

INVITED LECTURE H3

Targeted multiplexed protein expression profiling using MRM: studying biological variation of plasma protein levels using a twin cohort

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Multiple reaction monitoring (MRM) capabilities of triple quadrupole based MS systems have been used increasingly for quantitative monitoring of peptides/proteins in complex biological samples. Strategies for overcoming the key challenges (need for rapid assay development, need for higher multiplexing and need for assay robustness) are becoming critical. In this work, a triple quadrupole linear ion trap mass spectrometer has been utilized to create >1000 high quality, specific MRM transitions for multiple peptides to many plasma proteins. A non-isobaric chemical labelling strategy has been employed to create global internal standards to enable quantitative comparisons between clinical samples. This strategy has been applied to the study of 20 twin pairs, with the goal of understanding biological variation.

Plasma from 20 pairs of twins were collected and stored under identical conditions. Each sample was depleted (top 7 proteins), reduced/alkylated, and digested with trypsin. A global internal standard (GIS) was created from a pool of the digested patient samples by labelling it with the heavy mTRAQ reagent and added to each light labelled individual patient sample. By incorporating MRM transitions to this GIS in the assay, in addition to the MRMs to the sample, very high analytical reproducibility can be achieved for peptide ratios across measurements, typically 5% CV or better. MRM transitions (~800) to ~100 peptides for medium/high abundant plasma proteins were monitored. The protein ratios relative to the GIS were computed for all individual samples, providing an estimate of the biological variation across this small dataset. Proteins differed in the degree of variation, both low variation and higher variation in proteins. When comparing the set of protein ratios, the intra-twin correlation was typically higher than the inter-twin correlation. Understanding the biological variation of specific proteins across populations is an important consideration in determining the utility of protein biomarkers.