No evidence of hemoglobin damage by SARS-CoV-2 infection

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ABSTRACT

Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disease (COVID-19) has affected over 22 million patients worldwide as of August 2020. As the medical community seeks better understanding of the underlying pathophysiology of COVID-19, several theories have been proposed. One widely shared theory suggests that SARS-CoV-2 proteins directly interact with human hemoglobin (Hb) and facilitate removal of iron from the heme prosthetic group, leading to the loss of functional hemoglobin and accumulation of iron. Herein, we refute this theory. We compared clinical data from 21 critically ill COVID-19 patients to 21 non-COVID-19 acute respiratory distress syndrome (ARDS) patient controls, generating hemoglobin-oxygen dissociation curves from venous blood gases. This curve generated from the COVID-19 cohort matched the idealized oxygen-hemoglobin dissociation curve well (Pearson correlation R²=0.97, P<0.0001; a coefficient of variation of the root-mean-square deviation [CV(RMSD)] = 7.3%). We further analyzed hemoglobin, total bilirubin, lactate dehydrogenase, iron, ferritin, and haptoglobin levels. For all analyzed parameters, patients with COVID-19 had similar levels compared to patients with ARDS without COVID-19. These results indicate that patients with COVID-19 do not exhibit any hemolytic anemia or a shift in the normal hemoglobin-oxygen dissociation curve. We therefore conclude that COVID-19 does not impact oxygen delivery through a mechanism involving red cell hemolysis and subsequent removal of iron from the heme prosthetic group in hemoglobin.
have reported mortality rates for COVID-19 in the range of 25-32%, similar to the mortality rates for non-COVID-19 ARDS.\textsuperscript{9,10} While the exact pathophysiology of COVID-19 remains a topic of active investigation, growing evidence suggests that respiratory failure in COVID-19 patients behaves similarly to respiratory failure in patients with severe viral pneumonia that triggers ARDS.\textsuperscript{11,12} The uncertainty at the outset of this pandemic as well as some persistently unique features of the disease (e.g., increased thromboembolic risks), have led to a large number of proposed hypotheses regarding the pathophysiological mechanisms of SARS-CoV-2.

One widely proposed theory holds that viral proteins directly interact with human hemoglobin (Hb) and facilitate removal of iron from the protein’s heme prosthetic group, resulting in the loss of functional hemoglobin and the toxic accumulation of iron. This theory originated from a manuscript by Liu and Li\textsuperscript{13} posted in the preprint server ChemRxiv (over 1.89 million manuscript views as of August 2020). This work by Liu and Li,\textsuperscript{13} which has not been peer-reviewed and continues to be cited,\textsuperscript{14,15} uses \textit{in silico} approaches to model interactions between several viral proteins and hemoglobin. In brief, the manuscript suggests that viral proteins ORF1ab, ORF10, and ORF3a are derived from infected plasma cells and work in concert to remove heme from the β chain of hemoglobin, strip iron from heme, and sequester the resulting iron-free protoporphyrin IX (PPIX). The authors speculate that this coordinated “attack” of hemoglobin occurs in the plasma following immune hemolysis, resulting in the release of toxic amounts of iron, diminished functional hemoglobin levels, and disrupted heme metabolism. Finally, Liu and Li\textsuperscript{15} further purport that the consequences of such hemoglobin degradation account for some of the irregular clinical characteristics reported early in the pandemic (Figure 1). While this work has received significant attention from scientists, physicians, the press, and the general public, the study advances a theory of viral interaction with hemoglobin that is inconsistent with well-characterized mechanisms of physiological heme degradation,\textsuperscript{16} and, critically, advances a pathophysiological mechanism inconsistent with the clinical presentation of COVID-19 patients. Herein, we compare clinical laboratory data from confirmed COVID-19 patients admitted to the intensive care unit (ICU) at University of Pittsburgh Medical Center (UPMC) to patients with ARDS but without COVID-19. These empirical data challenge the theory that SARS-CoV-2 proteins directly remove iron from human hemoglobin as a pathophysiological mechanism of COVID-19.

**Methods**

We reviewed laboratory data from 21 patients with known COVID-19 (PCR positive for SARS-CoV-2) admitted to the ICU at UPMC and compared with 21 patients with non-COVID-19 ARDS from different etiologies, who had been enrolled in the Acute Lung Injury Registry (ALIR) and Biospecimen Repository at UPMC. Patient data were de-identified. This study was approved by the University of Pittsburgh Institutional Reviewer Board.

We recorded clinically available venous blood gas values of partial pressure of carbon dioxide (PvCO\textsubscript{2}), partial pressure of oxygen (PvO\textsubscript{2}), pH, and venous oxygen saturation of hemoglobin (SvO\textsubscript{2}). We recorded initial hemoglobin and total bilirubin levels. Values
Results

Oxygen-hemoglobin dissociation curves were generated using COVID-19 patient data and non-COVID-19 ARDS patient data and compared against the theoretical standard curve generated by the Severinghaus model.17 The average age of the COVID-19 cohort was 62 ± 9 years versus 47 ± 17 years for the ARDS cohort. The distribution of sex was 12 males and 9 females in the COVID-19 cohort and 10 males and 11 females in the non-COVID-19 ARDS cohort. The oxygen-hemoglobin dissociation curve generated from patients with COVID-19 was similar to the curve generated from patients with ARDS without COVID-19. The fitting generated from COVID-19 patient data matched the ideal oxygen-hemoglobin dissociation curve well (Pearson correlation $R^2=0.97$, $P<0.0001$; CV(RMSD)=$7.3\%$). The fitting generated from non-COVID-19 ARDS patients also matches the ideal oxygen-hemoglobin dissociation curve reasonably well (Pearson correlation $R^2=0.92$, $P<0.0001$; CV(RMSD)=$9.4\%$, Figure 2). This comparison of oxygen-hemoglobin dissociation curves suggests that hemoglobin oxygen affinity is not altered in patients with COVID-19 admitted to the ICU.

Patients with COVID-19 had similar total hemoglobin, total bilirubin, ferritin, iron and LDH levels compared to patients with ARDS without COVID-19 (none were significantly different, Figure 2). Few patients had haptoglobin data, but available COVID-19 values were similar to ARDS (mean 202 vs. 283, respectively). Error bars represent standard error of mean, except for age, which is described as standard deviation.

Discussion

Liu and Li13 generated computational results obtained by sequence analysis, molecular modeling, and docking approaches to propose a novel model of viral degradation of hemoglobin-derived heme. However, the authors employed methodologies and docking simulations that have been heavily criticized, as thoroughly discussed in a recent report by Read.18 It is important to note that Liu and Li13 do not present any experimental support for their theories, and even though they have revised the initial manuscript and vastly changed their calculated parameters (as of version 9), their new calculations are speculative at best, as Read has recently addressed in an addendum to his manuscript (version 2).18 Moreover, the hypotheses originally put forth by Liu and Li13 remain unchanged. Our work thus focuses on the unique mechanism of SARS-CoV-2 proposed by Liu and Li13, direct virus triggered hemoglobin degradation. We highlight that this hypothesis is not supported by existing evidence, known pathologies of coronaviruses, Liu and Li own docking calculations,18 or the clinical data presented herein.

Most nonstructural viral proteins of coronaviruses are not found in large amounts in plasma but rather localize in infected cells where they play important roles in RNA replication.19 Thus, these proteins are unlikely to access appreciable amounts of hemoglobin. There is no evidence suggesting that the virus enters erythrocytes, where heme concentrations are 15-20 mM, and these highly specia-
lized cells lack the requisite cellular machinery needed to produce viral proteins.\textsuperscript{20} As a result, intraerythrocytic hemoglobin is likely protected from exposure to viral proteins. Liu and Li\textsuperscript{13} posit that the interaction between viral proteins and hemoglobin may occur in the plasma after immune hemolysis, but significant hemolysis has not been documented in COVID-19 patients.\textsuperscript{8,21,22} Nor did we observe hemolysis in our patients. Any elevation in LDH levels seen in COVID-19 patients is likely derived from hepatocellular injury and not intravascular hemolysis. Liu and Li\textsuperscript{13} suggest that the virus could infect plasma cells via the ACE2 receptor and induce secretion of viral proteins from infected plasma cells. However, there is no evidence of SARS-CoV-2 infecting plasma cells.\textsuperscript{23,24} Further, secretion of viral proteins from any infected cells is extremely rare and does not occur in any viruses related to SARS-CoV-2.\textsuperscript{25}

The manuscript’s flawed\textsuperscript{18} and experimentally unverified docking models lead the authors to suggest that a non-structural viral protein, ORF10, binds to heme and releases heme-derived iron. In this putative mechanism, ORF1ab and ORF5a bind the hemoglobin protein and cause conformational changes that expose the heme to ORF10, which subsequently breaks down the cofactor into iron and PPIX; the latter is then theoretically captured by ORF1ab. This model would represent an entirely novel mechanism of hemoglobin degradation, as hemoglobin has not been documented to undergo large conformational shifts as a result of protein-protein interactions.\textsuperscript{26} Further, the removal of iron from PPIX by ORF10, a protein of only 38 amino acids, is unlikely considering that heme degradation is catalyzed by significantly larger and more complex, well-characterized heme-oxgenase proteins.\textsuperscript{15} As the authors’ hypothesis represents a completely novel and unexpected model of heme degradation, careful \textit{in vitro} and \textit{in vivo} studies would be required to confirm such a mechanism. It is worth noting that we do not observe a statistical difference in iron, bilirubin, or ferritin levels between COVID-19 patients and ARDS controls (Figure 2), indicating that heme breakdown is not occurring above typical catabolic levels in the COVID-19 cohort.

We further assert that the clinical syndrome observed in COVID-19 patients is not consistent with Liu and Li’s\textsuperscript{13} model of heme degradation. The clinical evidence presented here does neither suggest hemolysis, hemoglobin degradation, removal of iron from the heme molecule, nor altered oxygen affinity of hemoglobin (Figure 2). The oxygen dissociation curve calculated from real-life patient data fits quite well with the idealized standard curve in both the COVID and ARDS cohorts ($R^2=0.97$, $R^2=0.92$, respectively). There is minimal deviation from the idealized curve CV(RMSE) of 7.3 and 9.4\%, respectively. This finding is in agreement with another recent study that uses hemoglobin isolated from the red blood cells of COVID-19 patients demonstrating normal hemoglobin-oxygen dissociation properties \textit{ex vivo}.\textsuperscript{27}

The clinical data presented here suggests no significant hemolysis or abnormal hemoglobin-oxygen dissociation characteristics. As aforementioned, newer clinical reports of critically ill COVID-19 patients suggest both mortality rates and mechanical ventilation requirements similar to other forms of ARDS.\textsuperscript{2,7–10} There has been no evidence to suggest a unique hemoglobin-specific mechanism such as large-scale hemoglobin degradation. In addition to the work here, there has been no other evidence of significant anemia or iron overload.\textsuperscript{31,32} While the finding of elevated transaminase levels has been widely described, this condition exists without hyperbilirubinemia, which would be a signal of excessive hemolysis and release of hemoglobin into the plasma.\textsuperscript{26,28} Increased risk of thromboembolism is observed in COVID-19 patients;\textsuperscript{5,6} however, acute infections are known to increase the risk of thromboembolism\textsuperscript{26} and thromboembolism is not generally driven by a hemoglobin-based toxicity. Neither “silent hypoxia”,\textsuperscript{29} nor patients who maintain a normal work of breathing before rapid onset of ARDS’ suggest an undetected hemoglobin-based toxicity. Any removal of iron and/or heme from hemoglobin would not have an effect on pulse oximetry measurements, as the resulting heme-free hemoglobin does not absorb light in the wavelength range used by these detectors and thus would not interfere with such measurements.\textsuperscript{30,31} Hemoglobin desaturation correlates well with decreases in partial pressure of oxygen in patients with COVID-19 respiratory failure: in our cohort, we did not observe gross abnormalities in the partial pressure of oxygen \textit{versus} hemoglobin oxygen saturation.

The world community is rapidly working to understand the pathophysiology of COVID-19 in an effort to better prevent the spread of the disease, manage patients, and ultimately develop definitive therapies. There is no suggestion that patients with COVID-19 exhibit a hemolytic anemia or a shift in the normal hemoglobin-oxygen dissociation curve. Thus, COVID-19 does not impact oxygen delivery through a mechanism involving red cell hemolysis and removal of iron from the heme prosthetic group in hemoglobin.

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**References**