Top-Down Mass Spectrometry in Clinical Diagnosis? A Potentially Less Invasive Approach for Plasma Cell Disorders Classification

Author: Lidong He, Alan G. Marshall // **Date:** MAR.5.2019 // **Source:** AACC Academy

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Lidong He

Alan G. Marshall

Plasma cell disorders comprise a spectrum of monoclonal gammopathies including monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, AL amyloidosis, POEMS syndrome, and Waldenstrom's macroglobulinemia, all of which are characterized by a plasma cell clonal expansion. If there is clinical suspicion of one of these disorders, serum is tested for the presence of an elevated level of a monoclonal immunoglobulin (M protein, composed of two identical ~25 kDa light chains and two identical ~50 kDa heavy chains linked together by disulfide bonds) and monoclonal free light chains secreted by clonal plasma cells. Despite recent research advances, the systemic AL amyloidosis and multiple myeloma five-year survival rates are still ~51%, indicating the need for improved diagnosis and treatment plans.

To elucidate the mechanisms of plasma cell disorders, immunoglobulin gene usage preference has been studied by gene sequencing. For example, the predilection of M protein lambda light chain LV1 gene (germline sequence) usage (>95% of the time) was found in POEMS syndrome. Also, a monoclonal gammopathy patient with LV6-57 germline sequence should be comprehensively evaluated for systemic immunoglobulin light-chain amyloidosis (ALS), because it occurs more often in ALS than in the normal B-cell repertoire.

However, only a few patients were involved in those studies due to the use of time-consuming PCR-based sequencing and requirement of target gene-specific primer. In addition, invasive bone marrow aspiration is required prior to gene sequencing, and there is risk for potential false negatives if the plasma cell clone in a localized microenvironment is missed during bone marrow aspiration. In contrast, mass spectrometry approaches detect and characterize M protein from serum (less invasive) which represents the entire circulating immunoglobulin population (including M protein and normal polyclonal immunoglobulins). Furthermore, mass spectrometry detects M protein-specific post-translational modifications (PTMs) which cannot be identified by gene sequencing.

Traditional bottom-up MS/MS has been widely applied for targeted immunoglobin analysis, in which specific surrogate peptides are monitored.[1] However, that approach is difficult to implement in de novo sequencing of immunoglobulins in a serum matrix, because enzymatic digestion of polyclonal immunoglobulins background yields highly abundant peptides which overwhelm the signals from M protein variable region peptides. In contrast to bottom-up MS/MS, top-down (M protein light chain and heavy chain after 15 min reduction) LC-MS/MS selects specific M protein light chain/ heavy chain mass-to-charge precursor ion for fragmentation and thus avoids most of the polyclonal immunoglobulin interference.

Prior top-down MS/MS has been applied extensively to characterization of therapeutic monoclonal antibodies sequences based on database searching (requiring target protein sequences in the searched database). However, endogenous immunoglobulin variable region sequences are heterogeneous at the organism level, and the database cannot include all M protein variable region sequences. In such a case, top-down MS/MS de novo sequencing presents the only choice.

We have exploited 21 tesla Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (the highest-resolution mass analyzer) to benchmark top-down MS/MS de novo sequencing of M protein from serum.[2, 3] We first provided a model for plasma cell disorders by spiking normal serum with therapeutic monoclonal antibody adalimumab, so that multiply charged adalimumab light chain and heavy chain signals were observed well above the polyclonal immunoglobulin background. Furthermore, 72% adalimumab light chain sequence coverage was obtained from top-down MS/MS. Further, we recently developed an algorithm (implemented into data analysis software), in which database-aided de novo sequencing can accurately characterize M protein light chain and heavy chain variable region sequences. Our pilot study based on blind analysis of the M protein light chain sequences showed that MS/MS-determined sequences accurately matched those from gene sequencing for patients with AL amyloidosis. The KV1-33 germline

sequence identified in one sample turned out to be the most common kappa germline sequence identified in AL amyloidosis and is more likely to be associated with liver involvement. Moreover, the LV3-21 germline sequence identified in a second sample is less commonly involved in renal problems. In addition, M protein heavy chain glycoform profile was extensively characterized and differed from normal human polyclonal immunoglobulins glycoform profiles, and thus may serve as another disease classifier.

As a less invasive approach compared to conventional gene sequencing, top-down MS/MS de novo sequencing of M protein has the potential to provide invaluable information for plasma cell disorders classification and may aid future personalized therapy. Before wide adoption of this approach, the present method must be adapted to lower field FT-ICR or Orbitrap mass analyzers for implementation in clinical laboratories. In addition, top-down MS/MS de novo sequencing software needs to be further optimized for high-throughput processing.

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