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Plasma Proteomics of COVID-19-Associated Cardiovascular Complications

Implications for Pathophysiology and Therapeutics

Jason D. Roh, MD, MHS, a Robert R. Kitchen, PhD, a J. Sawalla Guseh, MD, a Jenna N. McNeill, MD, b Malika Aid, PhD, c Amanda J. Martinot, DVM, MPH, PhD, d,e Andy Yu, BSc, a Colin Platt, PhD, a James Rhee, MD, PhD, d,e Brittany Weber, MD, PhD, a Lena E. Trager, BA, a Margaret H. Hastings, PhD, a Sarah Ducat, BS, a Peng Xia, PhD, a Claire Castro, PhD, a Abhilasha Singh, PhD, a Bjarni Atlason, a Timothy W. Churchill, MD, f Marcelo F. Di Carli, MD, f,g Patrick T. Ellinor, MD, PhD, d, h Dan H. Barouch, MD, PhD, f,i Jennifer E. Ho, MD, a Anthony Rosenzweig, MD a

VISUAL ABSTRACT

HIGHLIGHTS

- Plasma proteomics identifies key biological processes associated with cardiac injury and stress in COVID-19.
- Among 4,996 analytes measured, ADAMTS13, the vWF-cleaving protease whose loss-of-function causes microvascular thrombosis, displays the most significant inverse association with myocardial injury in COVID-19. Mendelian randomization of ADAMTS13 cis-pQTLs supports a causal role of ADAMTS13 deficiency in myocardial injury.
- Increased Activin/TGFβ signaling is strongly associated with the heart failure biomarker NT-proBNP in COVID-19.
- SASP, a marker of biological aging, is the dominant process associated with disease severity and cardiac involvement in COVID-19. SARS-CoV-2 infection in hamsters induced a similar pattern of SASP expression, suggesting a potentially bidirectional interaction: senescence may enhance susceptibility to SARS-CoV-2, which may in turn induce premature senescence.
SUMMARY

To gain insights into the mechanisms driving cardiovascular complications in COVID-19, we performed a case-control plasma proteomics study in COVID-19 patients. Our results identify the senescence-associated secretory phenotype, a marker of biological aging, as the dominant process associated with disease severity and cardiac involvement. FSTL3, an indicator of senescence-promoting Activin/TGFβ signaling, and ADAMTS13, the von Willebrand Factor–cleaving protease whose loss-of-function causes microvascular thrombosis, were among the proteins most strongly associated with myocardial stress and injury. Findings were validated in a larger COVID-19 patient cohort and the hamster COVID-19 model, providing new insights into the pathophysiology of COVID-19 cardiovascular complications with therapeutic implications. (J Am Coll Cardiol Basic Trans Science 2022;7:425–441) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Since its discovery in December 2019, SARS-CoV-2 has resulted in over 300 million cases of COVID-19 and over 5.4 million deaths globally.1 Although viral pneumonia is the predominant clinical manifestation of severe COVID-19, cardiovascular complications occur frequently and are independently associated with morbidity and mortality. Among hospitalized COVID-19 patients, myocardial injury occurs in ~36%,2 vascular thrombosis in ~16%,3 and acute heart failure in ~2.5%.4 Acute heart failure in COVID-19 often occurs in those without pre-existing heart failure, and is associated with mortality rates approaching 47%.4 Myocardial injury, the most common cardiac complication in COVID-19, also remains a strong predictor for adverse outcomes and is associated with up to 3-fold increases in mortality.2 Multiple factors likely contribute to cardiac injury and dysfunction in COVID-19.2–8 While myocardial injury, indicated by increased circulating cardiac troponins, is common in COVID-19,2 both type I myocardial infarction caused by epicardial coronary artery occlusion and myocarditis appear to be relatively rare,5,7 suggesting other mechanisms drive most COVID-19-related cardiac complications. We systematically examined circulating proteins in control subjects and COVID-19 patients to identify those biological processes most strongly associated with biomarker evidence of myocardial injury and stress. These studies support inferences into the pathogenesis of COVID-19–induced cardiac complications and implicate pathways amenable to intervention in this process.

METHODS

DISCOVERY STUDY. To identify proteins that change with cardiac involvement in COVID-19, a case-control plasma proteomics study was performed in 80 subjects. Access to patient plasma samples was facilitated by the multi-institutional Massachusetts Consortium on Pathogen Readiness (MassCPR) and the Mass General Brigham (MGB) Biobank. COVID-19 subjects were recruited and plasma samples were collected with informed consent and IRB approval between March and June 2020 at Massachusetts General Hospital and Brigham and Women’s Hospital in Boston, Massachusetts. Subjects were chosen based on data from their electronic medical records to fill 5 prespecified groups representing a range of disease severity and cardiac involvement. To reduce the confounding influence of factors previously linked to COVID-19 severity, groups were matched as much as
possible for age, sex, and race. Patients enrolled in immunomodulator clinical trials or receiving such agents off-label were excluded. Study groups included non-COVID-19 control subjects; COVID-19 patients with moderate disease (hospitalized, but not requiring intensive care unit [ICU] level of care) with or without cardiac involvement; and COVID-19 patients with severe disease (requiring ICU care) with low or high degrees of cardiac involvement (Table 1). In patients with moderate COVID-19, cardiac involvement was defined as at least 1 of the following near time of plasma collection for proteomics: a high-sensitivity cardiac troponin T (hsTnT) >14 ng/L, N-terminal pro-B-type natriuretic peptide (NT-proBNP) >1,800 pg/mL, and/or cardiovascular dysfunction documented by transthoracic echocardiography (left ventricular ejection fraction [LVEF]<50%, regional wall motion abnormality, right ventricular systolic dysfunction, or >25% relative decrease from prior LVEF). The hsTnT threshold of 14 ng/L is consistent with the Fourth Universal Definition of Myocardial Infarction, which defines myocardial injury as a troponin concentration >99th percentile upper reference limit. For severe COVID-19 patients in the ICU, who have a high prevalence of mild troponin elevation, we used a hsTnT threshold of 20 ng/L to distinguish between low and higher levels of myocardial injury, whereas the remainder of the criteria were the same. Clinical outcomes included intubation, vasopressor requirement, neuromuscular blockade use, sedation, renal replacement therapy, pulmonary embolism, deep venous thrombosis, and in-hospital mortality. Adjudication and analysis of all clinical covariates and outcomes data was done by physicians who are board-certified in cardiology/advanced heart failure (J.H.) and pulmonary/critical care (J.M.).

PLASMA PROTEOMIC PROFILING. All blood samples obtained from subjects in the discovery study were anticoagulated with ethylenediaminetetraacetic acid. Collected plasma was stored at –80°C and underwent 2 freeze-thaw cycles before proteomic analysis. An aptamer-based proteomics platform (version 4, Somalogic) was used to measure relative levels of plasma proteins. 4,996 analytes, corresponding to 4,730 distinct human proteins.

CLINICAL VALIDATION STUDY. Key findings in the discovery proteomics study were validated in a publicly available plasma proteomics data set from the MGH Emergency Department COVID-19 cohort. This data set represents a larger cohort of 305 COVID-19 subjects who were recruited independently through the MGH Emergency Department by Filbin et al. In total, 12 of our 80 study subjects overlapped with this cohort. However, the larger validation cohort incorporated an additional 293 COVID-19 subjects and, importantly, used a different antibody-based proteomics platform (Olink) to measure relative levels of plasma proteins. Patients from the validation cohort who were hospitalized had serial plasma samples collected during their hospitalization on days 0, 3, and 7. For 36 patients, an additional sample was collected on day E, corresponding to a significant clinical deterioration event. Maximum disease severity for each subject within 28 days of presentation was determined according to the World Health Organization (WHO) Ordinal Outcomes Scale (1 = death within 28 days; 2 = intubated, ventilated, survived to 28 days; 3 = noninvasive ventilation or high-flow nasal cannula, 4 = hospitalized, supplemental O2 required; 5 = hospitalized, no supplemental O2 required; 6 = not hospitalized).

| TABLE 1 Clinical Characteristics of Subjects in Discovery Study |
|---------------------------------------------------------------|
| No COVID-19 | Moderate COVID-19 | Severe COVID-19 |
| --- | --- | --- | --- |
| Control | CV | CV | CV | CV |
| (n = 26) | (n = 13) | (n = 12) | (n = 14) | (n = 15) |
| Age, y | 62 ± 10 | 63 ± 8 | 72 ± 16 | 62 ± 12 | 63 ± 10 |
| Women | 10 (39) | 9 (69) | 6 (50) | 4 (29) | 4 (27) |
| Race | 0.174 | | | | |
| White | 19 (73) | 6 (46) | 8 (67) | 5 (36) | 7 (47) |
| Black | 1 (4) | 2 (15) | 2 (17) | 4 (29) | 5 (33) |
| Hispanic | 2 (8) | 4 (31) | 2 (17) | 4 (29) | 3 (20) |
| Other | 4 (15) | 1 (8) | 0 (0) | 1 (7) | 0 (0) |
| BMI, kg/m² | 33.1 ± 4.1 | 27.6 ± 4.1 | 31.1 ± 8.7 | 27.9 ± 4.3 | 31.9 ± 10.3 |

Past medical history

| Pulmonary disease | 8 (31) | 2 (15) | 3 (25) | 4 (29) | 3 (20) |
| Asthma | 0 (0) | 1 (8) | 0 (0) | 1 (7) | 2 (13) |
| ILD | 1 (4) | 2 (15) | 2 (17) | 2 (14) | 1 (7) |
| CAD | 0 (0) | 1 (8) | 0 (0) | 0 (0) | 0 (0) |
| MI | 0 (0) | 0 (0) | 3 (25) | 0 (0) | 2 (13) |
| Stroke | 0 (0) | 1 (8) | 1 (8) | 0 (0) | 2 (13) |
| Hypertension | 13 (50) | 6 (46) | 10 (83) | 7 (50) | 9 (60) |
| Hyperlipidemia | 11 (42) | 8 (62) | 7 (58) | 7 (50) | 9 (60) |
| Diabetes mellitus | 1 (4) | 2 (15) | 6 (50) | 6 (43) | 7 (47) |
| Cancer | 0 (0) | 3 (23) | 3 (25) | 0 (0) | 6 (40) |
| Liver disease | 2 (8) | 4 (31) | 1 (8) | 2 (14) | 2 (13) |
| Kidney disease | 0 (0) | 1 (8) | 6 (50) | 1 (7) | 1 (7) |

Baseline medications

| Anticoagulation | 1 (4) | 0 (0) | 5 (42) | 0 (0) | 3 (20) |
| NSAID | 9 (35) | 3 (23) | 5 (42) | 3 (21) | 8 (53) |
| Statin | 11 (42) | 5 (38) | 7 (58) | 6 (43) | 6 (40) |
| Immunosuppression | 0 (0) | 0 (0) | 1 (8) | 1 (7) | 1 (7) |

Values are mean ± SD or n (%). Pbest for analysis of variance for age, or Fisher exact for categorical tested differences between groups. *P < 0.05 on Fisher exact pairwise testing for categorical variables and Student’s t-test for age between COVID-19 patients and COVID-19 negative controls - groupings or between CV (low/high) within moderate and severe COVID-19 groups, respectively. BMI = body mass index; CAD = coronary artery disease; COPD = chronic obstructive pulmonary disease; CV = cardiac involvement; ILD = interstitial lung disease; MI = myocardial infarction; NSAID = nonsteroidal anti-inflammatory drugs.
MENDELIAN RANDOMIZATION OF ADAMTS13 CIS-pQTLs. Automated 2-sample Mendelian randomization (MR) was performed through an open-source application, MR-Base,11 that systematically supports causal inference interrogation with summary-level data.12 A multivariable instrument was constructed to genetically estimate protein levels, ADAMTS13 (A Disintegrin and Metalloproteinase with a Thrombospondin type I motif, member 13) using locus-wide significant pQTLs acquired from PhenoScanner.13,14

Given an experimentally derived hypothesis that COVID-19 results in cardiac injury without type I infarction, we sought the most proximate International Classification of Diseases-10th Revision code for acute myocardial injury that is independent of obstructive epicardial coronary disease (International Classification of Diseases code: I24.8, other forms of acute ischemic heart disease). Summary statistics were acquired from UKB-b:11309 (n = 463,010 with the data set containing 269 cases and 462,741 control subjects). Outcome summary statistics were accessed September 20, 2020, in MR-Base. See Supplemental Methods and Supplemental Figure 1 for a detailed description of the MR procedure.

HAMSTER COVID-19 MODEL. Cardiac and pulmonary tissue from 6 golden Syrian hamsters (Envigo), ages 10–12 weeks, from our prior work15 were used to further investigate candidate targets from the human discovery study. All animals were housed at Bioqual and studies were approved by their Institutional Animal Care and Use Committee. Animals were divided into 2 groups, naïve (uninfected) control subjects and SARS-CoV-2 infected (n = 3/group). SARS-CoV-2 infection was performed using previously published methods.15 Histological processing of lung and cardiac tissue, Carstair’s method, and hematoxylin and eosin staining protocols were performed using previously published methods.15,16 See the Supplemental Methods for a detailed description of the hamster COVID-19 model and additional methods used for processing explanted tissue samples.

CARDIOMYOCYTE EXPERIMENTS. Neonatal rat cardiomyocyte isolation was performed using previously published methods.17 Cardiomyocytes were cultured in serum-free medium and incubated for 18 hours with 10 ng/mL of various recombinant ligand proteins (Peprotech or R&D Systems) known to bind TGFβ receptors or Activin type II receptors. RNA was extracted using Trizol, and polymerase chain reaction was done using SYBR green and standard amplification protocols. Relative changes in gene expression were measured using the ΔΔCT method. Senescence associated β-galactosidase staining was performed using Cell Signaling Technology Kit (9860) and quantified using ImageJ software (National Institutes of Health).

BIOINFORMATIC AND STATISTICAL ANALYSES. For analysis of the SOMAscan proteomics discovery study, median normalized relative abundances of the 4,996 analytes were imported into R (version 3.6.1) using the SomaDataIO package. Principal component analysis was performed on log-transformed values using R’s prcomp function, differential protein abundance using limma (version 3.40.6), and protein enrichment using clusterProfiler (version 3.12.0). Pathway analysis was done using Gene Ontology data (MSigDB), appended with 2 curated senescence associated secretory phenotype (SASP) pathways. For gene-set enrichment analyses, proteins were ranked by t-statistic and 50,000 permutations used to compute the nominal enrichment P values. For all analyses, P values were adjusted for multiple comparisons using the Benjamini and Hochberg method.

For validation using the data released by Filbin et al,10 we obtained Olink Normalized Protein EExpression (NPX) data and associated patient metadata from Olink.18 Of the 383 individuals included in this study, we removed patients without documented COVID-19 positivity, leaving 305 patients. In assessing the relationships of ADAMTS13 and follistatin-like 3 (FSTL3) with days postdiagnosis, WHO severity, and 28-day survival, we performed repeated-measures analysis of variance (days postdiagnosis) and Kruskal-Wallis rank sum tests (for severity and survival) and provide the significances of these associations. For the analyses of WHO severity and 28-day survival we used only protein abundances in NPX units from D0 patient samples.

Between-group comparisons in patient demographics and outcomes were examined using analysis of variance for continuous variables and Fisher exact for categorical variables. If indicated, pairwise comparisons were conducted between those with and without COVID-19, as well as those with and without cardiac involvement within each disease severity stratum. Pearson correlations were used to measure correlation of candidate proteins with cardiac biomarkers.

Analysis of the golden Syrian hamster lung RNA sequence data was done using STAR version 2.7.3a with the MesAur1.0 (GCF_000349665.1) assembly and annotation of the hamster downloaded from NCBI. Transcript abundance estimates were calculated internal to the STAR aligner using the algorithm of htseq-count. DESeq2 was used for normalization, producing both a raw and normalized read count table. Differential expression at the gene level were
performed by DESeq2 implemented in the DESeq2 R package. An adjusted \( P \) value <0.05 was used to determine genes that were significantly up- or down-regulated by SARS-CoV-2 at day 4 postchallenge compared with naïve control subjects using the Benjamini and Hochberg method.

**RESULTS**

**CHARACTERISTICS OF DISCOVERY STUDY PARTICIPANTS.**

The cohort for the plasma proteomics discovery study had a mean age of 64 ± 11 years and was 41% women (Table 1). There were no significant differences in age or sex between COVID-19 patients and control subjects, although control subjects included more White subjects and there was a nonsignificant trend of higher age in moderate COVID-19 patients with cardiac involvement because of limited samples available for matching. Control subjects had higher body mass index, but less coronary artery disease, diabetes, cancer, and chronic kidney disease. There were no differences in baseline hypertension, hyperlipidemia, myocardial infarction, stroke, liver, or pulmonary disease. Among moderate COVID-19 patients, those with cardiac involvement had more chronic kidney disease and baseline antiangiogenesis. In severe COVID-19 patients, cancer was more prevalent in those with higher levels of cardiac involvement. Consistent with our definition of cardiac involvement, cardiac involvement in COVID-19 patients was associated with higher high-sensitivity cardiac troponin T (hsTnT) and NT-proBNP, but also higher C-reactive protein (CRP), and D-dimer levels (Supplemental Table 1). There was a significant difference in pulmonary embolism and deep venous thrombosis, although event rates were relatively low. Severe COVID-19 patients with high degrees of cardiac involvement had the highest in-hospital mortality (33%) (Supplemental Table 1).

**SENESCENCE-ASSOCIATED SECRETORY PROTEINS INCREASED WITH COVID-19 DISEASE SEVERITY AND CARDIAC INVOLVEMENT.**

To first determine if plasma proteomic profiles can distinguish subjects according to COVID-19 disease severity and/or cardiac involvement, we performed unsupervised principal component analysis in our discovery cohort. The first principal component (PC1), a compressed set of proteins representing the maximum variance in the data set, clustered most subjects into their prespecified group (Figure 1A). This suggests that the plasma proteome can not only distinguish between subjects with and without COVID-19, but can also accurately discriminate COVID-19 disease severity and cardiac involvement. Notably, cardiac TnT and NT-proBNP, proteins used to assign subjects to the different groups, were not amongst the most highly regulated proteins in PC1 (Supplemental Figure 2), suggesting that other biological processes likely underlie much of the variability in disease severity and cardiac involvement in COVID-19 patients. To gain insight into what these processes might be, we first performed pathway analysis using those proteins loaded in PC1. Interestingly, despite study subjects being age-matched, pathway analysis identified the senescence-associated secretory phenotype (SASP-1), a marker of biological aging (Supplemental Table 2), as the most highly enriched process (normalized enrichment score [NES] = 2.2; \( P_{\text{adj}} = 9.6 \times 10^{-5} \)) in PC1 (Figure 1B, Supplemental Table 3). To confirm this unexpected finding, a more comprehensive SASP-2 set was generated from a literature search of all published SASP proteins (Supplemental Table 2) and similarly was highly enriched in PC1 (NES = 2.0; \( P_{\text{adj}} = 9.6 \times 10^{-5} \)).

Because our initial pathway analysis only incorporated proteins loaded in PC1, to further investigate this dominant senescence signal, we performed additional pathway analysis in our various COVID-19 subgroups using all 4,996 protein analytes measured on the SOMAscan platform. SASP-2 emerged as the most significantly up-regulated process associated with cardiac involvement in both moderate (NES = 1.6; \( P_{\text{adj}} = 2.8 \times 10^{-2} \)) and severe (NES = 1.7; \( P_{\text{adj}} = 2.1 \times 10^{-2} \)) COVID-19 (Supplemental Tables 4-7). Furthermore, in the independent validation cohort of 305 COVID-19 patients, the 2 SASP sets were the pathways most significantly associated with disease severity (Figure 1B, Supplemental Table 8). Transcriptional profiles of lungs from hamsters infected with SARS-CoV-2 also showed marked enrichment of both SASP-1 (NES = 1.8; FDR q-value = 1.5 \times 10^{-3} \)) and SASP-2 (NES = 2.2; FDR q-value <0.001) compared with age-matched noninfected control subjects (Figure 1C, Supplemental Table 9), demonstrating that these age-related senescence genes can be induced by SARS-CoV-2 infection.

**MICROVASCULAR INFLAMMATORY PROTEINS ARE ASSOCIATED WITH INCREASING COVID-19 SEVERITY.**

Next, to identify specific plasma proteins that are differentially regulated in COVID-19, we compared all 4,996 protein analytes in COVID-19 patients (n = 54) with noninfected control subjects (n = 26). Consistent with the systemic microvascular pathology reported in COVID-19,\(^5\)\(^6\)\(^7\) our plasma proteomics analysis strongly suggested an element of endothelial injury in these patients. The most highly up-regulated plasma proteins in COVID-19 patients were extracellular histones (Figure 2A, Supplemental Table 10),
**FIGURE 1** Senescence Associated Secretory Phenotype Is Associated With COVID-19 Severity and Cardiac Involvement

(A) Unsupervised clustering of the 80 patient plasma samples based on principle component 1 (PC1). (B) Venn diagram displaying overlapping pathways associated with increasing COVID-19 disease severity in the discovery and validation cohorts. In the discovery cohort, enrichment analysis was done with PC1 proteins (Supplemental Table 3). In the validation cohort, pathway analysis was performed using Day 0 proteomic profiles regressed on maximum World Health Organization disease severity per patient (Supplemental Table 8). (C) Heat maps displaying differential expression of the 50 most significantly regulated genes in the SASP-1 or -2 gene sets in lungs from hamsters infected with SARS-CoV-2 vs naive control subjects. Tissue samples were collected 4 days after infection. n = 3/group. SASP = senescence associated secretory phenotype.
markers of neutrophil extracellular traps implicated in COVID-19-related microvascular inflammation and thrombosis.\(^\text{20}\) Furthermore, von Willebrand Factor (vWF), a clotting factor secreted by activated endothelial cells, was one of the most significantly elevated circulating proteins in COVID-19 (341\% higher; \(P_{\text{adj}} = 8.3 \times 10^{-18}\)) (Figure 2A, Supplemental Table 10).

To identify proteins correlated with COVID-19 severity, we limited our analysis to only COVID-19 positive subjects, comparing patients with moderate (\(n = 25\)) vs severe (\(n = 29\)) COVID-19, and found 171 plasma proteins differentially expressed between the groups (Figure 2B, Supplemental Table 11). The most significantly increased protein in severe COVID-19 was regenerating islet-derived protein 3 gamma (353\% higher; \(P_{\text{adj}} = 1.4 \times 10^{-5}\)), an antimicrobial protein secreted by pulmonary and gut epithelium.\(^\text{21,22}\) Interestingly, 2 of the 10 most significantly down-regulated proteins were neural cell adhesion molecules (NCAM1 and NCAM2), which were 38\% and 34\% lower in patients with severe COVID-19, respectively (\(P_{\text{adj}} = 6.6 \times 10^{-5}\) and \(9.0 \times 10^{-4}\)). NCAMs regulate axonal outgrowth and olfactory development,\(^\text{23}\) raising the possibility of a role in the transient anosmia frequently seen in COVID-19. The most significantly down-regulated protein in severe COVID-19, however, was A Disintegrin and Metalloproteinase with a Thrombospondin type I motif, member 13 (ADAMTS13) (44\% reduction; \(P_{\text{adj}} = 1.4 \times 10^{-5}\)). ADAMTS13 is a secreted protease that inhibits thrombosis by cleaving vWF multimers, and its deficiency is a known cause of spontaneous microvascular clot formation and thrombotic thrombocytopenia purpura (TTP).\(^\text{24}\) vWF/ADAMTS13 ratios progressively increased with worsening COVID-19 severity (Supplemental Figure 3), suggesting that a deficiency in ADAMTS13 levels relative to its known substrate occurs with increased COVID-19 severity. Furthermore, ADAMTS13 levels inversely correlated with clinical D-dimer levels in COVID-19 patients (\(r = -0.7\); \(P < 0.001\)) (Supplemental Figure 4).
suggesting that the overall thrombotic burden in COVID-19 patients is higher in those patients with lower ADAMTS13 levels.

**ADAMTS13 DEFICIENCY IN COVID-19 RELATED MYOCARDIAL INJURY.** Our initial analyses implicated senescence and microvascular inflammatory processes in overall COVID-19 pathophysiology. To gain further insight specifically into the cardiac complications of COVID-19, we performed targeted regression analysis using established clinical biomarkers of myocardial injury or stress. To identify circulating proteins most strongly associated with
myocardial injury in COVID-19, we regressed the entire data set for the 54 COVID-19 patients on cardiac troponin (TnT) SOMAmer levels, which strongly correlated with clinical hsTnT concentrations ($r = 0.8; P = 3.1 \times 10^{-13}$) (Supplemental Figure 5). A total of 1,143 proteins differed significantly after regression on TnT (Figure 3A, Supplemental Table 12). The proteins with the most significant positive association with cardiac TnT were mostly intracellular myocyte proteins, likely reflecting myocardial necrosis. Interestingly, the protein with the most significant negative association with cardiac TnT was ADAMTS13 ($\beta$ coefficient $= -0.4; P_{\text{adj}} = 8 \times 10^{-7}$). Given the marked and progressive decline in ADAMTS13 levels observed with increasing cardiac involvement along with its strong correlation with TnT (Figures 3B and 3C), we looked to see if a similar phenomenon was occurring in the COVID-19 patients from the MGH Emergency Department validation cohort. Indeed, in these 305 patients, plasma ADAMTS13 levels displayed a similarly robust inverse correlation with cardiac troponin I ($r = -0.4; P = 2 \times 10^{-16}$) (Supplemental Figure 6) and decreased further as COVID-19 progressed ($P = 4.4 \times 10^{-13}$) (Figure 4A). Moreover, lower ADAMTS13 levels on presentation were associated with overall disease severity ($P < 2.2 \times 10^{-15}$) and 28-day mortality ($P = 7.7 \times 10^{-5}$) (Figures 4B and 4C).

To assess a potential causal role of decreased ADAMTS13 in myocardial injury, we performed Mendelian randomization (MR) analysis in 463,010 subjects in the UK Biobank using intronic cis-pQTLs, genetic determinants of ADAMTS13 protein levels that map to the gene itself. Because these alleles are inherited independent of confounders, a positive association provides supportive evidence that ADAMTS13 levels may be in a causal pathway with cardiac injury, even outside the severe effects seen in TTP. Indeed, we found that genetically determined lower levels of plasma ADAMTS13 were associated with higher likelihood of clinically diagnosed myocardial injury in a large, general population (inverse-variant weighted $\beta = -2.34 \times 10^{-4}; P = 6.4 \times 10^{-5}$) (Figure 5, Supplemental Figure 7), suggesting that reduced ADAMTS13 levels increase vulnerability to cardiac injury.

To further investigate why circulating ADAMTS13 levels are lower in severe COVID-19 patients, we looked to see if ADAMTS13 gene expression changed in the SARS-CoV-2-infected hamsters. Similar to humans, hamsters display severe endothelialitis associated with SARS-CoV-2 infection. Additionally, we found evidence of vascular thrombus formation in the lungs and small vessel fibrin deposition and cardiomyocyte degeneration consistent with microvascular compromise and cardiac injury in infected animals (Figures 6A to 6F). Compared to naive control subjects, ADAMTS13 gene expression was 67% lower in SARS-CoV-2-infected animals.

**Figure 4** Association of Lower ADAMTS13 Levels With Disease Severity and Mortality in COVID-19

| Boxplots of ADAMTS13 plasma levels in the MGH Emergency Department validation cohort (n = 305 COVID-19 patients) according to (A) days from initial presentation or at event, E, marking significant clinical deterioration; (B) maximum COVID-19 severity (World Health Organization classification); or (C) 28-day survival. Boxplots display median and 25th and 75th percentiles. NPX = normalized protein expression. |
FIGURE 5 Mendelian Randomization Analysis of cis-pQTLs in ADAMTS13 Gene Infers Causality in Myocardial Injury

Scatterplot of SNP effects on ADAMTS13 plasma levels (pQTLs) and clinically diagnosed myocardial injury in the UK Biobank general population sample (n = 463,010; 269 cases and 462,741 control subjects). The slope of each line corresponds to the estimated Mendelian randomization effect per method. (Light blue = inverse variance-weighted method; dark blue = weighted median method; green = MR Egger method). This demonstrates that genetically determined decreases in ADAMTS13 levels are quantitatively associated with increasing risk of myocardial injury, and support a causal role of ADAMTS13 in this context.

ADAMTS13 showed an inverse relationship with NT-proBNP, the correlation was modest (r = −0.3; P = 2.6 × 10⁻²) (Figure 3C), suggesting that other processes are likely more substantial contributors to myocardial stress in COVID-19. To identify proteins strongly associated with myocardial stress, we regressed the entire data set of our 54 COVID-19 patients on NT-proBNP Somamer levels, which strongly correlated with the clinical assay (r = 0.8; P = 3.8 × 10⁻¹²) (Supplemental Figure 8). Of the 526 proteins that were differentially expressed after regression, FSTL3, a marker of TGFβ and Activin receptor signaling, displayed the most significant positive association with NT-proBNP (β coefficient = 0.4; P_{adj} = 4.6 × 10⁻⁷) (Figure 7A, Supplemental Table 13). FSTL3 levels correlated strongly with both NT-proBNP and cardiac TnT (r = 0.7; P < 0.001 for both), and were markedly higher in both moderate and severe COVID-19 patients with cardiac involvement compared with their respective control subjects (Figures 7B and 7C, Supplemental Tables 4 and 6).

The association between FSTL3 and NT-proBNP levels robustly validated in the MGH Emergency Department cohort. FSTL3 levels showed similarly powerful correlations with NT-proBNP (r = 0.7; P < 2 × 10⁻¹⁶) and cardiac troponin I (r = 0.6; P < 2 × 10⁻¹⁶) (Supplemental Figure 9) and increased with disease duration (P = 2.3 × 10⁻⁶) (Figure 8A). Moreover, presenting FSTL3 levels strongly associated with both disease severity (P < 2.2 × 10⁻¹⁶) and 28-day mortality (P = 3.9 × 10⁻¹⁰) in COVID-19 (Figures 8B and 8C).

MR analysis was unable to be performed for FSTL3 because only a single pQTL (rs2986335) has been identified for this protein and this pQTL did not reach genome-wide significance. However, because FSTL3 is an indirect marker of increased TGFβ superfamily signaling rather than part of the causal pathway, we would not anticipate a causal relationship between FSTL3 and heart failure. Because FSTL3 expression is induced by activation of TGFβ and Activin receptors, which can be initiated by multiple ligands of the TGFβ superfamily, including TGFβ, Activins, bone morphogenic proteins, and growth differentiation factors, we looked to see which of these ligands might be contributing to the increased circulating FSTL3 associated with myocardial stress in COVID-19. In total, 14 of the relevant ligands are measured on the SOMAscan platform, and 12 of these positively correlated with FSTL3 (Supplemental Figure 10). Of those ligands, only Activins and TGFβ robustly increased FSTL3 expression in cardiomyocytes (Supplemental Figure 11). Interestingly, we found that these FSTL3 inducers also increased the

(P_{adj} = 1.0 \times 10^{-5}) (Figure 6C), suggesting that at least part of the decrease in circulating ADAMTS13 in COVID-19 is through decreased mRNA levels resulting in reduced synthesis of this antithrombotic protein. This was associated with a 3.8-fold increase in vWF expression (P_{adj} = 1.2 \times 10^{-10}) (Figure 6C).

TGFβ Superfamily Signaling Is Associated with Increased Myocardial Stress in COVID-19. Although cardiac microvascular thrombosis is likely a major contributor to myocardial injury in COVID-19, it is only identified in a subset of patients. Nonischemic etiologies, such as right ventricular strain, myocarditis, and stress cardiomyopathy, are other sources of cardiac injury and dysfunction in COVID-19, which can result in elevated cardiac troponins as well as increases in the heart failure biomarker NT-proBNP. Although
percentage of senescent cardiomyocytes in culture (Supplemental Figure 12), consistent with the known biology of these ligands in other cell systems and the marked increase in SASP enrichment we observed in COVID-19 patients with cardiac involvement. Of these proteins, TGFβ1 was the most significantly increased in COVID-19 patients (74% higher than control subjects, $P_{adj} = 7.2 \times 10^{-3}$), suggesting that it may be the primary circulating ligand responsible for the increase in FSTL3 observed in COVID-19 patients with elevated NT-proBNP. Although we were unable to determine if TGFβ1 expression is directly increased in the hearts of those with COVID-19, RNAseq profiles of lungs from hamsters infected with SARS-CoV-2 indicated that TGFβ1 signaling is indeed up-regulated in animals infected with COVID-19 (NES = 1.5; FDR q-value = 2.7 × 10^{-3}) (Supplemental Figure 13).

**DISCUSSION**

This study systematically analyzed the plasma proteome of control subjects and COVID-19 patients
across a range of disease severity and cardiac involvement to gain mechanistic insights into the pathophysiology of myocardial injury and stress associated with COVID-19. Key findings from our 80-subject discovery cohort were validated in a larger cohort of 305 COVID-19 patients and investigated in the Syrian hamster model of COVID-19. Our results not only provide additional evidence supporting prior reports of dysregulated innate immune responses, microvascular inflammation, thrombosis, and cell death pathways in COVID-19, but also identify multiple new proteins and biological processes potentially involved in COVID-19 pathophysiology. Importantly, this is the first proteomics study to focus

![Figure 7: Association of TGFβ Superfamily Signaling With Heart Failure Biomarker in COVID-19](image)

(A) Volcano plot displaying differentially expressed plasma proteins in the discovery study after regression on NT-proBNP levels. Beta coefficient is plotted on the X-axis showing magnitude and direction of correlation with NT-proBNP. Red = significant (P_adj < 0.05). Green = nonsignificant. P values were adjusted for multiple hypothesis testing using the Benjamini and Hochberg method. (B) Boxplots displaying median and 25th and 75th percentiles of plasma FSTL3 levels according to disease severity and cardiac involvement in the discovery cohort. (C) Pearson correlations of FSTL3 levels with biomarkers for myocardial stress (NT-proBNP) and injury (cardiac TnT) in the discovery cohort. Solid line represents best fit line after simple linear regression, and dashed lines represent the 95% CI. TGFβ = transforming growth factor beta; other abbreviations as in Figure 3.
on cardiac complications in COVID-19. The a priori focus on cardiac involvement in our case-control study design led to the identification of 3 key biological processes strongly associated with COVID-19–related myocardial injury and stress: 1) a reduction in the antithrombotic protein, ADAMTS13; 2) an increase in TGFβ superfamily signaling, previously linked to cardiac dysfunction and injury, fibrosis, and senescence; and 3) an up-regulation of the SASP, a marker of biological aging. Notably, these pathways are potentially amenable to therapeutic intervention with agents already in clinical trials or approved by the U.S. Food and Drug Administration for other indications.

The first major finding from our analyses was the detection of a marked reduction in circulating ADAMTS13 that progressed with COVID-19 duration and severity. Of the 4,996 protein analytes assessed in our discovery cohort, ADAMTS13 not only was the most significantly decreased protein in severe COVID-19, but also displayed the strongest inverse association with myocardial injury. Autopsies have found evidence of microvascular thrombosis in multiple organs, including the heart, in COVID-19 patients. Given its role in thrombotic microangiopathies, the possibility has been raised that an acquired ADAMTS13 deficiency or local imbalance with its substrate, vWF, could be contributing to thrombotic complications in COVID-19. Our data provide new evidence supporting an important role for reduced ADAMTS13 in COVID-19–related myocardial injury, and Mendelian randomization of cis-pQTL variants within the ADAMTS13 gene suggest that deficiencies in its circulating levels may be causal in myocardial injury, independent of acute coronary syndromes. Although our MR analysis was not specific to COVID-19, reduced ADAMTS13 levels have been observed in other infectious processes, such as sepsis and influenza, which are also frequently associated with myocardial injury. It is thus plausible that the reduction in ADAMTS13 levels driven by severe infections contributes to a common immunothrombosis pathophysiology driving cardiac microvascular occlusion and myocardial injury in these contexts.

The degree to which reduced ADAMTS13 contributes to microvascular thrombosis is likely influenced by the extent of endothelial injury and inflammation in the affected microvascular networks, which are more severe in COVID-19 than other respiratory virus infections, such as influenza. Notably, data from the hamster model also showed marked down-regulation of ADAMTS13 transcription with SARS-CoV-2 infection, suggesting that the decreased circulating levels seen in COVID-19 are at least partially mediated by a primary decrease in its synthesis, as opposed to a purely consumptive secondary process. As more phenotypic data become incorporated into the rapidly developing COVID-19 genetic databases, additional ADAMTS13 pQTL analyses in COVID-19
patients could help confirm its specific role in COVID-19-related myocardial injury.

Importantly, given recent safety concerns of therapeutic systemic anticoagulation in critically ill COVID-19 patients, who notably also display the lowest ADAMTS13 levels and highest risk of cardiac complications, repletion of ADAMTS13 could provide a safer, targeted, and potentially more effective strategy for preventing vascular thrombosis and myocardial injury in these patients, and could be targeted to those with low ADAMTS13 levels. Recombinant ADAMTS13 has already demonstrated safety in patients with congenital TTP. Additionally, it has been shown to reduce myocardial injury and inflammation in animal models of cardiac ischemia, and recent reports suggest it can effectively reduce the heightened vWF activity and ultra-high molecular weight vWF multimers detected in blood samples from patients with severe COVID-19. Data from our group and others now show that extracellular and cardiac microvascular thrombosis also occurs in hamsters infected with SARS-CoV-2, suggesting it may represent an ideal preclinical model for testing the therapeutic efficacy of recombinant ADAMTS13 interventions in COVID-19. Based on our collective findings, we propose that targeted ADAMTS13 repletion warrants further evaluation as a tailored approach to preventing myocardial injury and possibly other thromboembolic complications in patients with severe COVID-19 and reduced ADAMTS13 levels.

In addition to microvascular thrombosis, other nonischemic etiologies, including myocarditis, stress cardiomyopathy, and right ventricular strain, also contribute to myocardial necrosis in COVID-19, often resulting in substantial increases in circulating levels of not just cardiac troponins, but also NT-proBNP. Indeed, in our analyses, we found that the protein most strongly associated with NT-proBNP was not ADAMTS13, but rather FSTL3, a marker of TGFβ superfamily signaling. FSTL3 is 1 of only 4 circulating proteins (of 1,310 assayed) that potentially distinguishes between patients with acute stress cardiomyopathy in comparison to acute myocardial infarction. Although these proteins can be acutely cardioprotective in the setting of ischemia or inflammation-mediated myocardial stress, chronically high levels likely become maladaptive, and induce reversible cardiac dysfunction, as is seen in some COVID-19 patients. Multiple TGFβ superfamily inhibitors, including those currently being investigated in clinical trials, have shown benefits in preclinical models of heart failure, as well as acute lung injury. Although these data suggest that this class of reagents could potentially attenuate adverse cardiac and pulmonary remodeling and dysfunction in COVID-19, careful patient selection and optimal timing of such interventions will be critical to maximizing therapeutic benefit while minimizing potential adverse effects.

TGFβ superfamily signaling could also contribute to the long-term sequelae seen in some survivors of COVID-19. A hallmark of TGFβ superfamily members, particularly the Activins and TGFβ ligands that increased FSTL3 expression in cardiomyocytes, is their ability to induce paracrine senescence, a process of irreversible cell cycle arrest closely linked to aging and cancer. Circulating factors secreted by senescent cells, collectively referred to as SASP, often amplify the inflammatory response and subsequent adverse remodeling from tissue injury, and have led to the hypothesis that a pre-existing burden of senescent cells and SASP in the elderly may explain their heightened risk for developing severe COVID-19. Recent work has intriguingly suggested that targeted elimination of senescent cells dramatically improves survival in aged animals with COVID-related disease; however, whether this age-related biology is associated with worse outcomes in humans is unclear. To the best of our knowledge, our data are the first to report an increase in SASP profiles in COVID-19 patients that tracks with disease severity. Also, because study subjects in our discovery cohort were mostly age-matched, this suggests that biological aging may represent a more accurate predictor for developing severe COVID-19 than chronological aging alone. Indeed, in the validation cohort, a subject’s SASP profile on presentation was the most significant pathway associated with progression to more severe disease over the subsequent 28 days. Conversely, the marked up-regulation of SASP genes seen in hamsters with severe COVID-19, suggests that SARS-CoV-2 infection itself induces an increase in SASP production. Taken together, these data support a bidirectional model of COVID-19 interaction with senescence biology. It seems likely that biological aging, reflected in circulating SASP proteins, puts patients at higher risk for severe COVID-19 and that SARS-CoV-2 infection also induces cellular senescence, further increasing SASP production, leading to a positive feedback loop. Determining whether COVID-19 accelerates the accumulation of senescent cells in patients and in which tissues will become increasingly important as the long COVID syndrome becomes more prevalent. Senescent cells, which can persist long after acute tissue injury, have been shown to contribute to chronic organ damage, including in the heart.
drivers of this process in COVID-19, which could be linked to surges of Activins and TGFβ during acute COVID-19, could provide new insights into some of the next stages of this pandemic and the enigmatic pathophysiology of the long COVID syndrome.

**STUDY LIMITATIONS.** First, observational data cannot definitively establish causality. In the case of ADAMTS13, however, additional inferences from genetics strongly support a pathogenic role. Further studies of SARS-CoV-2 infection in Syrian hamsters also mechanistically link viral infection to reduced ADAMTS13 expression. Second, although the subjects included in the discovery study, COVID-19 patients and control subjects, were well-matched for potential confounders, sample sizes were relatively small. In this context, the robust validation of our key findings in a larger data set generated using an independent proteomic technology is reassuring. Third, in patients with severe COVID-19, vasopressors and anti-coagulation use were more common in those with cardiac involvement. However, these medications seem unlikely to affect the major findings of our study, which were also observed in patients with moderate COVID-19 and cardiac involvement, who did not experience more exposure to these medications compared with their respective control subjects. Additionally, all 3 major biological findings were supported by data from the hamster COVID-19 model, which does not incorporate these medications.

Finally, although promising candidates were identified that might be targeted to mitigate COVID-19 cardiac complications, these hypotheses require rigorous additional testing. Importantly, our clinical observations strongly resonate with findings in the hamster model of COVID-19, further supporting an important role of these processes in COVID-19 pathophysiology. These findings also suggest this model could provide a powerful preclinical tool for testing the safety and therapeutic efficacy of currently available reagents targeting these candidates as a prelude to clinical trials.

**CONCLUSIONS**

Systematic analysis of circulating plasma proteins revealed important insights in COVID-19 cardiovascular pathophysiology and provides a foundation for novel therapeutic approaches to prevent or treat cardiac complications of COVID-19. Further investigation is warranted to determine if restoring ADAMTS13 levels, inhibiting TGFβ superfamily signaling, or modulating senescence can mitigate cardiovascular complications and improve outcomes in COVID-19 patients.
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APPENDIX For an expanded Methods section as well as supplemental tables and figures, please see the online version of this paper.