

Evaluation of different mass spectrometry data acquisition and analysis strategies for amyloidosis typing.

Han-Yin Yang, James G. Bollinger, Dao-Fu Dai, Andrew Hoofnagle, Christine C. Wu, Kelly Smith, Michael J. MacCoss

Department of Genome Sciences, University of Washington, Seattle, WA, 98195

Department of Pathology, University of Washington, Seattle, WA, 98195

Stratus Biosciences, Seattle, WA 98117

Introduction:

Laser microdissection couple with mass spectrometry (LDMS) has been successfully applied in amyloidosis diagnosis, in which amyloid types are confirmed based on spectral counts of amyloidogenic proteins in formalin-fixed paraffin-embedded tissue section. Although LDMS based methods appears to be a superior alternative to current immunohistochemical assay, several aspects needs to be considered in the assessment of a reliable diagnosis platform. One of the issues is what is the measure of “signal” used to discriminate different amyloidosis types. Because the different peptides respond very differently by mass spectrometry, some individual peptides might be a better diagnostic predictor rather than using the protein as whole. Here, we quantify the difference between samples using peptide and protein levels, and discusses the diagnosis ability of different quantification approaches. In addition, we apply different normalization approach to correct for the variances in LD capture quantities and sample preparation.

Method:

Forty-two Congo red regions were collected from fourteen patient and control cases. We extract proteins from LMD sample using a heat-mediated antigen retrieval method and add internal standard protein, heavy-labeled apolipoprotein A-I, in each sample. The resulting mixture is digested with trypsin. We collect spectral data on protein digests using both data-dependent and data-independent acquisition (DDA and DIA) methods on Q-Exactive HF, in which DDA datasets are used for peptide identification and spectral counting. DIA datasets are used for

extracting peak area of peptides. We quantify amyloidosis related peptides and /or proteins using different approaches including RAW spectrum counts, normalized spectral abundance factor (NSAF), and intensity-based absolute quantification (iBAQ).

Preliminary Data:

No matter which quantification approach we used to quantify proteins, we see serum amyloid P component in most of patient cases which meets the current criteria to confirm amyloidosis.

However, intensity based quantification method shows better ability to discriminate patient and control cases, especially in some patient cases there are only few spectral counts for serum amyloid P compare to zero count in control case. In addition, different amyloid types could also be determined by the abundance difference of proteins in different samples.