Proteomic profile of extracellular vesicles and lipoproteins contribute to hyperinflammation and coagulation disorders in severe COVID-19

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Individuals who develop severe COVID-19 often experience thrombotic events and metabolic disorders due to the hyperinflammatory state induced by SARS-CoV-2. Given the clinical importance of coagulation abnormalities and the necessity to investigate entities capable of reflecting molecular alterations caused by the virus, our study focused on analyzing the protein content of extracellular vesicles (EVs) and lipoproteins in COVID-19 patients. EVs and lipoproteins are biological nanoparticles that are part of the circulatory system and are recognized for their ability to transport biomolecules and facilitate cellular communication locally and systemically. These entities are evaluated as potential biomarkers of disease and mediators of immune responses. In COVID-19, studies have examined EVs as key players in sustaining the hyperinflammatory state and the pathophysiology of severe disease. Additionally, severe COVID-19 patients exhibit significant alterations in their lipid profiles, which are strongly associated with adverse clinical outcomes and a poor prognosis. There has been demonstrated a strong correlation between reduced levels of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) in these patients. The mechanisms underlying dyslipidemia in COVID-19 remain incompletely understood, but evidence suggests that viral infection induces functional modifications in lipoproteins. Here, we evaluate how the protein content of EVs may be altered by SARS-CoV-2 infection, as well as investigate proteins associated with HDL and LDL in individuals with COVID-19, to provide insights into the pathological processes associated with the progression and severity of the disease. For this, we used size exclusion chromatography to isolate plasma EVs, which were subsequently evaluated by flow cytometry. In parallel, we isolated plasma HDL and LDL through density gradient ultracentrifugation. We employed a label-free proteomics approach to identify changes in protein abundance and responses to SARS-CoV-2 infection. To assess differences in the proteome, we distinguished biological groups in critically ill patients with COVID-19 (survivors and non-survivors) and controls. Finally, computational analysis was applied in both EVs and HDL/LDL, to map biological pathways and processes correlated with the pathophysiology of COVID-19. Compared to control subjects, plasma exhibits elevated concentrations of total circulating EVs in COVID-19 patients. These vesicles, in addition to being secreted by platelets, have tissue factor (TF)-dependent procoagulant activity. Differentially expressed proteins in control versus COVID-19 (82) and survivors versus non-survivors (18) comparisons indicated that processes related to platelet activation, and neutrophil degranulation are strongly associated with the pathophysiology of severe COVID-19. In HDL and LDL, the proteome of COVID-19 patients is

altered compared to control, and the profile of these proteins is associated with complement system activation and coagulation cascade. ApoA-I and PON1 are less abundant in COVID-19, and proteins related to inflammatory response and tissue injury (SAA, Protein S100) were associated with mortality from the disease since they were predominant in non-surviving patients. Our dataset demonstrates that changes in the proteome of patients affected by severe COVID-19 are mediated by immunological events of inflammation which impact the progression and severity of the infection

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