Systematic evaluation of depletion and enrichment technologies for platelet-free plasma proteomics

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Abstract

Sepsis poses a significant global health threat, emerging as a leading cause of morbidity and mortality. My Ph.D aims to improve sepsis diagnosis by identifying crucial biomarkers for patient stratification, using advanced mass spectrometry and proteomics techniques. A core component of this work is the optimization of sample preparation workflows for next-generation mass spectrometry, with particular focus on SP3-based protocols and the influence of protein-to-bead ratios and bead chemistries on proteome coverage. These methodological investigations revealed differences in peptide identification, physicochemical properties, and quantification outcomes. To further enhance the detection of low-abundant proteins-key candidates for diagnostic biomarker discovery-we benchmarked multiple depletion and enrichment technologies, demonstrating that each method introduces distinct quantitative biases and dynamic-range effects that must be addressed through robust normalization strategies and appropriate reference standards. These method-dependent biases add to other sources of variability inherent to clinical proteomics, including technical differences in plasma preparation, storage duration, and hemolysis contamination, all of which can influence downstream cohort analyses and therefore require careful statistical planning. Developing strong knowledge in power calculations, assessment of inter-individual variability, and modeling of confounding factors such as age, comorbidities, and treatment history is essential for designing a statistically sound study capable of detecting clinically meaningful protein-level changes. Particular emphasis will be placed on characterizing the sources of systematic and random bias across the entire workflow-spanning sample preparation, LC–MS acquisition, and data processing-and implementing corrective measures such as batch-aware normalization, retention-time alignment harmonization, advanced missing-value imputation strategies, and statistical frameworks that appropriately capture multi-level structure within the cohort. Although the clinical cohort is not yet available, acquiring these skills will enable me to develop the necessary statistical expertise to analyze the plasma proteomics data once patient samples become accessible.

Keywords: Sepsis, Clinical cohort analysis, Plasma proteomics, Bias correction, Power analysis

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