HDL Proteomic Alterations in a Prospective ICU Cohort

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Introduction. Although known for their fundamental roles in lipid metabolism, lipoproteins perform other physiological and pathophysiological roles. In this context, the high-density lipoprotein (HDL) is considered a protective group of particles due to its pleiotropic actions in inflammatory and infectious diseases (*e.g.* regulation of inflammatory response, anti-thrombotic action, inhibition of adhesion molecules expression, LPS binding capacity, anti-apoptotic effects). Low HDL-cholesterol has been associated not only with increased risk of cardiovascular diseases, cancer, and overall mortality, but also with mortality related to infections. The mechanism by which HDL-cholesterol is inversely related to mortality remains debatable. Many of HDL functions come from its associated apolipoproteins. The HDL proteome is composed of dozens of proteins that play distinct roles in a subset of HDL particles. In this context, mass spectrometry-based proteomics plays an important role in their identification and quantification.

Methodology. We obtained serum samples from 205 patients immediately after intensive care unit (ICU) admission. We followed this cohort from the São Paulo Medical School Hospital and found that 54 of those patients died. More than half of them had infection-related deaths. We then isolated their HDL by ultracentrifugation and analyzed them using data-independent acquisition proteomics. We built a spectra library from DIA using DIA-NN software. Then, we analyzed extracted chromatograms in Skyline software using MS2 data for quantification. Peaks integration was manually verified and protein areas were calculated by summing up individual areas from two to five precursor ions (in turn, precursor ions were quantified as the sum of four to ten transitions). To reduce technical variability, we normalized protein abundance by the areas of four heavy labeled peptides spiked in all samples during protein digestion.

Results and Discussion. Deceased patients had lower HDL-cholesterol upon ICU entry (14 mg/dL [IQR 9 – 21] *versus* 22 mg/dL [IQR 15 – 28], $P = 0.0012$). We used a linear regression analysis to evaluate differences in protein abundance between both surviving and deceased patients. Out of the 65 quantified proteins, fifteen of them had altered abundance. Eight of those proteins were raised by more than 40% in deceased patients. L-selectin is one of those proteins, and previous reports showed enhanced levels of serum L-selectin in ICU patients. Conversely, seven of the proteins were reduced by more than 50% in deceased ICU patients. Among those proteins, apolipoprotein M (ApoM) was protective against overall mortality, with hazard ratio of 0.64 per 1-SD increase (95% CI 0.45 to 0.92, $P = 0.015$). ApoM is a carrier of the lipid signaling molecule sphingosine-1-phosphate. This correlates with our previous findings showing ApoM as a protective factor in COVID-19 mortality.

Conclusion. Our results point to a remodeling of the HDL proteome in deceased ICU patients when compared to surviving ones. We hope our results contribute to further understanding the role of HDL in infectious diseases and the development of therapies that lower ICU-related deaths.

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