Tacrolimus Measurement: Building a Better Immunoassay

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Tacrolimus (formerly known as FK506) is a macrolide immunosuppressant that is used for primary immunosuppression after organ transplantation (1). The landmark work of Starzl at the University of Pittsburgh paved the way for the clinical use of tacrolimus as a primary immunosuppressant, leading to a shift away from cyclosporine use (2–4). Today, tacrolimus is used in the majority of transplant patients (5). Tacrolimus binds to several cytosolic immunophilins of importance; binding to the FK506 binding protein leads to the formation of a complex that interacts with calcium-dependent calcineurin calmodulin translocation pathways that result in prevention of T-cell activation (6). Monitoring blood tacrolimus concentrations is essential in assessing organ rejection and toxicity because of its narrow therapeutic index, wide inter- and intraindividual pharmacokinetic variability, and susceptibility to cytochrome P450–mediated drug interactions (7).

Immunoassays play a major role in the analysis of many clinical laboratory analytes and in recent years have become more popular for measuring tacrolimus, replacing the mass spectrometric methods that were initially favored over the earlier immunoassay methods. Measurement of drugs by immunoassay offers the advantages of automation and ease of use (assays available around the clock 7 days a week). Furthermore, this approach does not require specialized trained staff, has the advantage of using commercially available calibrators and reagents, and permits random-access testing. Because of these factors, immunoassays allow for reasonably fast turnaround times, particularly for the afterhours testing often needed for assessment of the clinical status of a transplant patient. One of the main disadvantages of immunoassays is the lack of analytical specificity due to cross-reactivity with drug metabolites (active and inactive) and other analytes (8). For tacrolimus the cross-reactivity of the metabolites in most current immunoassays is still substantial (up to 60%), which can cause an unpredictable overestimation of the true drug concentrations (8, 9). In contrast, mass spectrometry can be less robust than immunoassay platforms and requires highly trained staff, but it is capable of measuring several compounds in a single run. However, in hospitals mass spectrometry is often not available for afterhours use. Although major improvements in mass spectrometry methods have simplified sample preparation and provided faster turn-around times, the above-mentioned limitations still persist (10).

In this issue of Clinical Chemistry, Wei et al. elegantly describe the measurement of tacrolimus using 2 antitacrolimus monoclonal antibodies in a sandwich assay that has minimal metabolite interference (11). Tacrolimus is a ring-structured hapten. The structure of such haptens makes interaction with 2 binding proteins (e.g., antibodies) very unlikely because of steric hindrance that occurs following binding of a hapten to one binding protein. Because of this phenomenon, the immunoassay platforms for the detection of such haptens are generally competitive immunoassays. Antibody specificity is a major concern for competitive immunoassays. Various metabolites, including M-I (13-O-demethyl), M-II (31-O-demethyl), M-III (15-O-demethyl), and M-V (15,31, di-O-demethyl) cross-react with immunoassays for tacrolimus (8). Many of the different immunoassays currently available from various manufacturers have been reported to produce tacrolimus values higher than those for HPLC–tandem mass spectrometry (MS/MS) (12). The microparticle ELISA (MEIA) produces results that are 15–20% higher, and EMIT (enzyme multiplied immunoassay) and CEDIA (cloned enzyme donor immunoassay) results are reported to be 17% and 19% higher, respectively (12). Low hematocrit values also have been reported to give false-positive tacrolimus concentrations using the MEIA assay (13). In addition, competitive immunoassays have a limited dynamic range. Yet Wei et al. demonstrate in this article that their newly developed sand-
wich immunoassay has a similar dynamic range (1–30 ng/mL) to liquid chromatography-MS/MS (LC-MS/MS) methods, with negligible bias and markedly less metabolite cross-reactivity compared to other commercially available competitive immunoassays (11). It should be noted on review of the Bland–Altman plots presented by the authors that there appears to be an increased deviation from LC-MS/MS values at concentrations greater than or equal to 16 ng/mL, although this is perhaps not of clinical significance.

The approach used by Wei and colleagues in this sandwich immunoassay for tacrolimus measurement opens the door to the development of similar assays for other drugs that have potentially cross-reactive metabolites. Examples of such drugs include cyclosporine, sirolimus, digoxin, and phenytoin.

Immunoassay technology has evolved from the original assays of 50 years ago. Many new technologies allow for improvement of immunoassays, with the development of more specific antibodies and with better affinity to the target antigen/hapten. The advent of in vitro antibody display technologies provides the ability to engineer new or augment specific attributes of interest, such as affinity, specificity, or stability, into antibody molecules (14). Software developments allowing for 3-D modeling of interactions between antibodies and the antigen also serve to improve immunoassay development. However, there remain challenges for immunoassays in areas beyond the quantification of drugs. These challenges include the measurement of total and free thyroid hormones and steroids. In general, immunoassays are unreliable when used for quantifying low concentrations of steroids such as cortisol, testosterone, and estradiol. Improvement in quantification using a similar approach to that employed by Wei et al. is urgently needed.

A longitudinal replicate study of immunosuppressive drugs performed by the College of American Pathologists showed substantial imprecision for the immunoassays, which accounted for 77% to 90% of the total variance. The major source of imprecision came from within-laboratory factors, with tacrolimus showing large between-year within-laboratory variation (15). This is likely to be a result of lot-to-lot changes in the immunoassay reagent. The development of more specific immunoassays does not ensure the prevention of lot-to-lot variability. The onus remains on the manufacturer to employ strict QC in the manufacture of the reagents as well as testing for minimal variability between lots and monitoring shifts in QC ranges among customers using the product. Nevertheless, the development of this novel sandwich assay for the measurement of tacrolimus by Wei and colleagues represents a milestone in immunoassay development that will hopefully assist in improving immunoassay measurement of drugs and other analytes.

References