Serum Proteomics in COVID-19 Patients: Altered Coagulation and Complement Status as a Function of IL-6 Level

Angelo D’Alessandro,* Tiffany Thomas, Monika Dzieciatkowska, Ryan C. Hill, Richard O. Francis, Krystalyn E. Hudson, James C. Zimring, Eldad A. Hod, Steven L. Spitalnik,*∥ and Kirk C. Hansen*∥

ABSTRACT: Over 5 million people around the world have tested positive for the beta coronavirus SARS-CoV-2 as of May 29, 2020, a third of which are in the United States alone. These infections are associated with the development of a disease known as COVID-19, which is characterized by several symptoms, including persistent dry cough, shortness of breath, chills, muscle pain, headache, loss of taste or smell, and gastrointestinal distress. COVID-19 has been characterized by elevated mortality (over 100 thousand people have already died in the US alone), mostly due to thromboinflammatory complications that impair lung perfusion and systemic oxygenation in the most severe cases. While the levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) have been associated with the severity of the disease, little is known about the impact of IL-6 levels on the proteome of COVID-19 patients. The present study provides the first proteomics analysis of sera from COVID-19 patients, stratified by circulating levels of IL-6, and correlated to markers of inflammation and renal function. As a function of IL-6 levels, we identified significant dysregulation in serum levels of various coagulation factors, accompanied by increased levels of antifibrinolytic components, including several serine protease inhibitors (SERPINs). These were accompanied by up-regulation of the complement cascade and antimicrobial enzymes, especially in subjects with the highest levels of IL-6, which is consistent with an exacerbation of the acute phase response in these subjects. Although our results are observational, they highlight a clear increase in the levels of inhibitory components of the fibrinolytic cascade in severe COVID-19 disease, providing potential clues related to the etiology of coagulopathic complications in COVID-19 and paving the way for potential therapeutic interventions, such as the use of pro-fibrinolytic agents. Raw data for this study are available through ProteomeXchange with identifier PXD020601.

KEYWORDS: SARS-CoV-2, serum, disease severity, clot, inflammation

INTRODUCTION

In late 2019, a newly identified RNA virus in the family of Coronaviridae was identified as the etiology of a form of severe acute respiratory syndrome (SARS). The 29,903 nucleotides comprising this viral genome share a 89.1% similarity with a group of SARS-like coronaviruses (genus Betacoronavirus, subgenus Sarbecovirus) that were previously isolated in bats in China. SARS-CoV-2 viral infection promotes the development of COVID-19 disease, which is characterized by a wide spectrum of clinical symptoms, including fever, persistent dry cough, shortness of breath, chills, muscle pain, headache, loss of taste or smell, and gastrointestinal distress. Although the mechanisms are incompletely understood, the severity of COVID-19 varies across subjects as a function of age and sex (worse in males and older subjects). Various comorbidities worsen the prognosis in COVID-19 patients, including obesity, diabetes, cardiovascular disease, and immunosuppression (e.g., cancer patients undergoing chemo- or radiotherapy or transplant patients).

Structural and protein–protein interaction studies show that SARS-CoV-2, similar to other beta coronaviruses, enters target cells through the interaction of the viral spike protein S with the host’s angiotensin converting enzyme receptor 2 (ACE2). ACE2 expression is particularly high in epithelial cells of the oral mucosa and lung. Single cell RNaseq studies suggest that heterogeneity in ACE2 expression may contribute to patient-specific and organ-specific responses to this infection. Blocking the interaction between the ACE2
The present study provides the first proteomics analysis of sera from COVID-19 patients, stratified by circulating levels of IL-6, and correlated to markers of inflammation and renal function. As a function of IL-6 levels, we identified significant dysregulation in serum levels of various coagulation factors, accompanied by increased levels of antifibrinolytic components, including several serine protease inhibitors (SERPINs). These were accompanied by up-regulation of the complement cascade and antimicrobial enzymes, especially in subjects with the highest levels of IL-6, which is consistent with an exacerbation of the acute phase response in these subjects. 

Although our results are observational and preliminary, they clearly demonstrate an increase in the levels of inhibitory components of the fibrinolytic cascade in severe COVID-19 disease. As such, our results support the rationale underlying the potential use of pro-fibrinolytic agents, such as tissue plasminogen activator or urokinase, in managing coagulopathic complications in severe COVID-19 disease.

**METHODS**

**Blood Collection and Processing**

This observational study was conducted according to the Declaration of Helsinki, in accordance with good clinical practice guidelines, and approved by the Columbia University Institutional Review Board. Subjects seen at Columbia University Irving Medical Center/New York–Presbyterian Hospital included 33 COVID-19-positive patients, as determined by SARS-CoV-2 nucleic acid testing of nasopharyngeal swabs; in this group, the severity of the disease was inferred from serum levels of IL-6, which were determined by CLIA-certified ELISA-based measurements. The control group included 16 subjects, all of whom were SARS-CoV-2 negative by nasopharyngeal swab at the time of the blood draw. Some patients in this group were “never positive” subjects and some were COVID-19 convalescent patients who were previously positive, but currently negative, and at least 14 days postresolution of symptoms, as determined by testing nasopharyngeal swabs. Serum was obtained from freshly drawn blood after an overnight hold at 4 °C. Sera were then extracted via a modified Folch method (chloroform/methanol/water 8:4:3), which completely inactivates other coronaviruses, such as MERS-CoV. Briefly, 20 μL of serum were diluted in 130 μL of LC-MS grade water, 600 μL of ice-cold chloroform/methanol (2:1) was added, and the samples vortexed for 10 s. Samples were then incubated at 4 °C for 5 min, quickly vortexed (5 s), and centrifuged at 14 000 g for 10 min at 4 °C. The top (i.e., aqueous) and bottom (lipid) phases were removed and the protein disk was further rinsed with methanol (200 μL) prior to centrifugation (14 000 g for 4 min) and air drying in a biosafety hood.

**Protein Digestion**

Protein pellets from serum samples were digested in an S-Trap filter (Protifi, Huntington, NY), following the manufacturer’s procedure. Briefly, ~50 μg of serum proteins were first mixed with 5% SDS. Samples were reduced with 10 mM dithiothreitol at 55 °C for 30 min, cooled to room temperature, and then alkylated with 25 mM iodoacetamide in the dark for 30 min. Afterward, phosphoric acid was added to the samples to a final concentration of 1.2% followed by 6 volumes of binding buffer (90% methanol; 100 mM triethylammonium bicarbonate (TEAB); pH 7.1). After gentle mixing, the protein solution was loaded onto an S-Trap filter, spun at 2000g for 1 min, and the flow-through collected and reloaded onto the filter. This step was repeated three times, and then the filter was washed with 200 μL of binding buffer 3 times. Finally, 1 μg of sequencing-grade trypsin and 150 μL of...
Figure 1. Proteomics analysis of serum from COVID-19 patients. Proteomics analyses were performed on serum from 49 subjects, of which 16 were not acutely affected by COVID-19 and 33 were SARS-CoV-2-positive patients, as determined by nucleic acid testing of nasopharyngeal swabs (A). Volcano plot analyses in (B) highlight the most significant proteomics changes between the two groups. Following this analysis, we performed a partial least-squares-discriminant analysis of the data (C). As part of this analysis, COVID-19-positive subjects were divided into subgroups based on IL-6 levels, which were determined during routine clinical care using a clinically validated ELISA assay (D); the patients were classified into groups with low ($\leq$10 pg/mL), medium (10–65 pg/mL), or high (>90 pg/mL) IL-6 levels. (E) Hierarchical clustering based on this classification highlighted a significant impact of COVID-19 and IL-6 levels on proteins involved in inflammation, the complement and coagulation cascades, and antimicrobial enzymes. A vectorial version of this information is provided in Supplementary Figure S1. (F,G) Of note, proteomics results correlated with clinical measurements of the same variable (e.g., creatine kinase; panel F).
digestion buffer (50 mM TEAB) were added onto the filter and digested at 47 °C for 1 h. To elute peptides, three stepwise buffers were applied, with 200 μL of each with one more repeat; these included 50 mM TEAB, 0.2% formic acid in water, and 50% acetonitrile and 0.2% formic acid in water. The peptide solutions were pooled, lyophilized, and resuspended in 0.1% formic acid.

Nano Ultra-High-Pressure Liquid Chromatography–Tandem Mass Spectrometry (MS) Metabolomics

A total of 200 ng of each sample was loaded onto individual Evotips for desalting and then washed with 20 μL 0.1% formic acid followed by the addition of 100 μL of storage solvent (0.1% formic acid) to keep the Evotips wet until analysis. The Evosep One system was coupled to the timsTOF Pro mass spectrometer (Bruker Daltonics, Bremen, Germany). Data were collected over an m/z range of 100 to 1700 for MS and MS/MS on the timsTOF Pro instrument using an accumulation and ramp time of 100 ms. Post processing was performed with PEAKS studio (Version X+, Bioinformatics Solutions Inc., Waterloo, ON). Pathway analyses were performed with the DAVID software and Ingenuity Pathway Analysis. Graphs and statistical analyses were prepared with GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA), GENE E (Broad Institute, Cambridge, MA, USA), and MetaboAnalyst 4.0.28

RESULTS

Serum Proteomics of COVID-19 Patients Reveals Significant Up-Regulation of IL-6 Targets

An overview of the experimental design is provided in Figure 1A. Briefly, proteomics analyses were performed on sera from 49 subjects, 33 of whom were actively infected with SARS-CoV-2 (all runs are available through ProteomeXchange identifier: PXD020601). Results are reported extensively in Supplementary Table S1, which identifies 493 proteins (including Uniprot IDs); the table also includes quantitative values for each protein, as calculated with the PEAKS software by integrating the areas under the curve of all the peptide identifications assigned to a given protein. Volcano plot analyses were performed to highlight proteins that significantly increased or decreased in COVID-19 patient sera (Figure 1B). Pathway analysis of these results confirmed IL-6 signaling as the most upstream up-regulated pathway in COVID-19 patients (p-value: 3.57 x 10^{-17}; Supplementary Figure S1A), as listed in Supplementary Figure S1B. Specifically, several
direct and indirect targets of IL-6 signaling were enriched in this data set, including JNK, STAT3, and p53 (Supplementary Figure S1C). Supplementary Figure S1D provides an overview of the protein targets of IL-6 (and downstream regulators), levels of which increase (red) or decreased (blue) in COVID-19 patient sera.

Serum Proteomics as a Function of IL-6 Levels

IL-6 is a marker of COVID-19 disease severity; indeed, clinical trials are underway to disrupt signaling through the IL-6 receptor.17,18,20,29 In this context, combined with the pathway analysis described above (Supplementary Figure S1), we grouped COVID-19 patients based on IL-6 levels prior to partial least-squares-discriminant analyses (Figure 1C). Specifically, we used a CLIA-certified clinical assay (Figure 1D) to separate COVID-19 patients into groups with low (<10 pg/mL), medium (10−65 pg/mL), and high (>90 pg/mL) IL-6 levels. Of note, both PLS-DA (Figure 1C) and hierarchical clustering analyses (Figure 1E) revealed a signature associated with SARS-CoV-2 positivity in the serum proteome (separated across Principal Component 1 (PC1), which explained 15.5% of the total variance; Figure 1C). Consistent with the distribution of samples in the PLS-DA analysis, a subset of proteins with the highest loading weights across PC1 (Supplementary Table S1) followed a trend toward progressive increases or decreases as a function of IL-6 levels. One such protein was identified as C-Reactive Protein (CRP; Figure 1F), a marker of the acute phase response for which clinical laboratory values were available and correlated well with proteomics quantification ($r = 0.769, p < 0.0001; Figure 1G).

Pathway analyses, performed on protein clusters in sera of COVID-19 patients classified by IL-6 levels, are shown in a heat map (Figure 1E and Supplementary Figure S2); these results indicate a significant enrichment of clusters of proteins related to the coagulation and complement cascades, immunoglobulins and antimicrobial enzymes, apolipoproteins, and other transporters.

Complement and Coagulation Cascades

Sera of COVID-19 patients, especially those with IL-6 levels >10 pg/mL, contained increased levels of multiple proteins in the acute phase response that is initiated by IL-6—specifically components of the coagulation and complement cascades (top enriched pathway, p-value: $1.6 \times 10^{-31}$; Figure 2). The
components that increased or decreased significantly across all groups in this data set (ANOVA) were annotated in red or blue, respectively, against KEGG pathway hsa04610 (Figure 2, center panel; a larger version of this panel is provided in Supplementary Figure S3). Specifically, several peptides assigned to coagulation factors (including Factors 2, 5, 7, 10) were increased COVID-19 patient sera, whereas only Factor XIIIb and gelsolin were significantly decreased (Figure 2). Interestingly, increases in pro-coagulant components (e.g., kininogen 1 (KNG1), fibrinogen (FGA)) were accompanied by increases in anticoagulant components (e.g., vitamin K-dependent protein S (PROS1); Figure 2). In contrast, sera from COVID-19 patients, especially those with the highest IL-6 levels, exhibited significant increases in serum levels of several SERPINS and carboxypeptidases (CPB2/TAFI) in the coagulation/fibrinolytic cascade, including SERPINA1, SERPINA3, SERPINF2. Specifically, SERPINA1—alpha-1 antitrypsin—plays a dual role by slowing down clot formation and inhibiting proteases released by inflammatory cells, like neutrophil elastase. Similarly, SERPINA3 (alpha-1 antichymotrypsin) can inhibit neutrophil cathepsin G and mast cell chymase, both of which can convert angiotensin-1 to the active angiotensin-2. SERPINF2 (alpha-1 antiprostasin) is an inhibitor of plasmin (and other proteases) and CPB2 otherwise known as thrombin-activatable fibrinolysis inhibitor (TAFI) both create a pro-thrombotic state by inhibiting the fibrinolytic pathway.

In parallel, several components of the complement cascade were increased in COVID-19 patient sera, including Complement Factor H and I (CFH and CFI, respectively)—that are upregulated to ensure complement targeting of pathogens and not host cells—and C5 (Figure 2); this suggests an enhanced innate immune response in these subjects. Of note, deficiency or defects in CFH and CFI are associated with complement-mediated hemolytic uremic syndrome, hemolysis, and thrombosis.

**Immunoglobulins and Antimicrobial Enzymes**

COVID-19 patient sera were significantly enriched in proteins of the adaptive and innate immune responses (Figure 3). Although no changes were observed in the levels of immunoglobulin heavy chain constant regions (Figure 3A), all the other components increased. In particular, there were increases heavy chain variable regions (Figure 3B), light chain constant and variable regions (Figure 3C,D), in some cases proportional to IL-6 levels (e.g., IGKV3; Figure 3C). In addition, several enzymes with antimicrobial activity were increased in COVID-19 patient sera, suggesting the possibility of a secondary bacterial infection or simply an exacerbation of the acute phase response (Figure 3E). This trend was particularly evident for cystatin C (CST3), defensin A1 (DEFA1), leucine-rich alpha2 glycoprotein (LRG1), and lysozyme C (LYZC) (Figure 3E).

**Markers of Hemolysis and Cell Lysis**

Sera of COVID-19 patients with the highest IL-6 levels exhibited increased protein markers of hemolysis, including hemoglobin alpha and beta (HBA and HBB) and carbonic anhydrase (CA1; Figure 4A). Other RBC-derived proteins (i.e., band 3, anion exchanger 1 (AE1; the most abundant RBC membrane protein), peroxiredoxins 2 and 6, catalase, and...
biliverdin reductase B) correlated significantly with HBA and HBB levels, despite not reaching significance when compared to COVID-19-negative subjects, suggesting that minimal hemolysis was present in a subset of the most severely ill patients in our study (Figure 4A), perhaps due to mechanical ventilation or other iatrogenic interventions—including the sample collection protocol adopted in this study. In addition, sera from COVID-19 patients with the highest IL-6 levels (>90 pg/mL) had increased levels of the metalloprotease inhibitor TIMP1 and creatine kinase M (CKM; a marker of cardiac tissue damage), but not of actin or histones (Figure 4B).

Proteomics Correlates to Clinical Laboratory Parameters of Inflammation, Cardiac Function, and Renal Function

CKM levels were the top positive correlate to clinical laboratory measurements of IL-6 (Figure 5A; $r = 0.544$, $p = 0.001$; Supplementary Table S1), followed by proteins involved in extracellular matrix remodeling, such as Inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3), retinol binding protein 4 (RBP4) and complex partner transthyrelin (TTR), collagen 12A1, and glucose phosphate isomerase (GPI; Figure 5A). In contrast, IL-6 levels were negatively correlated to thrombospondin 1 (THBS1; Figure 5A), which has fibrinogen and heparin-binding domains and participates in cell-matrix interactions. Of note, levels of SERPIND1 (heparin cofactor II), a thrombin inhibitor that also binds to, and is activated by, heparin or dermatan sulfate, negatively correlated with IL-6 (Figure 5A). In addition, protein correlates to other inflammatory markers, such as CRP (Figure 5B), showed stronger associations than those observed for IL-6. For example, IL-6, leptin-binding protein (LBP), and complement component C9 all showed strong correlations (>0.75, $p < 0.0001$) with clinical laboratory measurements of CRP levels (Figure 5B; full list in Supplementary Table S1), which also correlated well by CRP levels measured by proteomics. Similarly, clinical laboratory measurements of lactate dehydrogenase (LDH; a marker of cell lysis) positively correlated with the levels of LDHA (Figure 5C) and LDHB (Supplementary Table S1) as measured by proteomics, catalase (CAT), S100A12, and ezrin (EZR; Figure 5C); the latter is an epithelial cell marker, suggestive of epithelial cell damage, rather than hemolysis, which may also contribute to increased LDH in these patients. In addition, levels of CKM, a marker of cardiac tissue damage, correlated significantly with the proteomics measurements of the same parameter, as well as myoglobin (a marker of myocyte damage; Figure 5D). Finally, creatinine, a marker of renal function, showed significant positive correlations to complement factor D (CFD), a component of the alternative complement activation pathway, as well as LYZC, an antimicrobial enzyme (Figure 5E), suggesting interactions between kidney-related comorbidities and innate immunity in COVID-19 patients.

### DISCUSSION

To the best of our knowledge, this work is the first serum proteomics analysis of COVID-19 patients, stratified by the degree of inflammation, represented by IL-6 levels, as a marker of disease severity. Since the first submission of this manuscript, a handful of papers have been published on the impact of COVID-19 on the proteomes and metabolomes of patient sera, plasma, and red blood cells, or cells infected with SARS-COV-2 in vitro. In one of these manuscripts, disease severity and not IL-6 levels were used to classify COVID-19 patients, though overlapping findings were noted, especially with respect to acute phase response and complement components. Indeed, not surprisingly, in our study the degree of inflammation positively correlated with the circulating levels of coagulation components and inhibitors of the fibrinolytic cascade. Although thromboembolic complications have already been reported in the context of COVID-19, the etiology of this phenomenon in this context is unclear. Our results identify increased circulating levels of several coagulation factors, specifically Factor 5, 7, and 10, which could be targeted to prevent untoward clot formation in patients with the highest IL-6 levels. Of note, there is also upregulation of several SERPIN components in COVID-19 patient sera containing high IL-6 levels. A similar activation of the antifibrinolytic cascade in critically ill trauma patients is associated with the "fibrinolytic shutdown" phenotype. Thus,
this raises the possibility of intervening in COVID-19 patients displaying similar hypercoagulable phenotypes, by restoring the balance between pro- and antifibrinolytic cascades by administering pro-fibrinolytic agents, such as tissue plasminogen activator or urokinase.

Another interesting analogy between COVID-19 patients and trauma patients who develop acute lung injury is the observed increase, proportional to the degree of inflammation, in circulating levels of metalloproteinases involved in extracellular matrix remodeling. Neutrophil or macrophage infiltration in the lung secondary to shock, transfusion, hypoxia, and/or inflammation is a hallmark of acute lung injury following trauma/shock, and is a contributor to the early events driving lung fibrosis in pulmonary hypertension and/or chronic obstructive pulmonary disease.

Our study also identified up-regulation of several components of the complement cascade, along with several enzymes with antimicrobial activity, especially in patients with high IL-6 levels. These observations are directly explained by the role of IL-6 in stimulating acute phase responses—which also has significant crosstalk with the coagulation cascade. Alternatively, these results are suggestive of potential secondary bacterial infection in the severely ill COVID-19 patient, which could result in remote organ dysfunction (e.g., kidney), as correlative analysis of proteomics data and creatinine levels seems to suggest. Recent reports from Seattle identified septic shock in a high percentage of ICU patients that required therapeutic intervention to protect the heart and circulatory system. Of note, IL-6 is also known to stimulate the increase in the levels of several proteases, including matrix metalloproteinases (MMPs), matrilysin (MMP7), and stromelysin-1 (MMP3), which can cleave subclasses of IgG, a consideration with antimicrobial activity, especially in patients with high IL-6 levels. These observations are directly explained by the role of IL-6 in stimulating acute phase responses—which also has significant crosstalk with the coagulation cascade. Alternatively, these results are suggestive of potential secondary bacterial infection in the severely ill COVID-19 patient, which could result in remote organ dysfunction (e.g., kidney), as correlative analysis of proteomics data and creatinine levels seems to suggest. Recent reports from Seattle identified septic shock in a high percentage of ICU patients that required therapeutic intervention to protect the heart and circulatory system.

Of note, IL-6 is also known to stimulate the increase in the levels of several proteases, including matrix metalloproteinases (MMPs), matrilysin (MMP7), and stromelysin-1 (MMP3), which can cleave subclasses of IgG, a consideration that would contribute to explaining the observed increases in the variable chains of light and heavy immunoglobulins, in the absence of increases in the constant fragment of the heavy chain. Alternatively, coronaviruses have been shown to express papain-like enzymes, which is relevant in that papains are expressed by SARS-CoV-2 for epithelial cells expressing high levels of ACE2 receptor. Several of these findings are consistent with the COVID-GRAM scoring system that predicts development of critical illness based on neutrophil-to-lymphocyte ratio, lactate dehydrogenase, and direct bilirubin as 3 of 10 predictive factors.

Nonetheless, there are several limitations that affect the interpretation of the results of the present study. First, the analyses presented herein were performed on residual samples obtained for routine clinical laboratory testing. Serum samples were tested here, which clearly impacts any conclusion related to coagulation cascades. In this view, it is worthwhile to note that any conclusion on coagulation phenotype here is drawn on the basis of the measurement of protein levels, not direct determination of enzymatic activity. Although these samples were refrigerated and stored for <24 h before initial preparation for analysis, future studies on freshly collected samples will determine whether bias was introduced by these sample collection procedures. In addition, the COVID-19 patients studied herein were mostly male (75%) and older (median age 56), as compared to the controls (62% female, median age 33). Although these sex and age-related biases are consistent with what is observed in clinical cohorts, especially with respect to the most severely ill patients, the relatively limited number of younger, female COVID-19 patients studied here prevented any post hoc analysis based on age and sex as biological variables; nonetheless, samples are now being prospectively collected to perform these additional studies in newly enrolled cohorts. Moreover, all the COVID-19 patients in this study were inpatients and, as such, they were significantly ill. Future studies will investigate whether asymptomatic, COVID-19-positive patients have proteomic phenotypes comparable to healthy controls or are similar to patients with other, less severe, coronavirus infections. All the patients studied here exhibited symptoms severe enough to require hospital admission; therefore, the relatively small sample size did not allow an analysis focusing on comorbidities and complications (e.g., thromboembolism). To address this issue, longitudinal samples are currently being obtained as part of clinical trials at the Ernest E. Moore Shock Trauma Center at Denver Health (CO, USA) with the goal of investigating the potential for anticoagulants (e.g., heparin) or pro-fibrinolytic agents (e.g., tissue plasminogen activator) to be therapeutically useful in preventing thromboembolic complications in COVID-19 patients.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.0c00365.

Figure S1: Unsupervised Ingenuity Pathway Analysis of the data highlight a significant impact of IL-6-dependent regulation on protein targets in COVID-19 patients; Figure S2: Vectorial version of the heat map in Figure 1; Figure S3: Serum proteins increasing (red) or decreasing (blue) significantly in the serum of COVID-19 patients compared to controls—as mapped against the coagulation and complement cascade pathway (KEGG hsa04610) (PDF)

Table S1: Proteomics report and elaborations (XLSX)

AUTHOR INFORMATION

Corresponding Authors
Angelo D’Alessandro — Department of Biochemistry and Molecular Genetics, University of Colorado Denver, Anschutz Medical Campus, Aurora, Colorado 80045, United States; orcid.org/0000-0002-2258-6490; Phone: 303-724-0096; Email: angelo.dalesandro@ucdenver.edu
Steven L. Spitalnik — Department of Pathology & Cell Biology, Columbia University Irving Medical Center, New York, New York 10032, United States; Email: ss2479@cumc.columbia.edu

Authors
Tiffany Thomas — Department of Pathology & Cell Biology, Columbia University Irving Medical Center, New York, New York 10032, United States
Monika Dzieciatkowska — Department of Biochemistry and Molecular Genetics, University of Colorado Denver, Anschutz Medical Campus, Aurora, Colorado 80045, United States
Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acs.jproteome.0c00365

Author Contributions

SLS and KCH are equally contributing last/senior authors.

Author Contributions

TT, ROF, SLS, and EAH designed the study. TT, ROF, and SLS performed proteomics analyses and prepared the figures. MD, RCH, KCH, SLS, and EAH collected and processed the samples. TT, ROF, and SLS significantly revised the manuscript, which was significantly revised by SLS, TT, JCZ, and KEH and finally approved by all the authors.

Notes

The authors declare the following competing financial interest(s): Though unrelated to the contents of this manuscript, the authors declare that AD and KCH are founders of Omix Technologies, Inc. and Altis Biosciences, LLC. AD and SLS are consultants for Hemanext, Inc. SLS is also a consultant for Tioma, Inc. J CZ is a consultant for Rubius Therapeutics. All the other authors disclose no conflicts of interest relevant to the study. Raw data for this study are available through ProteomeXchange with identifier PXD020601.

ACKNOWLEDGMENTS

This research was supported by funds from the Boettcher Webb-Waring Investigator Award (ADA), RM1GM131968 (ADA and KCH) from the National Institute of General and Medical Sciences, and R01HL146442 (ADA), R01HL1497714 (ADA), R01HL148151 (ADA, SLS), R21HL150032 (ADA) from the National Heart, Lung, and Blood Institute.

REFERENCES

1. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.-M.; Wang, W.; Song, Z.-G.; Hu, Y.; Tao, Z.-W.; Tian, J.-H.; Pei, Y.-Y.; Yuan, M.-L.; Zhang, Y.-L.; Dai, F.-H.; Liu, Y.; Wang, Q.-M.; Zheng, J.-J.; Xu, L.; Holmes, E. C.; Zhang, Y.-Z. A New Coronavirus Associated with Human Respiratory Disease in China. Nature 2020, 579 (7798), 265–269.
2. Bendavid, E.; Mulaney, B.; Sood, N.; Shah, S.; Ling, E.; Bromley-Duffin, R.; Li, C.; Weissberg, Z.; Suavedra, R.; Tedrow, J.; Tversky, D.; Bogan, A.; Kupiec, T.; Eichner, D.; Gupta, R.; Ioannidis, J.; Bhattacharya, J. COVID-19 Antibody Seroreivalence in Santa Clara County, California. medRxiv, April 30, 2020. DOI: 10.1101/2020.04.14.20062463 (accessed August 8, 2020).
3. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; Cheng, Z.; Yu, T.; Xia, J.; Wei, Y.; Wu, W.; Xie, X.; Yin, W.; Li, H.; Liu, M.; Xiao, Y.; Gao, H.; Guo, L.; Xie, J.; Wang, G.; Jiang, R.; Gao, Z.; Jin, Q.; Wang, J.; Cao, B. Clinical Features of Patients Infected with 2019 Novel Coronavirus in Wuhan, China. Lancet 2020, 395 (10223), 497–506.
4. Yang, X.; Yu, Y.; Xu, J.; Shu, H.; Xia, J.; Liu, H.; Wu, Y.; Zhang, L.; Yu, F.; Fang, M.; Yu, T.; Wang, Y.; Pan, S.; Zou, X.; Yuan, S.; Shang, Y. Clinical Course and Outcomes of Critically Ill Patients with SARS-CoV-2 Pneumonia in Wuhan, China: A Single-Centered Retrospective, Observational Study. Lancet Respir. Med. 2020, 8, 475.
5. Gordon, D. E.; Jiang, G. M.; Bouhaddou, M.; Xu, J.; Obernier, K.; O’Meara, M. J.; Guo, J. Z.; Swaney, D. L.; Tummino, T. A.; Huettenthal, R.; Kaeke, R. M.; Richards, A. L.; Tutuncuoglu, B.; Foushard, H.; Batra, J.; Haas, K.; Modak, M.; Kim, M.; Haas, P.; Polacco, B. J.; Braberg, H.; Fabius, J. M.; Eckhardt, M.; Souchey, M.; Bennett, M.; Cakir, M.; McGregor, M. J.; Li, Q.; Naing, Z. Z. C.; Zhou, Y.; Peng, S.; Kirby, S. I.; Melnyk, J. E.; Chorba, J. S.; Lou, K.; Dai, S. A.; Shen, W.; Shi, Y.; Zhang, Z.; Barrio-Hernandez, I.; Memon, D.; Hernandez-Armenta, C.; Mathy, C. J. P.; Perica, T.; Pilla, K. B.; Ganesan, S. J.; Saltzberg, D. J.; Ramachandran, R.; Liu, X.; Rosenthal, S. B.; Calvillo, L.; Venkataramanan, S.; Libby-Lugo, J.; Liu, Y.; Wankowicz, S. A.; Bohn, M.; Sharp, P. P.; Trenken, M.; Young, J. M.; Cavero, D. A.; Haft, J.; Roth, T. L.; Rathore, U.; Subramanian, A.; Noack, J.; Hubert, M.; Roesch, F.; Vallet, T.; Meyer, B.; White, K. M.; Miorin, L.; Rosenberg, O. S.; Verba, K. A.; Agard, D.; Ott, M.; Emerman, M.; Ruggero, D.; Garcia-Sastre, A.; Jura, N.; von Zastrow, M.; Tauntton, J.; Ashworth, A.; Schwartz, O.; Vignuzzi, M.; d’Enfert, C.; Mukherjee, J.; Jacobson, M.; Malik, H. S.; Fujimori, D. G.; Ideker, T.; Craik, C. S.; Floor, S.; Fraser, J. S.; Gross, J.; Jali, S.; Akerete, A.; Kerem, T.; Beltrao, P.; Shokat, K.; Shoichet, B. K.; Krogan, N. J. A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. Nature 2020, 583, 459–468.
6. Andersen, K. G.; Rambaut, A.; Lipkin, W. I.; Holmes, E. C.; Garry, R. F. The Proximal Origin of SARS-CoV-2. Nat. Med. 2020, 26 (4), 450–452.
7. Shang, J.; Ye, G.; Shi, K.; Wan, Y.; Luo, C.; Ahara, H.; Geng, Q.; Auerbach, A.; Li, F. Structural Basis of Receptor Recognition by SARS-CoV-2. Nature 2020, 581, 221.
8. Xu, H.; Zhong, L.; Deng, J.; Peng, J.; Dan, H.; Zeng, X.; Li, T.; Chen, Q. High Expression of ACE2 Receptor of 2019-NCoV on the Epithelial Cells of Oral Mucosa. Int. J. Oral Sci. 2020, 12 (1), 1–S.
9. Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L.; Wang. X. Structure of the SARS-CoV-2 Spike Receptor-Binding Domain Bound to the ACE2 Receptor. Nature 2020, 581, 215.
10. Zou, X.; Chen, K.; Zou, J.; Han, P.; Hao, J.; Han, Z. Single-Cell RNA-Seq Data Analysis on the Receptor ACE2 Expression Reveals the Potential Risk of Different Human Organs Vulnerable to 2019-NCoV Infection. Front. Med. 2020, 14, 185.
11. Lei, C.; Qian, K.; Li, T.; Zhang, S.; Fu, W.; Ding, M.; Hu, S. Neutralization of SARS-CoV-2 Spike Pseudotyped Virus by Recombinant ACE2-Ig. Nat. Commun. 2020, 11 (1), 1–S.
12. Bloch, E. M.; Shoham, S.; Casadevall, A.; Sachais, B. S.; Shaz, B.; Winters, J. L.; van Buskirk, C.; Grossman, B. J.; Joyner, M.; Henderson, J. P.; Pekosz, A.; Lau, B.; Wesolowski, A.; Katz, L.; Shan, H.; Auwaerter, P. G.; Thomas, D.; Sullivan, D. J.; Paneth, N.; Gehrie, E.; Spitalnik, S.; Hod, E.; Pollack, L.; Nicholson, W. T.; Pirollo, L.-A.; Bailey, J. A.; Tobian, A. A. Deployment of Convalescent Plasma for Treatment of COVID-19. J. Clin. Invest. 2020, 130, 2757.
13. Shamamgara, B.; Siriwattananon, K.; Wangkanont, K.; Phoohachaoen, W. Perspectives on Monoclonal Antibody Therapy as Potential Therapeutic Intervention for Coronavirus Disease-19 (COVID-19). Asian Pac. J. Allergy Immunol. 2020, 38 (1), 10–18.
14. Callaway, E. The Race for Coronavirus Vaccines: A Graphical Guide. Nature 2020, 580, 576–577.
(15) Hu, Y.; Li, W.; Gao, T.; Cui, Y.; Jin, Y.; Li, P.; Ma, Q.; Liu, X.; Cao, C. The Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid Inhibits Type I Interferon Production by Interfering with TRIM25-Mediated RIG-I Ubiquitination. *J. Virol.* 2017, DOI: 10.1128/JVI.02143-16.

(16) Nguyen, K. B.; Watford, W. T.; Saloman, R.; Hofmann, S. R.; Pien, G. C.; Morinobu, A.; Gadina, M.; O'Shea, J. J.; Biron, C. A. Critical Role for STAT4 Activation by Type 1 Interferons in the Interferon-γ Response to Viral Infection. *Science* 2002, 297 ($SS89$), 2063–2066.

(17) Hadjadi, J.; Yatin, N.; Barnabei, L.; Corneau, A.; Bossiour, J.; Pere, H.; Charibit, B.; Bondet, V.; Chenevier-Gobeaux, C.; Brelillat, P.; Carlier, N.; Gauzit, R.; Morbieu, C.; Pene, F.; Marin, N.; Roche, N.; Szwebel, T.-A.; Smith, N.; Merkling, S.; Treluyer, J.-M.; Veyer, D.; Mouthon, L.; Blanc, C.; Tharaux, P.-L.; Rozenberg, F.; Fischer, A.; Dufy, D.; Rieux-Laucht, F.; Kerneis, S.; Terrier, B. Impaired Type I Interferon Activity and Exacerbated Inflammatory Responses in Severe Covid-19 Patients. *Science*, 2020, 369, 718–724.

(18) Blanco-Melo, D.; Nilsson-Payant, B. E.; Liu, W.-C.; Uhl, S.; Hoagland, D.; Moller, J.; Rordan, T. J.; Oshi, K.; Panis, M.; Sachs, D.; Wang, T. T.; Schwartz, R. E.; Lim, J. K.; Albrecht, R. A.; tenOever, B.; Kim, Y.-M.; Zink, E. M.; Nicora, C. D.; Weitz, K. K.; Webb-Sills, A. C.; Chauhan, S.; Thackray, L. B.; Halfmann, P. J.; Walters, K. B.; Estrada, J.; Murphy, G. W.; Mouthon, L.; Blanc, C.; Tharaux, P.-L.; Rozenberg, F.; Fischer, A.; Dufy, D.; Rieux-Laucht, F.; Kerneis, S.; Terrier, B. Impaired Type I Interferon Activity and Exacerbated Inflammatory Responses in Severe Covid-19 Patients. *Science*, 2020, 369, 718–724.

(19) Zhou, Q.; Wei, X.-S.; Xiang, W.; Wang, X.; Wang, Z.-H.; Chen, V.; Shannon, C. P.; Tebbutt, S. J.; Kollmann, T. R.; Fish, E. N. Interferon-A2b Treatment for COVID-19 Patient Sera. *Cell*, 2020, 182 (1), 59–72.

(20) Thomas, T.; Stefano, D.; Reisz, J. A.; Nemkov, T.; Bertolone, L.; Francis, R. O.; Hudson, K. E.; Zimring, J. C.; Hansen, K. C.; Hod, E. A.; Spitalnik, S. L.; D’Alessandro, A. COVID-19 Infection Results in Alterations of the Kynurenine Pathway and Fatty Acid Metabolism That Correlate with IL-6 Levels and Renal Status. *JCI Insight*, 2020, 5 (140327).

(21) Luo, P.; Liu, Y.; Qiu, L.; Liu, X.; Liu, D.; Li, J. Tocilizumab Treatment in COVID-19: A Single Center Experience. *J. Med. Virol.*, 2020, 92, 814–818.

(22) Scalpi, M.; Scalpi, S.; Piscioli, I.; Battista Scalera, G.; Longo, F. Pulmonary Thromboembolism in Critically Ill COVID-19 Patients. *Int. J. Infect. Dis.*, 2020, 95, 361.

(23) Grillet, F.; Behr, J.; Calame, P.; Aubry, S.; Delabrousse, E. Acute Pulmonary Embolism Associated with COVID-19 Pneumonia Detected by Pulmonary CT Angiography. *Radiology* 2020, 201544.

(24) Wang, J.; Hajizadeh, N.; Moore, E. E.; McIntyre, R. C.; Moore, P. K.; Veress, L. A.; Yaffe, M. B.; Moore, H. B.; Barrett, C. D. Tissue Plasminogen Activator (TPA) Treatment for COVID-19 Associated Acute Respiratory Distress Syndrome (ARDS): A Case Series. *J. Thromb. Haemostasis*, 2018, 16, 1752.

(25) Davitz-Castillo, P.; McMahon, B.; Aguila, S.; Bark, D.; Ashworth, K.; Allawzi, A.; Campbell, R. A.; Montenont, E.; Nemkov, T.; D’Alessandro, A.; Clendenen, N.; Shih, L.; Sanders, N. A.; Higa, K.; Cox, A.; Padilla-Romo, Z.; Hernandez, G.; Wachtow, E.; Tranah, G. D.; Nozik-Grayck, E.; Jones, K.; Pietras, E. M.; DeGregori, J.; Rondina, M. T.; Di Paola, J. TNF-α-Driven Inflammation and Mitochondrial Dysfunction Define the Platelet Hyperreactivity of Aging. *Blood*, 2019, 134 (9), 727–740.

(26) Narazaki, M.; Kishimoto, T. The Two-Faced Cytokine IL-6 in Response to Viral Infection. *J. Exp. Med.*, 2018, 219 (11), 3528.

(27) Burnum-Johnson, K. E.; Kyle, J. E.; Eisfeld, A. J.; Casey, C. P.; Stratton, K. G.; Gonzalez, J. F.; Haryarimana, F.; Negretti, N. M.; Sims, A. C.; Chauhan, S.; Thackray, L. B.; Halfmann, P. J.; Walters, K. B.; Kim, Y.-M.; Zink, E. M.; Nicora, C. D.; Weitz, K. K.; Webb-Roberson, B.-J. M.; Nakayasu, E. S.; Ahmer, B.; Konkel, M. E.; Motin, V.; Baric, R. S.; Diamond, M. S.; Kawasaki, Y.; Waters, K. M.; Smithers, B. D.; Metz, T. O. MPE:Es A Method for Simultaneous Pathogen Inactivation and Extraction of Samples for Multi-omics Profiling. *Analyst* 2017, 142 (3), 442–448.

(28) Chong, J.; Soufan, O.; Li, C.; Caraus, L.; Li, S.; Bourque, G.; Wishart, D. S.; Xia, J. *MetaboAnalyst 4.0: Towards More Transparent and Integrative Metabolomics Analysis*. *Nucleic Acids Res.* 2018, 46 (W1), W486–W494.
(42) D’Alessandro, A.; El Kasmi, K. C.; Plecitá-Hlavatá, L.; Ježek, P.; Li, M.; Zhang, H.; Gupte, S. A.; Stenmark, K. R. Hallmarks of Pulmonary Hypertension: Mesenchymal and Inflammatory Cell Metabolic Reprogramming. *Antioxid. Redox Signaling* 2018, 28 (3), 230–250.

(43) Heinrich, P. C.; Castell, J. V.; Andus, T. Interleukin-6 and the Acute Phase Response. *Biochem. J.* 1990, 265 (3), 621–636.

(44) Castell, J. V.; Gómez-Lechón, M. J.; David, M.; Andus, T.; Geiger, T.; Trullenque, R.; Fabra, R.; Heinrich, P. C. Interleukin-6 Is the Major Regulator of Acute Phase Protein Synthesis in Adult Human Hepatocytes. *FEBS Lett.* 1989, 242 (2), 237–239.

(45) Arentz, M.; Yim, E.; Klaff, L.; Lokhandwala, S.; Riedo, F. X.; Chong, M.; Lee, M. Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State. *JAMA* 2020, 323 (16), 1612–1614.

(46) Gearing, A. J. H.; Thorpe, S. J.; Miller, K.; Mangan, M.; Varley, P. G.; Dudgeon, T.; Ward, G.; Turner, C.; Thorpe, R. Selective Cleavage of Human IgG by the Matrix Metalloproteinases, Matrilysin and Stromelysin. *Immunol. Lett.* 2002, 81 (1), 41–48.

(47) Báez-Santos, Y. M.; St John, S. E.; Mesecar, A. D. The SARS-CoV-2 Coronavirus Papain-like Protease: Structure, Function and Inhibition by Designed Antiviral Compounds. *Antiviral Res.* 2015, 115, 21–38.

(48) Ziegler, C. G. K.; Allen, S. J.; Nyquist, S. K.; Mbano, I. M.; Miao, V. N.; Tzouanas, C. N.; Cao, Y.; Yousif, A. S.; Bals, J.; Hauser, B. M.; Feldman, J.; Muus, C.; Wadsworth, M. H.; Kazier, S. W.; Hughes, T. K.; Doran, B.; Gatter, G. J.; Vukovic, M.; Taliaferro, F.; Mead, B. E.; Guo, Z.; Wang, J. P.; Gras, D.; Plaisant, M.; Ansari, M.; Angelidis, I.; Alter, H.; Su, B.; Waghray, A.; Mitsialis, V.; Dwyer, D. F.; Buchheit, K. M.; Boyce, J. A.; Barrett, N. A.; Laidlaw, T. M.; Carroll, S. L.; Colonna, L.; Tkachev, V.; Peterson, C. W.; Yu, A.; Zheng, H. B.; Gideon, H. P.; Winchell, C. G.; Lin, P. L.; Bingle, C. D.; Snapper, S. B.; Kropski, J. A.; Theis, F. J.; Schiller, H. B.; Zaragosi, L.-E.; Barbry, P.; Leslie, A.; Kiem, H.-P.; Flynn, J. L.; Fortune, S. M.; Berger, B.; Finberg, R. W.; Kean, L. S.; Garber, M.; Schmidt, A. G.; Lingwood, D.; Shalek, A. K.; Ordoval-Montanes, J. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell* 2020, 181, 1016.

(49) Liang, W.; Liang, H.; Ou, L.; Chen, B.; Chen, A.; Li, C.; Li, Y.; Guan, W.; Sang, L.; Lu, J.; Xu, Y.; Chen, G.; Guo, H.; Guo, J.; Chen, Z.; Zhao, Y.; Li, S.; Zhang, N.; Zhong, N.; He, J. Development and Validation of a Clinical Risk Score to Predict the Occurrence of Critical Illness in Hospitalized Patients With COVID-19. *JAMA Int. Med.* 2020, 180, 1081.