Immunoglobulins: Expanding the Role for Mass Spectrometry in Protein Biomarker Quantification

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The impact of proteomics on clinical practice and laboratory medicine has been much anticipated, and many researchers have believed and predicted that the benefit would be primarily in the form of novel biomarker discovery. However, the most significant imminent improvement may be more straightforward: improved capability to measure clinical analytes and increased capacity for multiplexing to evaluate complex panels of disease biomarkers. Although discovery proteomics has revolutionized basic science in terms of defining protein complexes and molecular switching events determined by posttranslational modifications, the ongoing development of liquid chromatography–multiple reaction monitoring mass spectrometry (LC-MRM MS) techniques and their application to protein biomarker quantification continues to improve the capability of the clinical laboratory.

Building on the long history of use in small-molecule quantification applied both to drugs and metabolites, LC-MRM MS has been used to monitor peptide surrogates for protein biomarkers of specific clinical interest such as apolipoprotein A-I (1), C-reactive protein (2), and prostate-specific antigen (3). Furthermore, peptide immunoprecipitation and mass spectrometry quantification have recently been applied to monitor thyroglobulin (4), overcoming obstacles associated with traditional antibody-based techniques. These advances can be applied to numerous other analytes relevant to human disease.

One such critically important and active area of research is the measurement of immunoglobulins, both endogenous and therapeutic. As an example, the impact of this research can be made immediately in multiple myeloma (MM), which is a tumor of the immunoglobulin-producing plasma cells. Clonal expansion of the tumor cells produces a monoclonal immunoglobulin, which can be quantified as a direct biomarker of disease burden. The clinical paradigm for evaluating patients relies on evaluation of the immunoglobulin in serum and urine by protein electrophoresis and immunofixation as well as quantification by nephelometry. Other assays (e.g., serum free light chains) can also be applied in combination with these techniques. Patients are typically assessed at 2- to 4-week intervals during treatment and 1- to 4-month intervals during remission. The immunoglobulin measurements are used in patient care to evaluate disease severity, monitor response to therapy, determine when to discontinue chemotherapy, and detect disease relapse. Improvements of these approaches could be expected to significantly impact the ability to define complete responses to chemotherapy, potentially eliminate minimal residual disease (MRD), and provide earlier detection of disease relapse, opening an earlier window for patient treatment. All of these aspects could enhance the ability to treat MM patients and improve their outcomes.

A method for quantification of immunoglobulins using peptides derived from tryptic digestion of the constant regions was proposed by our research team at the Moffitt Cancer Center as part of a review article on the role of quantitative proteomics in developing personalized care for cancer patients (5). This method parallels the nephelometry measurements of the total immunoglobulin concentrations (e.g., IgG, IgA, and IgM) with slightly improved sensitivity and a trade-off in precision (6). Our view was that the impact of changing the platform for this measurement from protein electrophoresis to mass spectrometry may not initially be great, because the measurements were parallel to current clinical techniques. However, as the portfolio of clinical LC-MRM MS assays increases, this approach could become useful for implementation in the clinic.

Researchers at the Mayo Clinic have been working on the same problem of monitoring immunoglobulins in different disease settings, including MM, and have produced data for the feasibility and implementation of multiple mass spectrometry–based assays. These re-
searchers have developed methods quantifying light chains using electrospray quadrupole–time-of-flight mass spectrometry, which provides a rapid analysis with improved sensitivity and molecular specificity (due to the measurement of the intact molecular weight) compared to protein electrophoresis (7). In the most recent investigation, reported in this issue of Clinical Chemistry, Ladwig et al. have evaluated quantification of IgG subclasses and compared the results to isoform-specific nephelometry in the context of immune deficiency and IgG4-related disease (8). Both this and the earlier publications illustrate methods that can be readily applied to the automated analysis of clinical samples. Their thorough and systematic approaches to testing these assays with clinical samples set a high standard and consistently illustrate the utility of quantitative mass spectrometry for assessment of protein biomarkers.

Although all of the methods described above have been analogous to current clinical assays, both groups have also worked in parallel on disease-specific immunoglobulin quantification using peptides from the variable region of the monoclonal immunoglobulin secreted by MM tumor cells (6, 9). On the basis of existing literature describing proteomics experiments informed by RNA sequencing (10) and analysis of therapeutic antibodies (11, 12), proof-of-concept experiments have been performed to assess the utility of this disease-specific peptide-based approach to monitor the monoclonal immunoglobulin in serum. These methods will enter a very competitive space and must be compared to multiparameter flow cytometry (13) and genomic methods (14). However, retention of the current clinical paradigm of monitoring monoclonal immunoglobulin expression in serum has 2 benefits over genomic approaches for MRD detection using flow cytometry, allele-specific oligonucleotide PCR, or deep sequencing in serial bone marrow samples: lesser patient burden and systemic evaluation of disease. I fully expect that these methods will prove to have significant clinical value in MM. Regardless of how LC-MRM MS competes in this specific instance, the future is bright for quantitative proteomics to play a broader role in patient assessment as part of the clinical laboratory.

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References


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