Inflammation Profiling of Critically Ill Coronavirus Disease 2019 Patients

Douglas D. Fraser, MD, PhD\textsuperscript{1,2}; Gediminas Cepinskas, DVM, PhD\textsuperscript{1,3}; Marat Slessarev, MD, MSc\textsuperscript{1,4}; Claudio Martin, MD, MSc\textsuperscript{1,4}; Mark Daley, PhD\textsuperscript{1,5,6}; Michael R. Miller, PhD\textsuperscript{1,2}; David B. O’Gorman, PhD\textsuperscript{1,7}; Sean E. Gill, PhD\textsuperscript{1,4,8}; Eric K. Patterson, PhD\textsuperscript{1}; Claudia C. dos Santos, MD, MSc\textsuperscript{9,10}; on behalf of the Lawson COVID-19 Study Team

Objectives: Coronavirus disease 2019 is caused by severe acute respiratory syndrome-coronavirus-2 infection to which there is no community immunity. Patients admitted to ICUs have high mortality, with only supportive therapies available. Our aim was to profile plasma inflammatory analytes to help understand the host response to coronavirus disease 2019.

Design: Daily blood inflammation profiling with immunoassays.

Setting: Tertiary care ICU and academic laboratory.

Subjects: All patients admitted to the ICU suspected of being infected with severe acute respiratory syndrome-coronavirus-2, using standardized hospital screening methodologies, had daily blood samples collected until either testing was confirmed negative on ICU day 3 (coronavirus disease 2019 negative), or until ICU day 7 if the patient was positive (coronavirus disease 2019 positive).

Interventions: None.

Measurements and Main Results: Age- and sex-matched healthy controls and ICU patients that were either coronavirus disease 2019 positive or coronavirus disease 2019 negative were enrolled. Cohorts were well-balanced with the exception that coronavirus disease 2019 positive patients were more likely than coronavirus disease 2019 negative patients to suffer bilateral pneumonia. Mortality rate for coronavirus disease 2019 positive ICU patients was 40%.

We measured 57 inflammatory analytes and then analyzed with both conventional statistics and machine learning. Twenty inflammatory analytes were different between coronavirus disease 2019 positive patients and healthy controls (\(p < 0.01\)). Compared with coronavirus disease 2019 negative patients, coronavirus disease 2019 positive patients had 17 elevated inflammatory analytes on one or more of their ICU days 1–3 (\(p < 0.01\)), with feature classification identifying the top six analytes between cohorts as tumor necrosis factor, granzyme B, heat shock protein 70, interleukin-18, interferon-gamma-inducible protein 10, and elastase 2. While tumor necrosis factor, granzyme B, heat shock protein 70, and interleukin-18 were elevated for all seven ICU days, interferon-gamma-inducible protein 10 transiently elevated on ICU days 2 and 3 and elastase 2 increased over ICU days 2–7. Inflammation profiling predicted coronavirus disease 2019 status with 98% accuracy, whereas elevated heat shock protein 70 was strongly associated with mortality.

Conclusions: While many inflammatory analytes were elevated in coronavirus disease 2019 positive ICU patients, relative to healthy controls, the top six analytes distinguishing coronavirus disease 2019 positive ICU patients from coronavirus disease 2019 negative ICU patients were tumor necrosis factor, granzyme B, heat shock protein 70, interleukin-18, interferon-gamma-inducible protein 10, and elastase 2.

Key Words: coronavirus disease 2019, intensive care unit, host response, inflammation, biomarkers

Coronavirus disease 2019 (COVID-19) is caused by the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Confirmed cases of COVID-19 are
growing rapidly with spread to 188 countries and regions (1). The number of reported mortalities worldwide is more than 435,000 predicting a case-fatality rate of approximately 3.4% (2). Based on data from other centers, COVID-19 often results in acute respiratory distress syndrome (ARDS) with the leading cause of death in COVID-19 positive (+) patients being respiratory failure with or without multiple organ dysfunction (i.e., cardiac and/or renal) (3–6). Currently, there are no specific therapies for COVID-19, and patients are provided only supportive care.

Recent reports and commentaries have suggested that the severity of COVID-19 may be due to a “cytokine storm” (7), which is the excessive or uncontrolled release of cytokines in response to a pathologic event, such as a viral infection (8). These suggestions are due to increased inflammatory cytokine levels, such as interleukin-6 (IL-6), as well as fever, cytopenia, and hyperferritinemia (4, 9). Moreover, these commentaries have been accompanied by calls for the use of broad immunosuppression with steroids, IV immunoglobulin, and/or selective cytokine blockade as a therapeutic approach for COVID-19 (8, 10). While patient mortality could be improved with immunosuppressive therapies, the evidence for changes in specific cytokines is incomplete, and often observed at a single timepoint with limited comparison to control groups (4, 9). Additionally, as described in recent commentaries and reviews, the use of immunosuppressive therapies to treat critically ill patients, including those with ARDS, has often been challenging due to the potential to cause harm highlighting the need for rigorous data to support any proposed trials (11, 12).

The overall aim of this study was to characterize the inflammatory profile of critically ill COVID-19 patients over the first 7 days of ICU stay to potentially identify therapeutic targets. Our specific objectives were 1) to determine the inflammatory analytes changing between COVID-19+ ICU patients and healthy controls; 2) to determine the inflammatory analyte differences between COVID-19+ and COVID-19 negative (−) ICU patients; and 3) to determine the changes in relevant inflammatory analytes over time in COVID-19+ ICU patients.

METHODS

Study Participants and Clinical Data
This study was approved by the Western University, Human Research Ethics Board. We enrolled consecutive patients who were admitted to our level-3 academic ICU at London Health Sciences Centre-Victoria Campus (London, Ontario) and were suspected of having COVID-19 based on standard hospital screening procedures (13). We collected daily blood samples starting at admission and up to 3 days in COVID-19− patients, or up to 7 days in COVID-19+ patients. COVID-19 status was confirmed using reverse transcription polymerase chain reaction (RT-PCR) at the hospital lab (14). Our standard operating procedures were used to ensure all samples were treated rapidly and equally. Blood was obtained via indwelling catheters daily in the morning and placed immediately on ice. Once transferred to a negative pressure hood, blood was centrifuged and plasma isolated, aliquoted at 250 µL and frozen at −80°C. All samples remained frozen until use and freeze/thaw cycles were avoided.

Analyte Measurements
Levels of 57 inflammatory analytes were determined using multiplexed biomarker immunoassay kits according to manufacturers’ instructions (MilliporeSigma, 400 Summit Drive, Burlington, MA) or enzyme-linked immunosorbent assay (ELISA). For the former, plasma inflammatory analytes were measured using a Bio-PlexTM 200 Suspension Array system (Bio-Rad Laboratories, Hercules, CA), which used Luminex xMAPTM fluorescent bead-based technology (Luminex Corp, Austin, TX). Bioanalyte concentrations were calculated from standard curves using five-parameter logistic regression in Bio-Plex Manager 6.1 software. For the latter, plasma levels of TIMP1 (R&D Systems Duo Set #DY970-05, diluted 1:100 or 1:200), TIMP2 (R&D Systems Duo Set #DY971, diluted 1:100), and TIMP3 (R&D Systems Duo Set #DY973, diluted 1:3 or 1:4) were measured with ELISA.

Population Statistics
Medians (interquartile ranges [IQRs]) and frequency (%) were used to report ICU patient baseline characteristics for continuous and categorical variables, respectively; continuous variables were compared using Mann-Whitney U tests (or Kruskal-Wallis tests, as appropriate), and categorical variables were compared using Fisher exact chi-square, with p < 0.05 considered statistically significant. Daily analyte concentrations were also reported as medians (IQRs), and comparisons between groups were examined using Mann-Whitney U tests. Given the number of analytes analyzed and the risk of false positives, a p value of <0.01 was used as our standard for statistical significance. Receiver operating characteristic (ROC) curves were conducted to determine sensitivity and specificity of all continuous variables for predicting mortality. The area-under-the-curve (AUC) was calculated for each variable, and the coordinates of the curves were then analyzed to identify the cutoff values based on the highest sensitivity and specificity.
for predicting mortality. All analyses were conducted using SPSS version 26 (IBM Corp., Armonk, NY).

**Machine Learning**

COVID-19 analyte data were visualized with a nonlinear dimensionality reduction on the full data matrix using the *t*-distributed stochastic nearest neighbor embedding (*t*-SNE) algorithm (17). *t*-SNE assumes that the “optimal” representation of the data lies on a manifold with complex geometry, but low dimension, embedded in the full dimensional space of the raw data. For feature selection, we pooled analyte data across 1–3 ICU days for each of the COVID-19+ and COVID-19− cohorts and normalized observations within analyte. A random forest classifier was trained on the variables to predict COVID-19 status. A random forest is a set of decision trees and, consequently, we were able to interrogate this collection of trees to identify the features that have the highest predictive value (viz., those features that frequently appear near the top of the decision tree). We limited the decision trees to a maximum depth of five levels and constrained the forest to 50 trees to avoid overfitting the small dataset. We further explored the ability to perform automated classification of COVID-19+ versus COVID-19− patients from their analyte spectra, conservatively employing only a single decision tree and limiting the maximum tree depth to three levels. We trained and tested the classifier using a five-fold cross-validation approach.

**RESULTS**

We investigated 10 COVID-19+ ICU patients (median years of age = 61.0; IQR = 54.8–67.0), 10 age- and sex-matched COVID-19− ICU patients (median years of age = 58.0; IQR = 52.5–63.0), and 10 age- and sex-matched healthy controls (median years of age = 57.5; IQR = 52.8–62.8; *p* = 0.686). Baseline demographic characteristics, comorbidities, laboratory values, and chest radiograph findings are reported in Table 1. COVID-19−ICU patients had significantly higher unilateral pneumonia, whereas COVID-19+ ICU patients were more likely to have bilateral pneumonia. Sepsis was confirmed by infectious pathogen identification in only 20% of COVID-19− ICU patients, while sepsis was suspected in the remaining 80%. All other reported baseline measures were nonsignificant between patients, although a mortality rate of 40% was determined for COVID-19+ ICU patients.

We measured 57 inflammatory analytes in plasma using either fluorescent bead-based multiplex technology or ELISA. Table 2 shows that 20 inflammatory analytes were significantly different between COVID-19+ ICU patients and healthy controls (the remaining 37 nonsignificant analytes are shown in Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/CCX/A209). All significantly different analytes were elevated in COVID-19+ ICU patients relative to healthy controls except MMP2 that was decreased.

COVID-19+ and COVID-19− cohorts were then plotted in two dimensions following dimensionality reduction by stochastic neighbor embedding (Fig. 1A). The dimensionality reduction shows that the daily analyte measurements (ICU days 1–3) between the two cohorts were distinct and easily separable. To determine which analytes were most informative for COVID-19 status classification, we performed feature selection with a random forest classifier. The top six features were identified for the binary outcome of COVID-19+ versus COVID-19− in the following order: tumor necrosis factor (TNF), granzyme B, heat shock protein 70 (HSP70), interleukin-18 (IL-18), interferon-gamma-inducible protein 10 (IP-10), and elastase 2 (Fig. 1B). We then trained and tested a simple decision-tree classifier that yielded a classifier accuracy, or the ability of the analytes to predict COVID-19 status, of 98% (*p* < 0.001, five-fold cross-validation).

**DISCUSSION**

In this study, we measured 57 inflammatory analytes in plasma obtained from ICU patients, both COVID-19+ and COVID-19−, as well as age- and sex-matched healthy controls. Given the number of analytes measured, we used two complimentary methods to analyze the data, conventional population statistics and machine learning. Our data indicate the presence of a unique inflammatory profile characterized by early and sustained elevations in circulating TNF, granzyme B, HSP70, and IL-18. Circulating levels of IP-10 increased transiently on ICU days 2–3 and elastase 2 was consistently elevated on ICU days 2–7. Finally, the plasma levels of HSP70 in COVID-19+ ICU patients were associated with...
TABLE 1. Subject Demographics and Clinical Data

| Variable                                      | COVID-19+ Patients | COVID-19– Patients | Healthy Controls | p   |
|-----------------------------------------------|--------------------|--------------------|------------------|-----|
| n                                             | 10                 | 10                 | 10               | 1.000 |
| Age in years                                  | 61.0 (54.8, 67.0)  | 58.0 (52.5, 63.0)  | 57.5 (52.8, 62.8) | 0.686 |
| Sex                                           | 7 women:3 men      | 7 women:3 men      | 7 women:3 men    | 1.000 |
| Multiple organ dysfunction score              | 4.0 (2.5, 7.3)     | 6.0 (3.8, 8.0)     |                  | 0.251 |
| Sequential Organ Failure Assessment Score     | 4.5 (2.8, 9.3)     | 7.5 (4.8, 11.0)    |                  | 0.160 |
| Comorbidities, n (%)                          |                    |                    |                  |      |
| Hypertension                                  | 6 (60)             | 8 (80)             |                  | 0.628 |
| Diabetes                                      | 3 (30)             | 4 (40)             |                  | 1.000 |
| Chronic kidney disease                        | 2 (20)             | 1 (10)             |                  | 1.000 |
| Cancer                                        | 2 (20)             | 1 (10)             |                  | 1.000 |
| Chronic obstructive pulmonary disease         | 0 (0)              | 1 (10)             |                  | 1.000 |
| Baseline labs                                 |                    |                    |                  |      |
| WBC                                           | 8.5 (6.3, 16.1)    | 15.3 (11.1, 23.0)  |                  | 0.064 |
| Neutrophils                                   | 7.7 (5.7, 13.3)    | 12.2 (8.1, 15.2)   |                  | 0.197 |
| Lymphocytes                                   | 0.7 (0.6, 1.0)     | 1.6 (0.5, 2.3)     |                  | 0.141 |
| Platelets                                     | 206 (109, 294)     | 184 (159, 245)     |                  | 0.623 |
| Hemoglobin                                    | 122 (102, 136)     | 130 (104, 142)     |                  | 0.364 |
| Creatinine                                    | 107 (55, 288)      | 80 (54, 147)       |                  | 0.571 |
| Chest radiograph findings, n (%)             |                    |                    |                  |      |
| Bilateral pneumonia                           | 9 (90)             | 1 (10)             |                  | 0.001a|
| Unilateral pneumonia                          | 0 (0)              | 5 (50)             |                  | 0.033a|
| Interstitial infiltrates                       | 1 (10)             | 1 (10)             |                  | 1.000 |
| Normal                                        | 0 (0)              | 3 (30)             |                  | 0.211 |
| PaO₂/FIO₂ ratio                               | 124 (69, 202)      | 172 (132, 304)     |                  | 0.153 |
| Sepsis diagnosis                              |                    |                    |                  |      |
| Suspected                                     | 0 (0)              | 8 (80)             |                  | 0.001a|
| Confirmed                                     | 10 (100)           | 2 (20)             |                  | 0.001a|
| Interventions during study                    |                    |                    |                  |      |
| Antibiotics                                   | 10 (100)           | 10 (100)           |                  | 1.000 |
| Antivirals                                    | 3 (30)             | 0 (0)              |                  | 0.211 |
| Steroids                                      | 2 (20)             | 3 (30)             |                  | 1.000 |
| Vasoactive medications                        | 7 (70)             | 6 (60)             |                  | 1.000 |
| Renal replacement therapy                     | 2 (20)             | 1 (10)             |                  | 1.000 |
| High-flow nasal cannula                       | 5 (50)             | 2 (20)             |                  | 0.350 |
| Noninvasive mechanical ventilation            | 6 (60)             | 8 (80)             |                  | 0.628 |
| Invasive mechanical ventilation               | 7 (70)             | 8 (80)             |                  | 1.000 |
| Patient outcome, n (%)                        |                    |                    |                  |      |
| Survived                                      | 6 (60)             | 10 (100)           |                  | 0.087 |

COVID-19 = coronavirus disease 2019.

*p < 0.05.

Continuous data are presented as medians (interquartile ranges).
TABLE 2. Comparison of Coronavirus Disease 2019-Positive Patients on ICU Day 1 to Healthy Age- and Sex-Matched Control Patients

| Analyte                                      | Coronavirus Disease 2019-Positive Patients (n = 10) | Healthy Controls (n = 10) | p   |
|----------------------------------------------|----------------------------------------------------|---------------------------|-----|
| Elastase 2                                   | 40.2 (19.0, 69.9)                                  | 2.5 (1.7, 3.2)            | < 0.001 |
| Heat shock protein 70                        | 208135 (142253, 318061)                            | 26914 (24981, 30710)     | < 0.001 |
| IL-1RA                                       | 123.84 (24.43, 1037.93)                            | 4.30 (3.27, 4.77)        | < 0.001 |
| IL-6                                         | 88.13 (39.35, 306.70)                              | 0.70 (0.30, 1.56)        | < 0.001 |
| IL-8                                         | 8.84 (5.67, 18.64)                                 | 2.04 (1.48, 2.71)        | < 0.001 |
| Monocyte chemoattractant protein-1           | 696.6 (439.9, 1093.2)                              | 261.7 (209.0, 336.6)     | < 0.001 |
| Monokine induced by gamma interferon         | 10221 (6285, 41017)                               | 1717 (1126, 2294)        | < 0.001 |
| MMP8                                         | 2165 (1379, 4173)                                  | 255 (128, 301)           | < 0.001 |
| Resistin                                      | 39.15 (30.26, 118.81)                              | 11.88 (9.23, 14.09)      | < 0.001 |
| Tumor necrosis factor                        | 194.4 (124.3, 251.8)                              | 14.7 (10.3, 25.5)        | < 0.001 |
| IL-10                                        | 44.26 (17.80, 170.55)                              | 0 (0, 4.96)              | 0.001 |
| IL-18                                        | 141.4 (84.6, 2529)                                | 34.63 (16.16, 44.92)     | 0.001 |
| Macrophage colony-stimulating factor         | 184.2 (127.6, 288.2)                              | 21.7 (0, 38.0)           | 0.001 |
| Granzyme B                                   | 9.61 (5.33, 23.12)                                | 2.27 (1.65, 3.30)        | 0.002 |
| Thrombospondin-1                             | 1294 (565, 2185)                                  | 188 (132, 460)           | 0.002 |
| Macrophage inflammatory protein-1β           | 44.78 (35.88, 58.30)                              | 31.09 (24.13, 33.51)     | 0.003 |
| MMP2                                         | 71040 (58159, 88142)                               | 120458 (99649, 133271)   | 0.004 |
| Neutrophil gelatinase-associated lipocalin   | 117.5 (92.7, 506.7)                               | 74.90 (62.92, 90.64)     | 0.004 |
| IL-15                                        | 21.96 (12.78, 49.86)                              | 6.69 (4.79, 9.33)        | 0.005 |
| Interferon-γ                                 | 18.15 (7.82, 144.80)                              | 1.69 (0, 4.91)           | 0.006 |

IL = interleukin, MMP = matrix metalloproteinase.

Only statistically significant data are shown (all data are shown in Supplemental Fig. 1, Supplemental Digital Content 4, http://links.lww.com/CCX/A212; legend, Supplemental Digital Content 5, http://links.lww.com/CCX/A213). Data are presented as median (interquartile ranges). Data represent analyte concentration in pg/mL.

**Figure 1.** A. Subjects plotted in two dimensions following dimensionality reduction by stochastic neighbor embedding (tSNE). Purple dots represent coronavirus disease 2019-positive (COVID-19+) subjects, yellow dots represent COVID-19− subjects. The dimensionality reduction shows that based on daily plasma analyte concentrations, the two cohorts are distinct and easily separable. The axes are dimension less. B. Feature classification demonstrating the top 15 inflammatory analytes that classify COVID-19 status in ICU patients' days 1–3 with their % association. HSP = heat shock protein, IL = interleukin, IFN-γ = interferon-gamma, IP = interferon-gamma-inducible protein, M-CSF = macrophage colony-stimulating factor, MIG = monokine induced by gamma interferon, TNF = tumor necrosis factor.
mortality. Despite the exploratory nature of our study, the data generated suggest that these six inflammatory analytes could be considered for further investigation as potential biomarkers and/or therapeutic targets. While changes in some potentially useful inflammatory analytes were not identified in our study, larger cohorts will be necessary to elucidate their role in the host response (i.e., IL-6 required 47 patients per cohort to reach statistical significance based on 80% power and an alpha = 0.01).

Our COVID-19+ ICU patients were similar to those reported in earlier cohorts from China (4, 5), Seattle (3), and Italy (6) with respect to age, comorbidities, and clinical presentation. In contrast to COVID-19− ICU patients, and in keeping with findings from the Seattle cohort, our COVID-19+ ICU patients had a higher prevalence of bilateral pneumonia. COVID-19− ICU patients had higher illness severity scores than COVID-19+ ICU patients, although these differences were not statistically significant due to our small sample size. Given that ventilated COVID-19+ ICU patients are reported to have higher mortality (18) than comparable ARDS cohorts (19), and that MODS and SOFA scores have not been validated in COVID-19+ ICU patients, the lower median MODS and SOFA scores in these patients may not accurately represent their illness severity relative to the COVID-19− ICU patients. Indeed, mortality was 40% in our COVID-19+ ICU patients, whereas all COVID-19− ICU patients survived to discharge.

Compared with healthy controls, COVID-19+ ICU patients exhibit clinical and laboratory evidence of systemic inflammation. Increased circulating cytokine levels (e.g., TNF, IL-6, IL-8,
and IL-10), together with lymphopenia (in CD4+ and CD8+ T cells), characterize the purported “cytokine storm” associated with severe COVID-19. The mediator release pattern has been compared with that seen in secondary hemophagocytic lymphohistiocytosis, a hyperinflammatory syndrome commonly triggered by viral infections, and in a small percentage of severe sepsis patients, characterized by a fulminant and fatal hypercytokinaemia with multiple organ failure. However, when compared with COVID-19– ICU patients, the COVID-19+ ICU patients exhibited a pattern of cytokine elevation that was unique from previous reports in that elevations were sustained and dominated by TNF and the serine proteases granzyme B and elastase 2. These latter findings may be of particular clinical relevance as preclinical models suggest SARS-CoV-2 entry into cells may be blocked by protease inhibitors (20).

In contrast to other studies of sepsis and ARDS, we found persistently elevated levels of circulating TNF in our COVID-19+ ICU patients, a potent acute master regulator of the proinflammatory response. TNF is typically upregulated quickly and early following exposure to an invading pathogen or to tissue damage, after which secondary mediators propagate inflammation while circulating levels of TNF quickly normalize (21, 22). The persistently elevated levels of TNF in COVID-19+ ICU patients could be a potential target for anti-TNF therapy with either neutralizing antibodies or small molecule inhibitors (10).

Granzyme B is expressed specifically in the cytolytic granules of natural killer cells and cytotoxic T lymphocytes, and functions as a targeted cell death mediator traditionally considered to cause apoptosis of tumor and virally infected cells (23). Extracellular soluble granzyme B levels are elevated in autoimmune diseases and infections including the human endotoxemia model and in those with severe sepsis (24). Furthermore, granzyme B retains much of its proteolytic activity when exposed to plasma. Granzyme B can degrade several extracellular membrane components and is involved in the production, release, and/or processing of proinflammatory cytokines (25, 26). Granzyme B activates IL-18 through cleavage of pro-IL-18 (27, 28), which in turn promotes cellular apoptosis via induction of granzyme B. Activation of IL-18 also induces synthesis and release of the antiviral response mediator’s interferon-gamma (IFNy). Moreover, IL-18 is known to increase adhesion molecule expression in endothelial cells, both ICAM1 and VCAM1, thereby increasing microvascular leukocyte adhesion (29).

HSP70 was elevated in our COVID-19 ICU patients and associated with mortality. As a chaperone protein that is induced in response to environmental, physical, and chemical stresses, HSP70 is usually cytoprotective by limiting the consequences of damage and by facilitating cellular recovery via caspase inhibition. Conversely, HSP70 can also exacerbate the stress response, signaling tissue destruction, and aid in immunosurveillance by transporting intracellular peptides to distant immune cells (30). Extracellular HSP70 also promotes inflammation by activating Toll-like receptors and promoting entry of granzyme B into the cells initiating cellular apoptosis (23, 31). Interestingly, HSP70 is part of the receptor complex that interacts with the binding domain of the spike protein of infectious bronchitis virus, a member of the family Coronaviridae, enabling viral entry into lung and kidney cells (32).

IP-10 is an inflammatory chemokine released by monocytes and endothelial cells, which aids recruitment of activated T cells into sites of tissue inflammation. Either protecting or promoting infection, the actions of IP-10 depend on host immune status and genetic background (33). Previous studies suggest that IP-10 is protective in coronavirus-induced severe acute respiratory syndrome (SARS) (34, 35), whereas others have shown improved infectious disease outcomes after blocking IP-10 with neutralizing antibodies (33).

Elastase 2 is also a serine protease that slowly increases in COVID-19+ ICU patients over 7 ICU days. Neutrophil azurophilic granules, as well as monocytes/macrophages and mast cells, contain elastase 2 (36). Upon degranulation, elastase 2 is either released into circulation or mobilized to the leukocyte plasma membranes and subsequently deposited to vascular endothelium or subendothelial spaces (37, 38). In COVID-19+ ICU patients, elevated elastase 2 levels may contribute to increased pulmonary vascular permeability and injury. Elevated elastase 2 has been demonstrated under various severe inflammatory conditions and can contribute to the development of ARDS (39).

Our exploratory study has identified a unique pattern of inflammation in COVID-19+ ICU patients that could be considered for further study as biomarkers and/or therapeutic targeting (hypothesis-generating data); however, our study also has several limitations. First, we only studied critically ill patients and we cannot determine the inflammatory changes contributing to ICU admissions. Second, given the limited number of patients available for study, we used two complimentary methods to independently analyze our data and both methods arrived at similar conclusions. Third, our COVID-19 study population was relatively small; however, we still generated strongly significant data (e.g., true positives) and fulfilled an urgent need for exploratory data to focus future hypothesis-driven studies on larger cohorts. Finally, we report only mortality as a clinical outcome. Future studies with larger sample sizes can explore whether reported changes in inflammatory analytes correlate with additional clinical outcomes such as functional status in survivors.

In summary, we report sustained elevations in a unique combination of inflammatory analytes in COVID-19+ ICU patients. Our exploratory data are consistent with the slow, or absent improvement in COVID-19+ patients despite state-of-the-art ICU care, and could aid future hypothesis-driven research using larger ICU cohorts.

ACKNOWLEDGMENTS
We thank Ms. Shannon Seney for performing multiplex measurements and the entire Lawson COVID-19 Study Team for their support (Dr. Robert Arntfield, Dr. Ian Ball, Mr. Gordon Barkwell, Ms. Tracey Bentall, Dr. Karen Bosma, Ms. Saoirse Cameron, Ms. Eileen Campbell, Dr. David Carter, Dr. Carolina Gillio-Meina, Dr. Robert Hegele, Ms. Natalya Odoardi, Mr. Maitray Patel, Dr. Ram Singh, Dr. Kelly Summers, and Ms. Sue Tereschyn). We are grateful for the enthusiastic assistance of the frontline Critical Care Nursing Staff at London Health Sciences Centre.
Supplemental digital content is available for this article. Direct URL citations appear in the HTML and PDF versions of this article on the journal’s website (http://journals.lww.com/ccejournal).

We acknowledge funding from Western University (Research), the Department of Medicine and Pediatrics at Western University, the Lawson Health Research Institute (https://www.lawsonresearch.ca/), the London Health Sciences Foundation (https://lhf.ca/), and the AMOSO Innovation Fund.

The authors disclosed a patent pending (COVID-19 Therapy; #63012006). For information regarding this article, E-mail: douglas.fraser@lhsc.on.ca

REFERENCES

1. Johns Hopkins Coronavirus Resource Center: COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). 2020. Available at: https://coronavirus.jhu.edu/map.html. Accessed June 16, 2020

2. World Health Organization: WHO Director-General’s opening remarks at the media briefing on COVID-19. 2020. Available at: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---3-march-2020. Accessed June 16, 2020

3. Bhatraju PK, Ghassemieh BJ, Nichols M, et al: Covid-19 in critically ill patients in the Seattle region—case series. N Engl J Med 2020; 382:2012–2022

4. Tisoncik JR, Korth MJ, Simmons CP, et al: Into the eye of the cytokine storm. Microbiol Mol Biol Rev 2012; 76:16–32

5. 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;e200994

6. Grasselli G, Zanirrolo A, Zanella A, et al: Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. JAMA 2020; 323:1574–1581

7. Tisoncik JR, Korth MJ, Simmons CP, et al: Into the eye of the cytokine storm. Microbiol Mol Biol Rev 2012; 76:16–32

8. Ritchie AJ, Singanayagam A: Immunosuppression for hyperinflammation in COVID-19: a double-edged sword? Lancet 2020; 395:1054–1062

9. 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;e200994

10. Feldmann M, Maini RN, Woody JN, et al: Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. Lancet 2020; 395:1045–1062

11. Ritchie AJ, Singanayagam A: Immunosuppression for hyperinflammation in COVID-19: a double-edged sword? Lancet 2020; 395:1054–1062

12. Morel JC, Park CC, Woods JM, et al: A novel role for interleukin-18 in adhesion molecule induction through NF kappa B and phosphatidylinositol (PI) 3-kinase-dependent signal transduction pathways. J Biol Chem 2001; 276:37069–37075

13. Liu M, Guo S, Hibbert JM, et al: CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev 2011; 22:121–130

14. Chen J, Subbarao K. The Immunobiology of SARS*. Annu Rev Immunol 2007; 25:443–472

15. Hsieh YH, Chen CW, Schmitz SF, et al: Candidate genes associated with susceptibility for SARS-coronavirus. Bull Math Biol 2010; 72:122–132

16. Korkmaz B, Horvitz MS, Jenne DE, et al: Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol Rev 2010; 62:726–759

17. Young RE, Voisin MB, Wang S, et al: Role of neutrophil elastase in LTBE-induced neutrophil transmigration in vivo assessed with a specific inhibitor and neutrophil elastase deficient mice. Br J Pharmacol 2007; 151:628–637

18. Suzuki T, Yamashita C, Zemans RL, et al: Leukocyte elastase induces lung epithelial apoptosis via a PAR-1-, NF-kappaB-, and p53-dependent pathway. Am J Respir Cell Mol Biol 2009; 41:742–755

19. Bellani G, Laffey JG, Pham T, et al; LUNG SAFE Investigators; ESICM Trials Group: Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA 2016; 315:788–800

20. Hoffmann M, Kleine-Weber H, Schroeder S, et al: SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–280.e8

21. Pinsky MR, Vincent JL, Deviere J, et al: Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest 1993; 103:565–575

22. Abraham E, Anzueto A, Gutierrez G, et al: Double-blind randomised controlled trial of mononcocal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group. Lancet 1998; 351:929–933

23. Cullen SP, Brunet M, Martin SJ: Granymes in cancer and immunity. Cell Death Differ 2010; 17:616–623

24. Lauw FN, Simpson AJ, Hack CE, et al: Soluble granymes are released during human endotoxemia and in patients with severe infection due to gram-negative bacteria. J Infect Dis 2000; 182:206–213

25. Wensink AC, Hack CE, Bovenschen N: Granymes regulate proinflammatory cytokine responses. J Immunol 2015; 194:491–497

26. Buzza MS, Zamurs L, Sun J, et al: Extracellular matrix remodeling by human granzyme B via cleavage of vitronectin, fibronectin, and laminin. J Biol Chem 2005; 280:23549–23558

27. Zafar H, Murugupula S, Topik L, et al: Granzyme B is a novel interleukin-18 converting enzyme. J Dermatol Sci 2010; 59:129–135

28. Akeda T, Yamanaka K, Tsuda K, et al: CD8+ T cell granzyme B activates keratinocyte endogenous IL-18. Arch Dermatol Res 2014; 306:125–130

29. Bellani G, Laffey JG, Pham T, et al; LUNG SAFE Investigators; ESICM Trials Group: Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. JAMA 2016; 315:788–800

30. Calderwood SK, Mambula SS, Gray PJ Jr, et al: Extracellular heat shock proteins in cell signaling. FEBS Lett 2007; 581:3689–3694

31. Suzuki T, Yamashita C, Zemans RL, et al: Leukocyte elastase induces lung epithelial apoptosis via a PAR-1-, NF-kappaB-, and p53-dependent pathway. Am J Respir Cell Mol Biol 2009; 41:742–755

32. Morel JC, Park CC, Woods JM, et al: A novel role for interleukin-18 in adhesion molecule induction through NF kappa B and phosphatidylinositol (PI) 3-kinase-dependent signal transduction pathways. J Biol Chem 2001; 276:37069–37075

33. Liu M, Guo S, Hibbert JM, et al: CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev 2011; 22:121–130

34. Chen J, Subbarao K. The Immunobiology of SARS*. Annu Rev Immunol 2007; 25:443–472

35. Hsieh YH, Chen CW, Schmitz SF, et al: Candidate genes associated with susceptibility for SARS-coronavirus. Bull Math Biol 2010; 72:122–132

36. Korkmaz B, Horvitz MS, Jenne DE, et al: Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol Rev 2010; 62:726–759

37. Young RE, Voisin MB, Wang S, et al: Role of neutrophil elastase in LTBE-induced neutrophil transmigration in vivo assessed with a specific inhibitor and neutrophil elastase deficient mice. Br J Pharmacol 2007; 151:628–637

38. Voisin MB, Leoni G, Woodfin A, et al: Neutrophil elastase plays a non-redundant role in remodeling the venular basement membrane and neutrophil diapedesis post-ischemia/reperfusion injury. J Pathol 2019; 248:88–102

39. Suzuki T, Yamashita C, Zemans RL, et al: Leukocyte elastase induces lung epithelial apoptosis via a PAR-1-, NF-kappaB-, and p53-dependent pathway. Am J Respir Cell Mol Biol 2009; 41:742–755