinical trials aimed at quelling a dysregulated host immune response and hyper-inflammatory pathophysiologic cascade.

The role of IL-6 in the pathophysiology of COVID-19-associated respiratory failure is of significant interest (21, 22), and multiple trials are underway to evaluate IL-6 receptor blockade. However, prior to enrolling or identifying patients who may benefit from IL-6 receptor blockade, the association between IL-6 and severe acute lung injury must be clarified (23). This should be sought in conjunction with potential therapies directed toward IL-1β (24) or TNFα (25) receptor antagonism. Notably, all measured cytokines were elevated in the ICU control and COVID-19 groups compared with healthy controls, but only IL-6 was significantly associated with adverse pathophysiologic sequelae pertaining to pulmonary gas exchange and static lung compliance in COVID-19 patients (Fig. 1). This important finding demonstrates that while all critically ill patients with respiratory failure exhibit a pronounced inflammatory response, the role of IL-6 appears specific for COVID-19-induced lung injury. In other words, it is not the graded elevations in inflammation reported across the broadly defined disease severity classifications of COVID-19 (26) per se that are relevant (11, 19, 20, 27), but rather that within the specific disease classification of severe COVID-19, there is a significant correlation between IL-6 and the severity of pulmonary dysfunction (13). In a single patient example, we found tissue structural changes representing florid and persistent DAD and fibrosis (Fig. 3 *D–F*) with corroborating radiological evidence of diffuse opacification of the lung parenchyma (Fig. 3, *B* and *C*). In this patient, there was a precipitous elevation in serum IL-6 within 24 hours of endotracheal intubation and a subsequent deterioration in Pao_2/Fio_2 in the ensuing days (Fig. 3*A*). Further, in patients with COVID-19 compared with healthy community controls, the magnitude of increased serum IL-6 (~100-fold) was significantly greater than the magnitude of increased IL-1β (~2.5-fold) or TNFα (~four-fold) (sFig. 1, http://links.lww.com/CCX/A344). Only IL-6 and IL-1β, but not TNFα, were significantly increased in ARDS (Fig. 2). Last, the dynamic range of response of IL-6 in COVID-19 patients requiring mechanical ventilation was much greater than IL-1β or TNFα (21-fold vs 2-4 fold) (sFig. 2, http://links.lww.com/CCX/A345).

Importantly, given the current upward trajectory of the COVID-19 pandemic, accelerating the bench to bedside application of research findings is paramount to meaningful translation of COVID-19 research. Hence, our cross-platform validation of IL-6 assays between the research use only Simoa HD-1 and the clinically approved Siemens ADVIA Centaur XP platforms provides the means for other academic and community hospital settings to corroborate and extend our results, as IL-6 is not a routine clinical assay.

Our longitudinal data suggest a highly dynamic response of IL-6 that is mediated in part by disease severity, as evidenced by a deterioration in pulmonary gas exchange or death (sFig. 3, http://links.lww.com/CCX/A346). These observations are supported by recent literature demonstrating that increased IL-6 levels are associated with the need for mechanical ventilation (19). Given the wide, and often rapid, fluctuations of serum IL-6 concentration, it is imperative to consider daily assays, as such variation could otherwise be overlooked.

Our study has important strengths. First, comparing associations between inflammatory cytokines in COVID-19 patients with respiratory failure compared with a non-COVID-19 control cohort allows specificity to SARS-CoV-2 infection to be determined and provides considerable support to the concept that acute lung injury is a heterogeneous dysfunctional state. Our study builds upon previous research focused on group differences, based

on the classification of a clinical diagnosis of ARDS or adverse outcome (11, 19, 20), and demonstrates that the considerable data spread of IL-6 within severe COVID-19 patients is related to the pathophysiological parameters of respiratory function in the critical care setting, with implications for ICU resource allocation and clinical trial design. Second, our primary and secondary outcome (Pao_2/Fio_2, static lung compliance) are objective measures of pulmonary pathophysiology and readily reproducible endpoints in future clinical trials. Third, we have validated IL-6 assay methodology on a high-volume analytical platform available in many hospital laboratories. Finally, we used a highly granular approach to examine the longitudinal relationships between inflammatory cytokines, specifically IL-6, and Pao_2/Fio_2, thereby capturing the dynamic relationship and considerable daily variation in the host inflammatory immune response.

Our study has important limitations. First, not all of our patients had serum sampling from day 1, as the first eight patients were collected between days 4–7 post ICU admission. Therefore, we may have missed peak IL-6 levels in patients. Second, our small sample size limits the strength of our conclusions. Third, the observational study design does not establish causation, and our results only provide associations between cytokines and physiologic outcomes. Specifically, it is possible that increased IL-6 and its relationship with physiologic parameters of pulmonary dysfunction represents an association with disease severity or magnitude of the inflammatory response, instead of a direct role in the pathophysiology of tissue damage and dysfunction. IL-6 is a pleiotropic cytokine, with roles in both the innate and adaptive immune response, the acute phase response, and hematopoiesis, and cannot be classified as simply pro- or anti-inflammatory (28). Although our group and others have shown associations between IL-6 receptor blockade and amelioration of cytokine storm or improvements in clinical outcomes (29–33), contrary evidence provides cautionary messages of IL-6 receptor blockade in severe COVID-19 (34–38). Collectively, the current available evidence and conflicting reports suggest that the role of IL-6 in COVID-19 pathophysiology requires additional investigation as we await the results of upcoming interventional trials.

CONCLUSIONS

Elevated serum IL-6 is inversely related to Pao_2/Fio_2 and static lung compliance in critically ill COVID-19 patients but not with non-COVID-19 critically ill controls. Similar observations were not seen with serum IL-β or TNFα.

ACKNOWLEDGMENTS

We acknowledge the dedication of our multidisciplinary ICU staff and colleagues for their support of this study and thank the Vancouver General Hospital Foundation for their support.

Drs. Stukas, Hoiland, and Cooper are co-first authors. Drs. Wellington and Sekhon are co-senior authors.

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

Supported, in part, by grant from the Vancouver General Hospital Foundation. Dr. Sekhon is also supported by the Vancouver Coastal Health Research Institute Clinician Scientist Award. The remaining authors have disclosed that they do not have any potential conflicts of interest.

Trial Registration: ClinicalTrials.gov (NCT04363008).

For information regarding this article, E-mail: mypindersekhon@gmail.com

REFERENCES

1. World Health Organization: Coronavirus Disease (COVID-19) Situation Report-176 Highlights. 2020. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports. Accessed July 14, 2020
2. Chen T, Wu D, Chen H, et al: Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. *BMJ* 2020; 368:m1091
3. Wu C, Chen X, Cai Y, et al: Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* 2020; 180:1–11
4. Ruan Q, Yang K, Wang W, et al: Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* 2020; 46:846–848
5. Mehta P, McAuley DF, Brown M, et al; HLH Across Speciality Collaboration, UK: COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet* 2020; 395:1033–1034
6. Zhou F, Yu T, Du R, et al: Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet* 2020; 395:1054–1062
7. England JT, Abdulla A, Biggs CM, et al: Weathering the COVID-19 storm: Lessons from hematologic cytokine syndromes. *Blood Rev* 2020 May 15. [online ahead of print]
8. Zumla A, Hui DS, Azhar EI, et al: Reducing mortality from 2019-nCoV: Host-directed therapies should be an option. *Lancet* 2020; 395:e35–e36
9. Leisman DE, Deutschman CS, Legrand M: Facing COVID-19 in the ICU: Vascular dysfunction, thrombosis, and dysregulated inflammation. *Intensive Care Med* 2020; 46:1105–1108
10. Qin C, Zhou L, Hu Z, et al: Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis* 2020; 71:762–768
11. Chen G, Wu D, Guo W, et al: Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020; 130:2620–2629
12. Wang Z, Yang B, Li Q, et al: Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis* 2020; 71:769–777
13. Wu Z, McGoogan JM: Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; 323:1239–1242
14. Alhazzani W, Møller MH, Arabi YM, et al: Surviving sepsis campaign: Guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). *Intensive Care Med* 2020; 46:854–887
15. Ranieri VM, Rubenfeld GD, Thompson BT, et al; ARDS Definition Task Force: Acute respiratory distress syndrome: The Berlin definition. *JAMA* 2012; 307:2526–2533
16. Mcgonagle D, Sharif K, Regan AO, et al: The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. *Autoimmun Rev* 2020; 19:102537
17. Ye Q, Wang B, Mao J: The pathogenesis and treatment of the 'Cytokine Storm" in COVID-19.' *J Infect* 2020; 80:607–613
18. Wu Y, Xu X, Chen Z, et al: Nervous system involvement after infection with COVID-19 and other coronaviruses. *Brain Behav Immun* 2020; 87:18–22
19. Herold T, Jurinovic V, Arnreich C, et al: Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol* 2020; 146:128–136.e4
20. Huang C, Wang Y, Li X, et al: Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395:497–506

21. Jose RJ, Manuel A: COVID-19 cytokine storm: The interplay between inflammation and coagulation. *Lancet Respir* 2019; 2019:2019–2020
22. Moore JB, June CH: Cytokine release syndrome in severe COVID-19. *Science* 2020; 368:473–474
23. Aziz M, Aziz M, Fatima R, et al: Elevated interleukin-6 and severe COVID-19: A meta-analysis. *J Med Virol* 2020 Apr 28. [online ahead of print]
24. Aouba A, Baldolli A, Geffray L, et al: Targeting the inflammatory cascade with anakinra in moderate to severe COVID-19 pneumonia: Case series. *Ann Rheum Dis* 2020 May 6. [online ahead of print]
25. Feldmann M, Maini RN, Woody JN, et al: Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. *Lancet* 2020; 395:1407–1409
26. World Health Organization: Clinical Management of Severe Acute Respiratory Infection (SARI) When COVID-19 Disease Is Suspected. 2020. Available at: https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed July 6, 2020
27. Chi Y, Ge Y, Wu B, et al: Serum cytokine and chemokine profile in relation to the severity of coronavirus disease 2019 (COVID-19) in China. *J Infect Dis* 2020; 222:746–754
28. Choy EH, De Benedetti F, Takeuchi T, et al: Translating IL-6 biology into effective treatments. *Nat Rev Rheumatol* 2020; 16:335–345
29. Hoiland RL, Stukas S, Cooper J, et al: Amelioration of COVID-19-related cytokine storm syndrome: Parallels to chimeric antigen receptor-T cell cytokine release syndrome. *Br J Haematol* 2020; 190:e150–e154
30. Xu X, Han M, Li T, et al: Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci* 2020; 117:10970–10975
31. Guaraldi G, Meschiari M, Cozzi-Lepri A, et al: Tocilizumab in patients with severe COVID-19: A retrospective cohort study. *Lancet Rheumatol* 2020; 2:e474–e484
32. Price CC, Altice FL, Shyr Y, et al: Tocilizumab treatment for cytokine release syndrome in hospitalized COVID-19 patients: Survival and clinical outcomes. *Chest* 2020. [Epub ahead of print]
33. Somers EC, Eschenauer GA, Troost JP, et al: Tocilizumab for treatment of mechanically ventilated patients with COVID-19. *Clin Infect Dis* 2020;ciaa954
34. Antinori S, Bonazzetti C, Gubertini G, et al: Tocilizumab for cytokine storm syndrome in COVID-19 pneumonia: An increased risk for candidemia? *Autoimmun Rev* 2020; 19:102564
35. Morena V, Milazzo L, Oreni L, et al: Off-label use of tocilizumab for the treatment of SARS-CoV-2 pneumonia in Milan, Italy. *Eur J Intern Med* 2020; 76:36–42
36. Giacobbe DR, Battaglini D, Ball L, et al: Bloodstream infections in critically ill patients with COVID-19. *Eur J Clin Invest* 2020;e13319
37. Remy KE, Brakenridge SC, Francois B, et al: Immunotherapies for COVID-19: Lessons learned from sepsis. *Lancet Respir Med* 2020; 2:2–5
38. Radbel J, Narayanan N, Bhatt PJ: Use of tocilizumab for COVID-19-induced cytokine release syndrome: A cautionary case report. *Chest* 2020; 158:e15–e19