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Editorials

Dynamics of Clinically Important Proteins: Measuring Turnover of Drug Targets and Biomarkers

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As the concepts of personalized medicine gain increasing attention, the differences between patients, both in their presentation of disease and response to therapies, emerge as important factors in the overall success of healthcare. This is reflected in the fact that many drugs appear to be effective in a minority of patients with the disease for which the Food and Drug Administration approves the drug. Similarly, in the clinical laboratory, it has long been known that healthy individuals exhibit differences, in some cases large differences, in the concentrations of various serum proteins used as clinical biomarkers *(1)*. However, in both diagnosis and therapy, individuals are typically treated as if they were random representatives of the general population—i.e., diagnosed on the basis of population reference intervals and treated on the basis of responses observed in large clinical trials. The goal of personalized medicine is to improve outcomes for individual patients through access to detailed phenotypic and genomic information revealing their individual differences from the population average. The study by Farrokhi et al. *(2)*, published in this issue of *Clinical Chemistry*, illustrates an important but currently disregarded aspect of personal phenotype: the lifetime of clinically important proteins.

Most of the approximately 120 serum proteins detected by Food and Drug Administration–cleared tests measure the concentration of the protein in circulation *(3)*. These equilibrium concentrations result from a balance of a protein's appearance in the blood (by secretion, leakage, or generation by modification of a precursor) and disappearance (by kidney filtration, capture by tissues, or proteolytic destruction). Essentially all human proteins, except those in protected sites like the lens, turn over in the course of normal life, some very rapidly (e.g., human growth hormone in about 15 min) and some very slowly (e.g., hemoglobin in a matter of months). In many cases it appears that these rates can have a substantial impact on the clinical utility of proteins as diagnostic biomarkers. The short-lived growth hormone fluctuates dramatically during the course of the day *(4)*, while inducing a longer-lived protein (insulin-like growth factor 1, with a half-life of approximately 12 h) that proves to be a more practical clinical analyte. The long lifespan of red cells and their hemoglobin (Hb) content is also variable in diseases associated with increased destruction of red blood cells, which greatly alters the interpretation of HbA1c values. Nevertheless, the rates of turnover for most of the diagnostically important proteins remain unknown at this point, both for the general population and for individual patients.

With the realization that most drug targets are proteins, protein turnover has come to play an important role in the understanding of a drug's pharmacokinetic and pharmacodynamic effects. If, for example, a therapeutic antibody drug binds very tightly to a protein target and both are thus inactivated with respect to further physiological actions, then the total amount of the target produced over time directly influences the drug dose and/or frequency of administration. However, the total amount produced over time is not the same as concentration; rapid turnover of a low concentration protein can lead to more total protein being produced than would be the case for a high concentration protein that turns over slowly. Protein turnover thus emerges as a distinct feature of clinical importance that is not directly obtainable from the concentration measurements obtained in conventional clinical assays.

Measuring protein turnover generally requires administration of a pulse of some protein precursor that is labeled with a detectable isotope *(5)*. The introduction of mass spectrometry has allowed the replacement of radioactive labels with stable isotopes, while the application of various sample fractionation techniques has allowed access to low-abundance proteins.

The report by Farrokhi et al. provides an excellent case study combining a series of advanced methods to provide accurate turnover data for 4 proteins in 3 human volunteers. In this work, each volunteer received a total of approximately 1.75 g of deuterated leucine ([5,5,5-$^{2}H_{3}$]-L-leucine) infused over 18 h to label proteins synthesized during that period, followed by a washout (chase) period of a further 18 h during which labeled proteins gradually disappeared. Mean half-lives of these proteins ranged from 5 to 15 h with real but relatively small intrasubject variation (CV $<$ 30%). A fifth protein showed no incor-

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poration of label and thus presumably turned over very slowly.

Two key features of this work point to general applicability for measuring turnover in clinically important proteins. First is the use of straightforward multiple-reaction monitoring mass spectrometry, which is commonly used for small molecules, to measure unlabeled and labeled forms of a single tryptic peptide for each target protein, together with a very simple correction for label interference. With use of a relatively inexpensive triply deuterated leucine as the labeled amino acid, a mass increment of 3 atomic mass units (amu) is added to the mass of each tryptic peptide that contains a labeled leucine residue. However, due to the natural occurrence of some ^{13}C in all peptides, there is already a small amount of natural peptide that is 3 amu heavier than the nominal mass, in which all C is assumed to be ^{12}C. In the pulse-chase experimental design used here, the authors show that by measuring the +3 amu peak in a target peptide before administration of the labeled leucine, this background can be directly obtained and then subtracted from the signal at later time points to yield an accurate measure of the labeled leucine contribution.

A second key feature is the use of specific affinity enrichment of low-abundance proteins by means of antibodies on magnetic beads, followed by tryptic digestion, to obtain measurable amounts of a unique target peptide for each protein. Since the affinity enrichment removes only the target protein from a serum sample, leaving the others behind, the authors show that one protein after another can be enriched by its corresponding antibody from the same set of sample aliquots. The samples thus constitute a long-term resource rather than being consumed in one experiment. This offers the attractive possibility of measuring half-lives for the common diagnostic proteins in such an existing sample set with little additional cost or effort. The same multiple-reaction monitoring measurement approach could be applied to enrich a single leucine-containing peptide after tryptic digestion with use of an antipeptide antibody, when such a reagent is available. While the relatively sophisticated nanoflow chromatography the authors used to achieve maximum analytical sensitivity is not common in clinical laboratories, typical high-flow or microflow clinical liquid chromatography systems would be sufficient to apply the method to high- and medium-abundance serum proteins.

The main limitation of the approach for diagnostic purposes stems from the need to infuse an amount of a labeled amino acid costing almost $700 over 18 h and then to collect a series of 200 μL blood samples over the following 30–40 h. While this could be reasonably straightforward for hospitalized patients, it is unlikely to become routine.

In the future an increasing proportion of new drugs will themselves be proteins, most frequently antibodies aimed at endogenous protein targets. Given the high cost of protein drugs, it may become important to measure blood drug concentrations in individual patients (and possibly track these values over time) to ensure efficacy without waste. The other side of this coin—measurement of the turnover of the targets themselves in individual patients—is likely to become increasingly important to understand personal differences in efficacy and the declines in efficacy over time that are observed with many antibody drugs.

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