

Toxicology News

March 2008

An AACC/CAP Educational Newsletter for Toxicology Laboratories

Immunosuppressant TDM Has Successes and Challenges

By Amir M. Abushamaa

Organ transplantation is typically the last resort for patients with end-stage disease or organ failure. It may provide hope for patients who would otherwise succumb to deadly diseases, such as diabetic nephropathy, chronic active hepatitis, coronary artery disease, and emphysema. Fortunately, the field of transplantation has seen many advances over the past few decades. These advances come with certain considerations that can be ethical, emotional, religious, legal, or financial in nature. In addition, organ rejection and drug toxicity are other important considerations. This article provides an overview of advances in this field, with an emphasis on diagnostic implications.

History

The history of organ transplantation stretches back about 60 years. In 1954, Dr. Joseph Murray performed the first kidney transplant, between identical twins, at Brigham and Women's Hospital in Boston. He won the Nobel Prize in Medicine in 1990 for his efforts. Since 1954, there have been many "first-time" successful transplants, including lung (1963), pancreas (1966), liver (1967), heart (1967), heart/lung (1981), and small intestine (1988). At least 21 different organs or tissues, such as bone marrow, skin, and cornea, have been transplanted, and even artificial parts have been implanted.

The need for transplants is growing. There are more than 250,000 transplant patients worldwide and that number is expected to grow 3–5% every year. U.S. data from 2004, including over 25,000 patients, showed that the majority of transplants have been for kidney (59%), followed by liver (22%), heart (7%), and lung (4%) (1).

The historical success of transplantation and

growth in the number of transplant patients present both good and bad news. The good news is that the rates of first-year organ rejection have been decreasing while survival rates have been increasing (2). The bad news is that the number of organs available cannot keep up with the demand (2).

Successes

Progress has occurred in all phases of transplantation. First, there has been an improvement in organ procurement. For instance, the United States has a centralized system for organ procurement that allows for the prioritization and matching of patients with available organs. Improvements in human leukocyte antigen (HLA) typing methods have led to better matching of donors and recipients. Improvements in infectious disease testing (for example, hepatitis B, hepatitis C, human immunodeficiency virus, and cytomegalovirus) have played an important role in organ and patient screening, because an infection in a donor may affect a transplant decision.

Second, surgical techniques have improved so dramatically that the list of transplanted organs has grown to include organs that would have never been considered decades ago.

Third, the post-transplant phase has seen tremendous improvements in immunosuppressant drugs and corresponding diagnostic assays. The development of assays to monitor immunosuppressant drugs has been key because drug toxicity still leads to complications. Many other diagnostic tests, such as liver function, renal function, and other chemistry tests, also aid in the monitoring of patient status.

Drug therapy

Patients who undergo organ transplantation are generally placed on immunosuppressant drug ther-

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Unexpected Metabolites May Complicate Morphine Results

By Jennifer A. Collins

Although somewhat controversial, the use of opioid analgesics to treat chronic pain has become more common. Because these compounds have a high potential for abuse, many pain management programs incorporate urine drug screening to monitor compliance with the prescribed regimen and to deter patients from not taking any illicit or unreported medications. Patients may be asked to sign a contract agreeing to strict adherence to the treatment regimen and acknowledge that they may be terminated from the program for failure to comply.

Because self-reporting of drug use is notoriously unreliable (1), urine drug screening is used to assess compliance and identify illicit use. The consequences of noncompliance can be severe, so careful, informed interpretation of test results in light of the individual clinical situation is important. Although interpretation of drug screening results is fairly straightforward in most situations, recent evidence suggests that individuals receiving chronic high doses may exhibit unexpected metabolite patterns.

Hydromorphone

As urine drug screening has become more prevalent in chronic pain treatment programs, various laboratories have reported anecdotal findings of low levels of hydromorphone in the presence of high urine morphine concentrations. In a recent review of results of comprehensive urine drug screens in our laboratory we found hydromorphone present in 52% (11 of 21) of the specimens that had urine morphine concentrations in excess of 10,000 ng/mL. Although controlled administration studies have not yet been published, at least two studies based on drug screening of pain patients have concluded that hydromorphone may be produced as a minor metabolite of morphine under certain conditions.

In the first study, urine specimens were collected from chronic pain patients being treated with daily high-dose oral morphine and other opioids in an outpatient setting (2). All the patients denied hydromorphone use, and none had demonstrated any history of opiate misuse or abuse. Specimens were screened by immunoassay and presumptive positives were confirmed by gas chromatography/mass spectrometry. Hydromorphone was detected in the urine of 10 of the 13 patients studied. Hydromorphone-to-morphine ratios ranged from 0.015 to 0.024 in those samples, and there was an apparent correlation be-

tween urine morphine concentrations and the presence of measurable hydromorphone. The authors contrasted the relative hydromorphone/morphine concentrations in the study patients with those in a patient prescribed hydromorphone and methadone in addition to morphine, whose ratios ranged from 1.06 to 1.97.

The second was a retrospective study of chronic pain patients taking only morphine (3). In a total of 53 reviewed cases, hydromorphone was present in 21 cases and absent in 32, demonstrating a 66% prevalence rate. There was a significant correlation between the presence of hydromorphone and higher urine morphine concentrations. The urine morphine concentration was the only significant predictor of the presence of the hydromorphone metabolite in a logistic regression model. None of the subjects had any history of aberrant drug use.

Previous studies using controlled dosing conditions have identified hydrocodone as a minor metabolite of codeine (4,5). There appeared to be biotransformation of codeine to hydrocodone, albeit by poorly defined metabolic pathways. Biotransformation of morphine to hydromorphone has been demonstrated in several species; however, it has not been definitively demonstrated in humans (6).

Morphine metabolism

Morphine is metabolized primarily through glucuronidation to morphine-3-glucuronide and morphine-6-glucuronide, with morphine-3-glucuronide representing about 75% of the total (7). A small percentage of a dose is N-demethylated to form normorphine, and small amounts of sulfate conjugates are also present. A proposed pathway for the in-vivo transformation of morphine to hydromorphone involves a morphinone intermediate (2,5), similar to the mechanism describing the biotransformation of morphine to hydromorphone in bacterial systems used for biological production of hydromorphone (Figure 1) (8).

Although the studies published thus far do not provide definitive evidence of this minor metabolic pathway in humans, they certainly suggest that biotransformation of morphine to hydromorphone can occur. Thus, the presence of hydromorphone may not be definitive evidence of hydromorphone use, and results should be interpreted in the context of patient history and other clinical information.

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Despite First Impressions, Lamotrigine Requires TDM

By John M. Wilson

Lamotrigine (Lamictal, Glaxosmithkline) is primarily used to treat epilepsy, but has received approval for use in other conditions, such as bipolar disorder. Clinical trials demonstrated its efficacy as adjunctive therapy in treating refractory epilepsy.

Trial clinical target concentrations were originally in the range of 1–4 mcg/mL, and it was suggested that therapeutic drug monitoring might not be necessary, although the benefits and risks of elevated doses had not been evaluated (1). These initial assessments were questioned in a paper by Morris et al. (2). Based on their experience, these authors suggested that the therapeutic targets for lamotrigine needed to be higher, with a reference range of 3–14 mcg/mL. They corroborated earlier indications that patients also on carbamazepine and phenytoin experienced lower concentrations and had a smaller range of values for a given dose, while patients also on valproic acid showed higher concentrations and a wide range of values. The dose:concentration ratio differed almost fivefold in patients co-medicated with lamotrigine and valproic acid compared with patients on lamotrigine and inducing agents.

Trials vs. treatment

The differences in target concentrations are rooted in the differences between clinical trials and clinical management. A 50% reduction in an individual seizure rate may be considered a success in a pharmaceutical trial, but in a clinical setting a physician is likely to regard that as inadequate and attempt to lower the rate further by stepping up the dose or adding an additional medication.

The addition of a second drug may produce pharmacologic and/or pharmacokinetic interactions, requiring further modification of the dose. Therapeutic drug monitoring has traditionally played a significant role in managing these adjustments.

Pregnancy

Additional variability occurs during pregnancy. Lamotrigine clearance increases 40–60% in the first trimester, and remains high throughout the pregnancy (3,4). Following delivery, lamotrigine clearance returns to baseline within two weeks. The lamotrigine dose must be increased in women with epilepsy during pregnancy and decreased rapidly postpartum. De Haan et al. reported breakthrough seizures in nine of 12 pregnancies (4). In nonpregnant

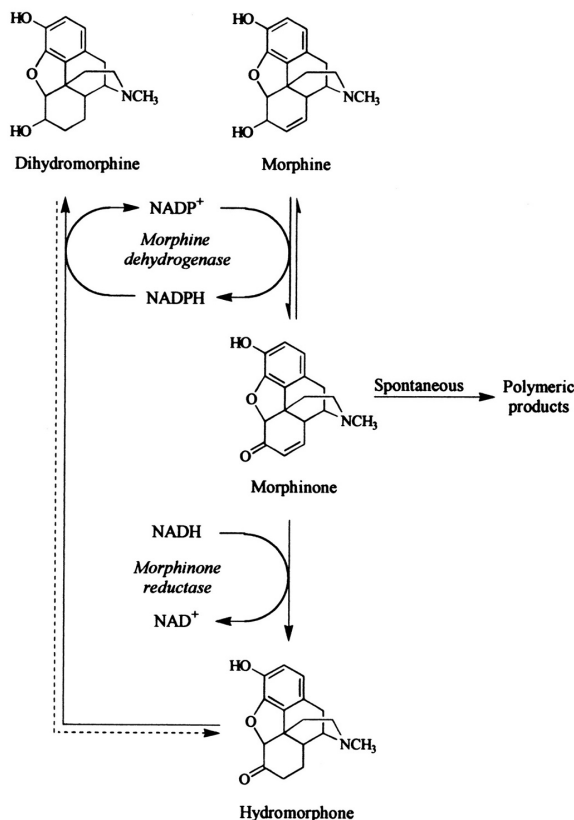


Figure 1. Biotransformation of morphine to hydromorphone by pseudomonas enzymes (8)

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women, the use of birth control pills also increases lamotrigine clearance, and the clearance returns to baseline when use is ceased.

Lamotrigine concentrations in breast milk are 50–60% of concentrations in maternal serum, so the neonate receives significant doses if the mother chooses to nurse. An abstract presented at the recent International Association of Therapeutic Drug Monitoring and Clinical Toxicology Congress in Nice noted that in utero exposure led to neonatal concentrations as high as 13.6 mcg/mL after delivery, and regular breastfeeding produced concentrations of 12.7 mcg/mL (5).

Metabolism and toxicity

It is considered an attractive feature that lamotrigine does not undergo oxidative metabolism, but is metabolized via conjugation. This route, controlled by uridinediphosphateglucuronyltransferase (UDPGT) in the liver, is also influenced by metabolic inducing and inhibiting drugs. This effect is compounded in the neonate because clearance by glucuronidation is poorly developed, so the newborn can achieve significant concentrations. The toxicological manifestations of lamotrigine in susceptible patients are frequently rash and central nervous system depression, symptoms that can cause a medical emergency in a newborn and sometimes mask the presence of another underlying condition. Monitoring of the lamotrigine level provides important information to ensure that the patient receives appropriate treatment.

Toxicity in adults appears to be concentration-related, and caution should be exercised as the dose increases. Werz et al. investigated high-dose therapy with daily doses from 600 to 1900 mg. Of 10 patients, three displayed toxicity at concentrations of 16, 29, and 29 mcg/mL (6). Hirsch et al. reviewed 811 patient charts for toxicity. About 60% of patients displayed toxicity at concentrations above 20 mcg/mL and one-third at concentrations between 15 and 20 mcg/mL (7).

TDM

With all new drugs, laboratory methodologies and proficiency testing lag behind the clinical need. Liquid chromatography (LC) is employed by most laboratories providing therapeutic drug monitoring of lamotrigine, and LC products are commercially available in the United States and Europe through companies such as ChromSystems and Recipe. The U.S. Food and Drug Administration recently approved an automated product employing immunoturbidimetry, a product likely to be widely used (8, 9).

Although clinical trials provide considerable in-

sight into the possible issues presented by each new drug, new information from clinical practice, if properly documented and reported, can supplant initial observations and improve care. The value of therapeutic drug monitoring for lamotrigine was underestimated during the early days of the drug's use but is now becoming more fully appreciated.

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Immunosuppressant TDM

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apy for life. These drugs suppress the normal immune response of the organ recipient (or host) against the donor's organ (or graft) to avoid the response known as "graft vs. host disease" or "rejection." The types of drug therapy include biological and chemical agents. Biological agents include some of the newer monoclonal antibodies (basiliximab and daclizumab) and polyclonal antibodies (antithymocyte globulin and antilymphocyte globulin). Chemical agents include corticosteroids, anti-proliferative agents (azathioprine and mycophenolate mofetil), calcineurin inhibitors (cyclosporine and tacrolimus), and mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus).

Most common agents

This article will primarily focus on the calcineurin and mTOR inhibitors because they are considered to be core/maintenance therapy, as well as the commonly administered immunosuppressant drug, mycophenolate. Transplant patients typically require combination therapy that consists of steroids, core/maintenance therapy (calcineurin and/or mTOR inhibitors), and adjunct therapy (anti-proliferative agents or mTOR inhibitors).

Some of the characteristics of calcineurin and mTOR inhibitors are shown in Figure 1. Cyclosporine, a calcineurin inhibitor, revolutionized the field of organ transplantation in 1983. The drug has some major side effects, such as neurotoxicity, hypertension, hirsutism, and post-transplant diabetes mellitus. Nephrotoxicity is one of its major drawbacks.

Tacrolimus is a calcineurin inhibitor that is more potent

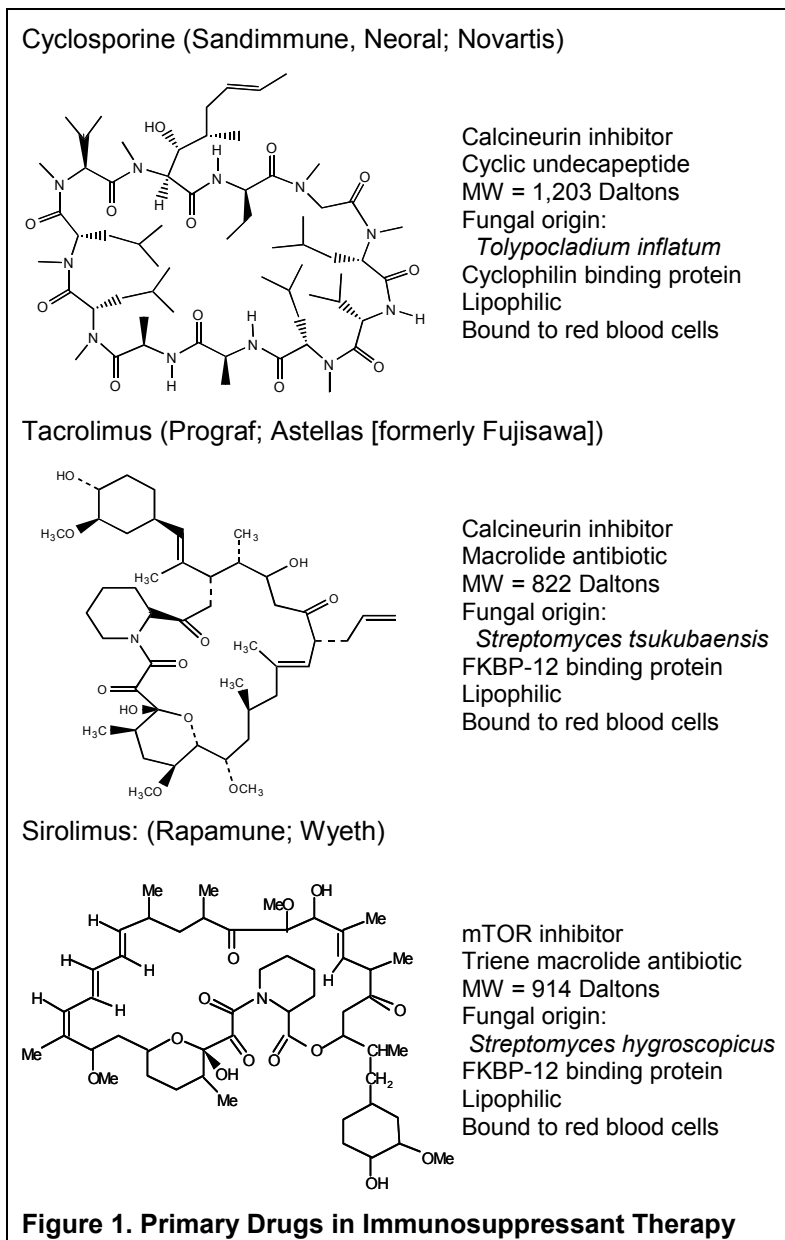


Figure 1. Primary Drugs in Immunosuppressant Therapy

and less toxic than cyclosporine. First discovered in a soil sample from Japan in 1984, its side effects are similar to, but milder than, cyclosporine's. It causes milder hypertension and post-transplant diabetes mellitus, but more neurotoxicity and hyperlipidemia. It is currently the most widely prescribed immunosuppressant drug.

Sirolimus is an mTOR inhibitor that was first discovered in the soil of Rapa Nui (Easter Island). It was previously known as rapamycin. Its side effects are even milder than those of calcineurin inhibitors, and include delayed wound healing, mouth ulcers, and hyperlipidemia. However, it does cause more pneumonitis and interstitial lung disease. Another mTOR inhibitor, for which U.S. Food and Drug Administration approval is currently pending, everolimus (Certican), is a chemical derivative of sirolimus. All of these drugs share several key characteristics: a relatively low molecular weight, complex chemical structure, fungal origin, lipophilic nature, and tendency to bind to red blood cells.

Mycophenolate mofetil (CellCept) is an anti-proliferative agent that is quite potent and has mild toxicity. Its primary side effects include gastrointestinal symptoms (diarrhea) and mild anemia. Its active form, mycophenolic acid, is also available under the trade name Myfortic.

Calcineurin inhibitors inhibit the calcium-calmodulin-calcineurin pathway that leads to interleukin-2 (IL-2) production, thereby inhibiting subsequent T-cell activation (3). The mTOR inhibitors inhibit the mTOR pathway that leads to the IL-2 mediated effect of T-cell proliferation (3). To achieve the synergistic effect of inhibition of both IL-2 production and IL-2's effects on T-cell proliferation, drug regimens often combine either cyclosporine or tacrolimus with sirolimus. There is no benefit to administering both cyclosporine and tacrolimus because they act by inhibiting the same pathway. Mycophenolate is a milder drug with a more selective effect on T cells because it inhibits the salvage pathway of nucleotide synthesis at the cell cycle of T cells, which is the more dominant nucleotide synthesis pathway (3).

The use of immunosuppressant drugs has changed over time as new agents with improved efficacy and/or reduced toxicity have been introduced. For instance, between 1995 and 2004, cyclosporine use for new kidney transplants decreased, while tacrolimus and sirolimus use increased. The use of azathioprine has almost ended, whereas mycophenolate use has increased to high levels (4).

Drug monitoring

The goals for immunosuppressant drug monitoring are to prevent organ rejection; avoid toxicity or side effects, especially nephrotoxicity; and monitor compliance with the treatment regimen (5). Nephrotoxicity is a particular concern because it is one of the major toxic effects of calcineurin inhibitors, which have historically dominated the immunosuppressant field. The clinical intent is to maintain the patient at the lowest effective drug level in order to minimize toxicity or side effects.

Because too little drug can lead to organ rejection and too much drug can lead to toxicity, a patient needs to be maintained within the drug's therapeutic range. The therapeutic ranges for immunosuppressant drugs have not been precisely defined. A variety of factors (such as individual differences in clinical state, pharmacokinetics, combination therapy, and time post-transplant) affect the drug levels in blood. The need for monitoring of mycophenolate has been debated in the scientific literature. Although it is a potent drug with milder toxicity, some clinicians believe that monitoring is required because of the low levels at which it is given, and, consequently, its variable pharmacokinetics.

Sample timing

Immunosuppressant drug monitoring is typically performed with trough monitoring (concentration at baseline or C₀), in which a single sample is obtained on the day of testing. Trough monitoring is easier to comply with, less painful, less costly, and of sufficient clinical efficacy compared with area-under-the-curve (AUC) monitoring (in which multiple samples are obtained over several hours).

Another method of drug monitoring, known as peak level monitoring (concentration at 2 hours post-dose or C₂), is not common. It is used at some centers for cyclosporine monitoring because studies have shown that a C₂ level for cyclosporine is more predictive of clinical outcome than trough level monitoring, primarily due to the presence of fewer metabolites at that early time compared with the trough level time. However, peak level monitoring is a compliance challenge from the standpoint of obtaining the sample at the correct time.

The drug monitoring frequency depends on the patient's stage post-transplant (6). Immediately post-transplant, inpatient monitoring is performed every 24 to 48 hours due to variable pharmacokinetics and the potential for acute infection. About 3–6 months post-transplant, outpatient monitoring is performed two or three times per week to ensure that the patient is stable. Beyond six months post-transplant, outpatient monitoring is performed every few months to

monitor compliance and adjust dosage. These are just guidelines and can vary based on the patient's clinical status, active infections, and rejection.

Diagnostic methodology

The diagnostic methods used for immunosuppressant drug monitoring are primarily based on high performance liquid chromatography (HPLC) or immunoassay. Chromatographic separation methods are based on analyte retention time, followed by either ultraviolet (UV) or mass spectrometric (MS) detection. HPLC with UV detection has been in use for cyclosporine for many years, as well as more recently for sirolimus. However, it lacks the desired sensitivity for tacrolimus, which does not have good UV absorbance at trough blood levels. Cyclosporine blood levels (150–300 ng/mL) are also higher than tacrolimus levels (5–12 ng/mL). HPLC/UV methodology and instrumentation require a considerable level of skill and maintenance.

HPLC with MS detection is a newer methodology that relies on a two-dimensional separation based on both retention time and mass-to-charge ratio. One can also perform tandem MS (LC/MS/MS), in which a "parent" ion can be further degraded into "daughter" ions, which are extremely definitive of the analyte or parent drug without its metabolites. This method provides greater specificity and allows for the simultaneous detection of multiple analytes or ions in the same sample. For this reason, it has gained popularity as a powerful research tool.

One of the major challenges of LC/MS is that, although it offers excellent specificity and is often known as the gold standard or reference method, there can be significant variability between different LC/MS methods. For instance, a recent study on sirolimus showed that slopes of LC/MS methods across several labs ranged from 0.6 to 1.2 (7). It requires a high level of skill, expensive instruments, and frequent maintenance, including daily calibrations. A back-up LC/MS system is often needed in the event of instrument downtime. Chromatographic methods generally have low to moderate throughput.

Immunoassays rely on antibodies for biological recognition of the analyte in conjunction with a reporter molecule, such as a tracer or conjugate, for detection. Detection methods include radioactivity, absorbance, fluorescence polarization, chemiluminescence, and enzymatic reaction rates. They are often performed on automated platforms that offer lower cost, higher throughput, and less maintenance than LC/MS. Precision and sensitivity can be comparable to LC/MS, depending on the technology. Although instrument cost may be lower than LC/MS, the reagent cost is often higher.

One of the major challenges with immunoassays is their metabolite cross-reactivity, even for assays that use monoclonal antibodies. The effect of this cross-reactivity is unclear because the role of metabolites is often not fully understood. For instance, more than 30 metabolites of cyclosporine have been identified to date, and it is not clear whether these metabolites confer efficacy or toxicity. A recent study showed that the levels of key metabolites of sirolimus remained constant over a period of about two years (8). A review of selected proficiency testing programs showed that immunoassay methods were dominant (9).

Challenges

A good immunosuppressant drug monitoring assay needs offer good sensitivity, precision, accuracy, dynamic range, and calibration stability (10, 11, 5). These characteristics present analytical challenges, which can be overcome by improvements in technology and assay design. Representative immunoassay technologies are shown in Table 1.

First, assays need high sensitivity because the analytes are present at lower blood concentrations due to the lower dosages given in combination therapy. Tacrolimus and sirolimus require particular sensitivity because their therapeutic ranges are typically 5–12 ng/mL, compared with cyclosporine's range of 150–300 ng/mL. Combination therapy with sirolimus has driven the therapeutic range down to 3–8 ng/mL for tacrolimus and 100–200 ng/mL for cyclosporine. The Abbott Architect (chemiluminescence) tacrolimus and sirolimus assays claim a functional sensitivity of 2 ng/mL.

Second, assays require improved low-end precision because of lower drug levels and narrower therapeutic ranges. As indicated above, tacrolimus and sirolimus have lower levels and narrower therapeutic ranges than cyclosporine. This translates into greater challenges in obtaining not only good sensitivity, but

also good low-end precision. The Abbott Architect assays imprecision is up to 10% CV.

Main challenge: whole blood required

Third, because these drugs are both lipophilic and tightly bound to red blood cells, they require whole blood assays, extraction by organic solvents (such as methanol), and precipitation of other cellular components and debris. This means that there is a requirement for some type of sample pretreatment step in both chromatography and immunoassay methods. The use of organic solvents also presents a challenge with regard to sample evaporation and timing, both in the pretreatment phase and within an instrument platform, and could lead to front-to-back effects.

Attempts to perform serum or plasma assays for drugs like tacrolimus did not succeed due to the tight binding of the drug to red blood cells. In fact, the drug's temperature sensitivity is such that the whole-blood-to-plasma ratio can vary considerably depending on storage temperature. This is another reason why whole blood assays have been the only option.

Obviously, the inclusion of a pretreatment step, whether offline (manual) or online (onboard), increases variability and affects assay precision. The Dade Dimension (ACMIA) cyclosporine and tacrolimus assays do not require manual pretreatment; pretreatment is performed onboard the instrument. Mycophenolate does not share some of these challenges because it can be measured in plasma.

Fourth, good accuracy provides reliable assays comparable to other reference methods. Accuracy can be assessed by various parameters, such as recovery, interference, correlation, and cross-reactivity. As mentioned earlier, two major challenges affecting accuracy are the variability of different LC/MS reference methods and metabolite cross-reactivity of immunoassays. Variability has been partially addressed by the adoption of gravimetrically prepared standards during the manufacturing process and good quality control procedures, using third-party controls for clinical lab testing, and subscribing to proficiency testing schemes. Appropriate test method validations must be performed before switching methods because results may not always be commutable between methods. Cross-reactivity can be addressed by various factors, such as assay format, antibodies, or technology. For instance, increasing the number of washes (that is, two-step instead of one-step) can decrease the cross-reactivity, albeit at the expense of sensitivity and precision. However, cross-reactivity is a controversial issue because the impact of metabolite measurement is not clear.

Fifth, in order to provide convenient assays and improve lab efficiency, it is important to have a wide

Table 1. Immunosuppressant Immunoassays

Cyclosporine	Tacrolimus	Sirolimus
ACMIA ¹	ACMIA ¹	Chemiluminescence ³
CEDIA ²	Chemiluminescence ³	MEIA ³
EMIT ¹	ELISA ⁴	
FPIA ³	EMIT ¹	
RIA ⁴	MEIA ³	

ACMIA=antibody-conjugated magnetic immunoassay; CEDIA=cloned-enzyme donor immunoassay; EMIT=enzyme-multiplied immunoassay technique; FPIA=fluorescence polarization immunoassay; RIA=radioimmunoassay; ELISA=enzyme-linked immunosorbent assay; MEIA=microparticle enzyme immunoassay

¹Siemens (formerly Dade Behring); ²Microgenics; ³Abbott;

⁴Diasorin

dynamic range with good high-end precision. This is especially relevant for cyclosporine, for which C2 monitoring may be performed. Manufacturers have addressed this issue in different ways. For example, the Abbott TDx (FPIA) Cyclosporine Monoclonal Whole Blood Assay has a dynamic range of up to 1,500 ng/mL, whereas the Microgenics CEDIA Cyclosporine Plus Assay uses both a low (up to 450 ng/mL) and a high (up to 2,000 ng/mL) range kit.

Sixth, calibration stability is a useful feature that allows for continuous access to the instrument platform, as well as better precision. Automated immunoassay platforms usually offer greater calibration stability (two to four weeks) than chromatographic methods, which require daily calibration.

Summary

In summary, there have been significant improvements in the field of organ transplantation. These improvements, although constrained by organ availability, have been translated into improved patient survival and quality of life. There has been an increase in the availability of both immunosuppressant drugs and their corresponding diagnostic assays. Our current challenges with diagnostic testing will continue into the future because of the trend toward combination therapy leading to lower doses of immunosuppressant drugs. However, it is important to remember that immunosuppressant drug assays are an aid in patient management, a piece of the patient's clinical puzzle, and not the sole indicator of organ rejection or drug toxicity.

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