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Inflammation Profiling of Critically Ill Coronavirus Disease 2019 Patients

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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

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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 as part of standard hospital testing by detection of two SARS-CoV-2 viral genes using polymerase chain reaction (14). Patient baseline characteristics were recorded at admission and included age, sex, comorbidities, laboratory values, arterial partial pressure to inspired oxygen (P/F) ratio, and chest radiograph findings. Although ICU severity of illness scores have not been validated in COVID-19+ patients, we calculated multiple organ dysfunction score (MODS) and Sequential Organ Failure Assessment (SOFA) score for both COVID-19+ and COVID-19– patient groups to enable objective comparison of their illness severity. We also categorized both patient groups as having confirmed or suspected sepsis diagnosis using Sepsis 3.0 criteria. Clinical interventions received during the observation period were included and consisted of antibiotics, antiviral agents, systemic corticosteroids, vasoactive medications, renal replacement therapy, high-flow oxygen therapy, and mechanical ventilation (invasive and noninvasive). Final participant groups were constructed by age- and sex-matching COVID-19+ ICU patients with COVID-19– ICU patients, as well as healthy controls that had blood samples previously banked in the Translational Research Centre, London, ON, Canada) (directed by Dr. D. D. Fraser; https://translationalresearchcentre.com/) (15, 16).

Blood Draws

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. 1*A***). 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. 1*B***). 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).

Supplemental Table 2 (Supplemental Digital Content 2, http://links.lww.com/CCX/A210) lists 17 inflammatory analytes that were significantly different between COVID-19+ and COVID-19– patients on any or all of ICU days 1–3 (the remaining 40 nonsignificant analytes for ICU days 1–3 are shown in **Supplemental Table 3**, Supplemental Digital Content 3, http://links.lww.com/CCX/A211). All significant analytes were elevated in COVID-19+ ICU patients relative to COVID-19– ICU patients. While many analytes were significantly different between COVID-19+ and COVID-19– patients over time, the top six analytes determined by feature classification over ICU days 1–3 are listed first, and were TNF, granzyme B, HSP70, and IL-18; IP-10 and elastase 2 were also significantly different between COVID-19+ and COVID-19– patients, but starting on ICU day 2. A time course for these six markers is shown in **Figure 2** over ICU days 1–3 for COVID-19– patients and over ICU days 1–7 for COVID-19+ patients. The mean values for these six analytes remained elevated in COVID-19+ patients across all seven ICU days. The remainder of the analytes measured over time are shown in **Supplemental Figure 1** (Supplemental Digital Content 4, http://links.lww.com/CCX/A212; **legend**, Supplemental Digital Content 5, http://links.lww.com/CCX/A213), with some analytes increasing (e.g., MMP1) and some decreasing (e.g., IFNγ and IL-1RA) over seven ICU days.

The feature matrix for day 1 COVID-19+ ICU patients was classified for mortality using a Random Forest classifier (1,000 trees) and three-fold cross-validation. As HSP70 was the leading analyte associated with COVID-19+ death, a ROC curve was then conducted to determine the sensitivity and specificity of HSP70 for predicting mortality. The AUC for HSP70 was 1.00, indicating perfect sensitivity and specificity for our 10 COVID-19+ ICU patients. Using Youden's Index, the HSP70 cutoff value for predicting mortality was >264,380 pg/mL. Of note, with the addition of the 10 COVID-19– cases to the analysis, the AUC and the cutoff for HSP70 remained the same.

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)		0.001[a]
Unilateral pneumonia	0 (0)	5 (50)		0.033[a]
Interstitial infiltrates	1 (10)	1 (10)		1.000
Normal	0 (0)	3 (30)		0.211
Pao_2/Fio_2 ratio	124 (69, 202)	172 (132, 304)		0.153
Sepsis diagnosis				
Suspected	0 (0)	8 (80)		0.001[a]
Confirmed	10 (100)	2 (20)		0.001[a]
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.
[a]$p < 0.05$.
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)	251.7 (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, 4.95)	0.001
IL-18	141.4 (84.6, 252.9)	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.

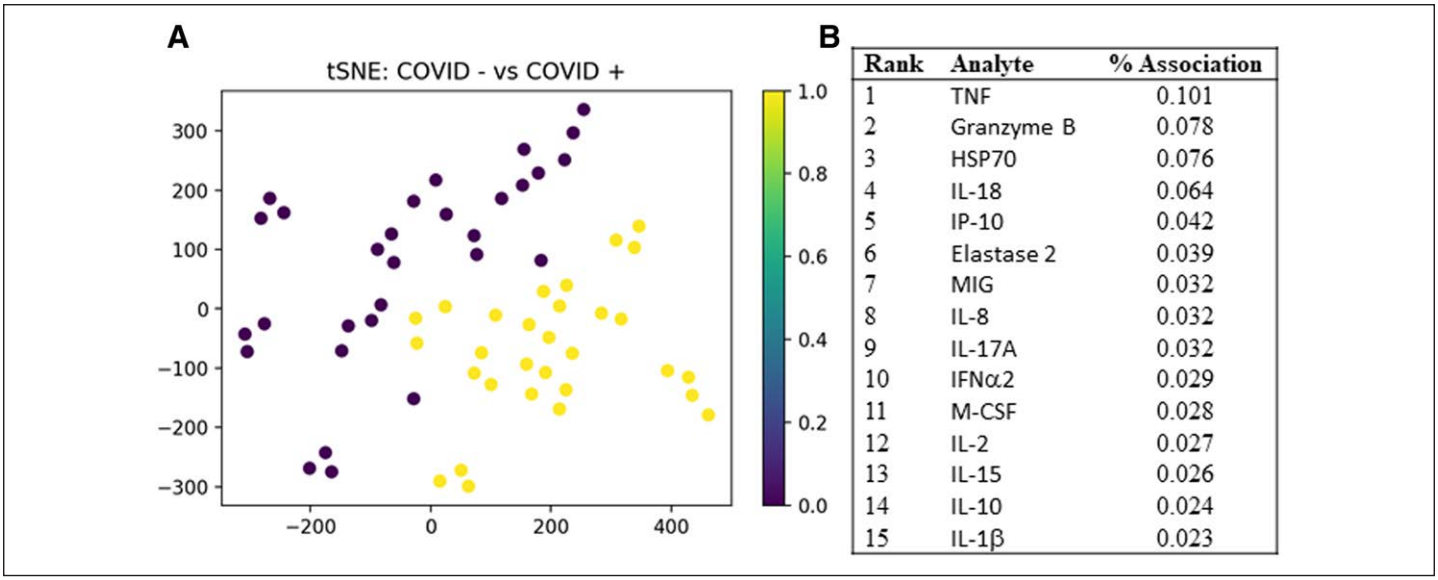

Figure 1. A, Subjects plotted in two dimensions following dimensionality reduction by stochastic neighbor embedding (tSNE). *Purple dots* represent coronavirus disease 2019-positive (COVID-19+) subjects, *yellow dots* represent COVID-19− subjects. The dimensionality reduction shows that based on daily 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.

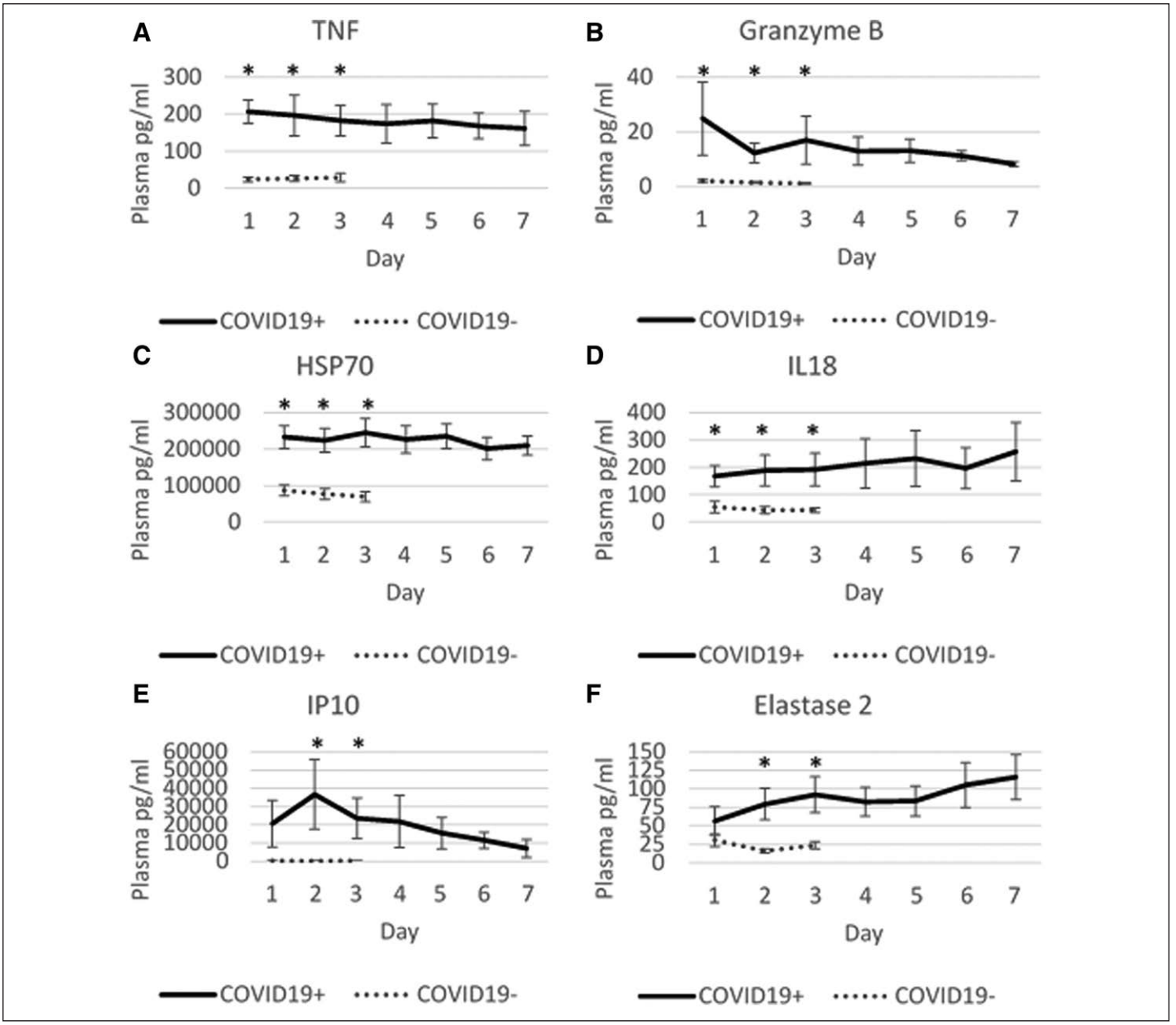

Figure 2. Time course for the top six inflammatory analytes between coronavirus disease 2019 (COVID-19)+ and COVID-19– ICU patients. Daily values are represented as mean (± SEM). *$p < 0.01$. HSP = heat shock protein, IL = interleukin, IP = interferon-gamma-inducible protein, TNF = tumor necrosis factor.

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 (IFNγ). 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.

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