Enhancing Light Chain Amyloid Detection via Decellularization and Data Independent Mass Spectrometry Acquisition

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Light Chain Immunoglobulins (LCIs) play an important role in deposition diseases, especially light chain amyloidosis (AL). These immunoglobulins, originating from a clonal disorder of plasma cells, form aggregates in the extracellular matrix of tissues, resulting in significant and life-threatening organ damage. The major challenge in characterizing and detecting LCIs amyloid deposition diseases lies in their diversity of subtypes and high abundance in the plasma. Distinguish between normal plasma soluble LCIs and those involved in disease-related aggregates is intricate. To address this, we devised a strategy that enriches fibrillar amyloid tissue, followed by an analysis pipeline for semiquantitative assessment of curated peptides. This approach aims to improve the diagnosis of amyloid light chain deposits.

Laser microdissected formalin-fixed paraffin-embedded segments of biopsied tissue segments were obtained from biopsied tissues of amyloidosis patients. These tissues included kidney, heart, skin and adipose tissue. The patient samples were de-identified and confirmed positive for Congo Red and Thioflavin T staining. Following decellularization, the samples underwent a series of steps: detergent treatment, heat-induced epitope reactivation, reduction, alkylation, and trypsin digestion. The processed samples were analyzed using nanochromatography coupled to Data-Independent Acquisition (DIA) mass spectrometry. Specifically, we employed the nanoUltimate-Exploris 240 quadrupole-orbitrap hybrid mass spectrometer. The analysis was performed with a 45-minute run time, a precursor mass range (m/z) of 310-1010, and a DIA isolation window (m/z) of 25. Data analysis was conducted using Skyline software.

The decellularization protocol was adapted and improved from a method previously described. Briefly, biopsy fragments were treated with detergent, high pH and temperature to differentially remove soluble plasma proteins keeping fibrillar proteins attached to the PEN polymer from microdissection slides. This promotes the removal of a large portion of plasma LCIs enrichment while retaining and enriching monoclonal LCIs that mark amyloid deposits. A DIA method was developed to cover the range of polarity of the selected peptides used to determine the specificity of the deposit. A workflow in the Skyline software was created to identify the type of LCI. There are two main types of LCIs: Kappa and Lambda. The Kappa light chain is encoded by a single gene, whereas the Lambda light chain by multiple genes. The peptides used to differentiate these Lambda chains were selected by combining information from immunoglobulin Lambda protein alignment (BLASTP) and peptide detectability. Thus, seven peptides were selected, some common to all LCIs (IGLC1, IGLC2, IGLC3, and IGLC7), such as YAASSYLSLTPEQWK. We established a metric to determine their reliability and reproducibility (idotp ? 0.90, dotp ? 0.70 and precursor and fragment mass errors ? 10 ppm).

Our research demonstrated the benefits of the decellularization strategy, enabling enrichment of LCI amyloidogenic aggregates from the plasma fraction. This approach enhances the detection of both constant and variable peptides of LCI, along with other amyloidogenic signature proteins deposited as fibrillar aggregates. By utilizing DIA, we achieve greater confidence in identifying the specific type of LCI amyloid deposits, while improves sensitivity and coverage of targeted peptides.

Agradecimentos: