The Increasing Role of Mass Spectrometry in Quantitative Clinical Proteomics

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Protein biomarker research has been burgeoning over the last decade because of the tremendous potential for impact in the clinic. Biomarkers are either in current use or envisioned for virtually every area of medicine, including risk stratification of the population, early diagnosis of disease (1), predictors of therapeutic response, pharmacodynamic markers for determining the dose and schedule of therapy (2), monitoring for the recurrence of disease, and surrogate end points for use in early clinical trials (3). Despite the ability of “omics” technologies to generate long lists of candidate markers, however, few new biomarkers have been introduced for clinical use in recent years (4). This state of affairs is due in large part to the lack of efficient technologies for verification and validation of these long lists of candidate markers (5). This key piece of technology must be able to make reliable and affordable measurements of proteins in large numbers of samples with moderate to high throughput (6). In short, we need a versatile way to build quantitative assays for any protein of interest without a major investment in time, money, and other resources.

In recent years, momentum has been growing around the use of quantitative mass spectrometry–based assays for protein measurement (7). This analytical technique measures a surrogate peptide of the target protein (assuming molar equivalence between the protein and peptide) via stable isotope dilution internal standardization. The mass spectrometer is capable of providing high selectivity and low limits of quantification, especially when it is coupled with immunoaffinity enrichment (8). Indeed, mass spectrometry has been used for decades for the quantitative analysis of a number of analytes, such as vitamin D, and for neonatal metabolite screening (9, 10), but it has only recently been extended to the measurement of proteins.

In this issue of Clinical Chemistry, Neubert et al. make an important contribution to the development of mass spectrometry–based assays (11). The authors present results from an assay that couples peptide immunoaffinity enrichment with mass spectrometry for quantifying pepsin in saliva. The study is notable because it demonstrates the capabilities of the technology and highlights the advantages of using a mass spectrometry–based approach in designing protein assays.

Previous work has shown that coupling peptide immunoaffinity with mass spectrometry provides low limits of quantification, robust assay performance, and moderate throughput (12). The authors extend the approach to an online high-flow immunoaffinity column format. The assay is capable of measuring pepsin concentrations in the low picomolar range with good reproducibility. Throughput is accomplished by performing the immunoaffinity enrichment step at high flow. The online enrichment is directly coupled to nanospray, conserving the capability for quantifying low pepsin concentrations. This study is a notable example of obtaining good throughput, acceptable performance metrics, and dependability with a nanospray configuration.

Another compelling feature of immunoaffinity–based mass spectrometry assays is the versatility in design and performance. For example, in assay construction, the choice of surrogate peptide determines which form of protein may be measured, making it possible to build assays to most modifications, variants, or isoforms of interest by choosing the appropriate peptide. The authors highlight this versatility by targeting the C-terminal fragment peptide to provide a combined measurement of pepsin and pepsinogen. Versatility in performance is shown in the scalability of the assay to larger volumes of initial material, allowing for an increase in the sensitivity, depending on the range of the target.

Finally, it is worth noting that these assays can be developed at lower cost and with less time compared with traditional immunoassays. Depending on the desired configuration of the final assay, the specificity of the mass spectrometer allows the specificity constraints on the antibody to be relaxed, making the antipeptide antibodies relatively easier to generate. Technologies for generating affordable peptide and protein standards also continue to improve. The time required to choose a surrogate peptide, obtain reagents, and characterize an assay, is typically well under 1 year. In addi-
tion, the mass spectrometer makes it possible to multiplex the detection of many analytes and thereby generate assays for large numbers of targets in a short amount of time.

The extent to which peptide immunoaffinity enrichment with mass spectrometry can be used in routine clinical measurement remains to be seen. There are still many hurdles to adopting such an assay on a wide scale (13, 14). As nicely demonstrated in this issue, however, the technology is poised to provide reliable quantitative measurements with the performance, capacity, and limits of quantification necessary to verify candidate biomarkers. Implementing this approach on a large scale, as proposed in a human proteome detection and quantification project (15), would make the proteome accessible to clinical chemists and undoubtedly narrow the gap between biomarker discovery and validation.

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References


