A Step Toward Simplicity for a Complex Analyte
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α1-Antitrypsin (A1AT) deficiency is a serious, life-threatening disorder driven by abnormally low circulating concentrations of A1AT, which lead to protease–antiprotease imbalance. This imbalance accentuates protease activity and can lead to tissue damage, the most important of which leads to impairment of lung function (1). The apparent simplicity of diagnosing this disorder by a single measurement of the A1AT concentration is belied by the variety of A1AT tests offered by every reference laboratory. Physicians frequently evaluate genotyping, phenotyping, and concentration measurements in toto or through results produced via an algorithm that involves reflexing from one test to another (2). The need for this complex approach reflects the intricate relationships between genotype, phenotype, protein function, and concentration. The challenge in arriving at appropriate diagnosis and treatment comes from the variable penetrance of A1AT genetic defects and the limitations of each test. Typical genotyping tests detect only the most common disease alleles, S and Z, unless full exon sequencing is used. Phenotyping by isoelectric focusing can detect known and unknown polymorphs, but only in cases in which the amino acid substitution leads to a change in the protein’s isoelectric point. Finally, A1AT is an acute-phase protein, and circulating concentrations can increase dramatically under conditions of stress, infection, and inflammation, perhaps providing transiently “normal” circulating A1AT concentrations, even in the presence of disease. Therefore, a correct diagnosis of deficiency requires that highly specific molecular information be used in concert with concentration measurements.

In this issue of Clinical Chemistry, Chen et al. (3) describe a liquid chromatography–tandem mass spectrometry (LC-MS/MS) approach for determining both the phenotype and the A1AT concentration in a single assay, in contrast to the usual approach of running 2 independent assays involving different techniques. This LC-MS/MS method is clearly an improvement for any laboratory seeking to improve productivity. Although splitting samples is a common and often necessary activity in many laboratories, it adds costs and increases the chances for error. The mass spectrometry approach has more to offer because it takes advantage of the fact that it can provide quantitative information at a level of molecular detail that is very difficult to achieve by any other method.

Determinations of A1AT phenotype have typically been performed via inspection of isoelectric focusing gels. For polymorphs leading to amino acid substitutions that change the protein’s charge, differences in migration are observed. Despite improvements in gel-based approaches, which originally were used to discover the disease in 1963 (4), and the availability of electrophoresis test platforms cleared by the US Food and Drug Administration, phenotyping remains a subjective, interpretive exercise. Although gel-based techniques for protein separation are tremendously powerful, the observation of protein bands reflects a complex set of interactions between protein, ampholytes, buffer composition, gel composition, and pore size. Protein migration within a gel can also be influenced by the protein load and the presence of nonprotein interferences, such as lipid and salt. Under these circumstances, it is no surprise that the analysis of gels is occasionally complicated, and Chen et al. (3) describe a discordant gel result that was rectified by sample dilution. In contrast, a mass spectrometry method that responds to rationally selected tryptic peptides containing the polymorphic sites of interest is unaffected by any of these factors. Direct detection of the molecular feature of interest is tremendously selective. Additionally, the interpretation of the mass chromatograms for phenotype determination appears to be a dramatic improvement over gel interpretation, with essentially no chance for ambiguity. As designed, the LC-MS/MS method detects only the most common phenotypes, but conceptually, expanding the panel requires only updating the list of peptides to be detected by the mass spectrometer and providing additional internal standards.

A1AT concentrations obtained by LC-MS/MS correlated well with those obtained by traditional nephelometric methods but were substantially higher to a degree that was inconsistent between variants. This finding very likely reflects fundamental differences in A1AT antibody binding as a function of the particular
amino acid substitutions in the different variants. To the extent that such variant-related differences are superimposed on abundance changes that are not directly associated with A1AT deficiency, further investigation is warranted. The appeal of using mass spectrometry is that it reveals features of assay performance that may have previously been unrealized and provides the tool by which the relevance of these differences can be assessed.

The emergence of protein assays in the clinical laboratory that use mass spectrometry appears to be growing in earnest, and this report makes another substantial contribution to the field. The descriptions of a number of validated assays have been published in Clinical Chemistry (5–8), and some of these assays are now being offered commercially. Although these assays may not reflect the lofty expectations the proteomics research community has established in tackling the problem of discovering new biomarkers, these assays demonstrate a clear, positive progression of the technology in the clinical laboratory. With assays being transferred from older platforms to mass spectrometric approaches, the clinical diagnostics community gains confidence and the necessary expertise to insure that protein and peptide mass spectrometry will have the same positive influence on clinical diagnostics that small-molecule mass spectrometry has had in the areas of endocrinology, newborn screening, and therapeutic drug monitoring.

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