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COVID-19 and Anemia-Interactions at the Hematological Frontier

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About the Study

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has predominantly been recognized for its respiratory complications. However, its systemic impact, particularly on hematological parameters, has emerged as a critical area of study, in a special issue for SARS-CoV-2 virus. Recent studies have highlighted a relationship between anemia and increased mortality rates in COVID-19 patients, especially in cases where there is an immune-mediated disruption of iron homeostasis. Moreover, reductions in hemoglobin levels have been observed among critically ill patients although the exact relationship between anemia and severe COVID-19 complications remains elusive.

D'Alessandro and our laboratory team analyzed the results from our Institutional Proteomics Unit derived from a study involving 73 patients with viral infections, including 20 individuals diagnosed with COVID-19 and concurrent anemia. This research corroborated the previously observed alterations in the structural and functional proteins of Red Blood Cells (RBCs) across a cohort of COVID-19 patients, noting variability associated with the severity of the disease as reflected by inflammation markers such as interleukin-6 and increased serum creatine. These findings suggest that Chronic Kidney Disease (CKD) may play a role in the onset of anemia in COVID-19, particularly among elderly patients with comorbidities. Our research study provides significant insights into the complex interactions between COVID-19 and anemia, focusing on changes in Red Blood Cell (RBC) biomechanics, enzyme activities and proteomics. Moreover, it expands the understanding of COVID-19 beyond respiratory symptoms, addressing the broader implications on RBCs and hematological parameters

The experimental design was based on a prospective observational study involving a single cohort of 74 participants, comprising 63 patients and 11 controls. The inclusion criteria specified participants over 18 years of age who had provided signed informed consent and had a documented SARS-CoV-2 infection confirmed by RT-PCR from at least one nasal/pharyngeal swab. To ascertain the specific effects of the COVID-19 virus, the study also included individuals with viral infections primarily caused by the influenza virus and respiratory syncytial virus, who presented with aclinical phenotype similar to COVID-19 but tested negative by RT-PCR on nasal/pharyngeal swabs. Advanced omics techniques were employed to analyze the proteomic alterations in RBCs, alongside traditional methods to assess RBC defects such as the measurement of enzyme activities, hemoglobin stability and RBC deformability. This comprehensive approach allowed for a nuanced understanding of the specific impacts of COVID-19 on blood cells compared to other viruses. The study was conducted upon admission, prior to any treatment and patients were divided into five groups based on their anemia status and RT-PCR results: Group 1 included COVID-19 positive patients without anemia (13 patients), group 2 comprised COVID-19 positive patients with anemia (20 patients), group 3 consisted of patients with other viral infections but without anemia (10 patients) and group 4 included patients with other viral infections and anemia (20 patients). Additionally, a control group (Group 5) of 11 healthy blood donors was included. Anemia was defined according to the World Health Organization criteria (<120 g/L for women and <130 g/L for men). For classifying the severity of disease, guidelines from the European Centre for Disease Prevention and Control and the National Health Commission of the People's Republic of China, alongside with WHO recommendations, were utilized. The study received approval from the local Ethics Committee and was conducted in accordance with the Declaration of Helsinki. Routine clinical data were collected in an anonymized manner, following the acquisition of signed informed consent from all participants and controls. Complete Blood Count (CBC) and basic serum biochemistry parameters have been tested in all SARS-CoV-2 positive patients, in several other viral infection and in the control group. In all patients with anemia, iron study, vitamin B_{12} and folate, C-Reactive Protein (CRP) and hepcidin were also tested. The hemoglobin stability was measured using the isopropanol test and RBC enzyme activities were measured according to Beutler with slight modifications RBC deformability was determined with the Laser optical rotational red cell analyzer (Lorrca®), a new-generation viscometer (ektacytometer) that, through its osmoscan module, measures the deformability of a RBC population exposed to an increasing osmotic gradient under a constant

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shear stress. This deformability parameter is known as Osmotic Gradient Ektacytometry (OGE).

The OGE profile is a characteristic bell-shaped curve from which three main rheological parameters can be obtained a) The Maximum Elongation Index (EImax), or the value of osmolality at maximum EI (deformability), b) The Minimum Osmotic Index (Omin), or the value of osmolality at which RBCs have attained their critical hemolytic value. This value corresponds to osmotic shifting of water into the cell in a hypotonic environment (osmotic fragility) and c) The osmolality at value at which the EI is midway between EImax and Omin (cell hydration).

Of the total cohort of 74 patients, RBC samples were available from 32 subjects for proteomics analyses. Specifically, proteomics analyses were performed on 11 healthy control subjects, patients with COVID-19 and patients with other viral infections. Proteomic analysis was performed on the day of collection using the standard procedure.

Anemia significantly influences the etiology and prognosis of various clinical conditions, including the respiratory challenges presented by COVID-19 infection. Comparing patient groups with different viral infections reveals additional variables that may influence anemia in COVID-19 patients.

The study identified a significant correlation between anemia and severe COVID-19 outcomes, characterized by increased levels of D-dimer, serum procalcitonin, creatinine and Blood Urea Nitrogen (BUN). These findings suggest a link between anemia and acute systemic complications in COVID-19, particularly involving renal function. Previous studies have similarly noted that anemia in COVID-19 patients could predict poorer outcomes, emphasizing the role of systemic inflammation and iron metabolism disruption in these patients.

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