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Editorial

Are Immunoassays Good Enough for Therapeutic Drug Monitoring of Tacrolimus?

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Tacrolimus (FK506), a macrolide lactam containing a 23-membered lactone ring with excellent calcineurin inhibition activity was first isolated in 1984 from fermentation broth of a soil bacterium, Streptomyces tsukubaensis. The inhibitory effect of tacrolimus on calcineurin is due to blocking of the transcription of interleukin-2. As a result, T-cell stimulation is inhibited, thus preventing organ rejection. Tacrolimus was approved by the FDA in 1994 as a prophylactic to prevent organ rejection in liver transplant recipients. Later due to efficacy of tacrolimus in preventing organ rejection, FDA further approved tacrolimus for preventing organ rejection in patients with kidney, heart, lung, intestinal and bone marrow transplantation. Therapeutic drug monitoring of tacrolimus is essential for two reasons; reducing adverse effects, primarily nephrotoxicity but also immunosuppression, neurotoxicity, malignancies, diabetes, and gastrointestinal complaints and also preventing underexposure which increases risk of organ rejection [1]. Tacrolimus is administered orally and available in two forms; immediate release (for example Prograf) and extended release formulations (Envarsus® and Astagraf®). Immediate release tacrolimus formulation should be administered twice daily but extended release can be administered once daily. Tacrolimus is a lipophilic drug which is extensively bound to erythrocytes (95%) and plasma proteins (4%). As a result, unbound fraction represents only 1% of total dose administered. Because of extensive binding with erythrocytes, therapeutic drug monitoring must be conducted using whole blood. Tacrolimus is metabolized by gastrointestinal and hepatic CYP-450 mixed function oxidase enzyme system, primarily by CYP3A5 and to some extent by CYP3A4 isoenzyme. Polymorphism of CYP3A5 has significant effect on metabolism of tacrolimus. Individuals carrying one or more copies of the wild-type allele *1 express CYP3A5, which increases tacrolimus clearance. These patients are called expressors and require 1.5 to 2 fold higher doses of tacrolimus compared to patients who carry CYP3A5*3 allele. Individuals with homozygous *3/*3 genotype are termed as CYP3A5 nonexpressers, which is the most frequent phenotype in most ethnic populations, except blacks [2]. The major variant of CYP3A5 is a single-nucleotide polymorphism (SNP) affecting intron 3 of CYP3A5 (6986A > G; rs776746 SNP) which is associated with reduced CYP3A5 activity [3].

Therapeutic range of whole blood tacrolimus was originally considered 4–15 ng/mL where tacrolimus induced acute nephrotoxicity can be observed with tacrolimus concentration exceeding 20 ng/mL in whole blood. However, a more recent guidelines based on a consensus report by the Immunosuppressive Drugs Scientific Committee of the International Association of Therapeutic Drug Monitoring and Clinical Toxicity (IATDMCT) was published in 2019. This consensus guidelines recommend trough concentration of 4–12 ng/mL in immunological low-risk kidney transplant recipients and preferably >7 ng/mL, if another immunosuppressant is used. When tacrolimus is prescribed in combination with mycophenolate or everolimus and corticosteroids in liver transplant recipients, the recommended target trough concentration is 6–10 ng/mL during the first 4 weeks after transplantation and 5–8 ng/mL after 4 weeks. However, higher target may be needed in heart and bone marrow transplant recipients [4].

Tacrolimus is used off-label in the treatment of glomerular diseases, Crohn disease, myasthenia gravis, and rheumatoid arthritis. Topical tacrolimus cream is used for treating moderate to severe atopic dermatitis and related dermatological conditions.

Therapeutic drug monitoring of tacrolimus can be conducted using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) or immunoassays. LC-MS/MS based methods are considered as the gold standard for therapeutic drug monitoring of tacrolimus because these methods are free from tacrolimus metabolites interference. However, tacrolimus cannot be monitored using high performance liquid chromatography combined with ultraviolet detection because tacrolimus does not have a good absorption peak in the ultraviolet regions. For most immunoassays, tacrolimus must be extracted from whole blood using extraction reagent supplied by the diagnostic company along with immunoassay kit. After centrifugation, tacrolimus concentration is measured in the supernatant. Older immunoassay showed over 30–40% positive interference compared to tacrolimus concentrations obtained by LC-MS/MS due to significant cross-reactivities with tacrolimus metabolites (eight metabolites; major metabolites; 13-O and 15-O-desmethyl tacrolimus). However, later specific immunoassays for tacrolimus using specific monoclonal antibody against tacrolimus were introduced which showed significantly less cross-reactivities with tacrolimus metabolites. In a recent study, the authors observed a proportional positive bias of 26% using Elecsys tacrolimus immunoassay (Roche) when compared with a reference LC-MS/MS method [5]. Parant et al. reported a significant 37% positive bias in liver transplant recipient using QMS tacrolimus assay (Thermo Fischer) in comparison to a reference LC-MS/MS method and this bias was even higher in patients who suffered from cholestasis with hyperbilirubinemia. In contrast, the chemiluminescence microparticle immunoassay (CMIA, Abbott Laboratories using Architect analyzer) showed acceptable analytical performance in patients with hyperbilirubinemia (positive bias less than 10%) [6]. Saitman et al. reported an average 18.5% positive bias with CMIA tacrolimus assay on the Architect analyzer compared to a reference LC-MS/MS (MassTrak from Waters Corporation) [7]. In another published report, the authors observed significantly less bias with CMIA tacrolimus assay (Architect analyzer, Abbott Laboratories), compared to a reference LC-MS/MS method, when patients received extended release tacrolimus. In contrast, bias was higher when patients took immediate release formulation [8].

The CMIA immunoassay using Alinity i analyzer showed no significant bias with a reference LC-MS/MS method (regression equation: $y = 0.9721x + 1.005$, $n = 101$) [9]. The antibody-conjugated magnetic immunoassay (ACMIA) from Siemens Healthcare Diagnostics is the only tacrolimus immunoassay where extraction of tacrolimus from whole blood is automated, thus reducing assay time. However, this assay is subjected to interference from heterophilic antibodies and related endogenous compounds. In one study, the authors reported elevated tacrolimus concentration in a 45-year-old white man who received kidney transplant due to end-stage renal disease as a result of ANCA-associated vasculitis. His tacrolimus concentration using ACMIA assay on day 5 after transplant was 20.4 ng/mL. In contrast CMIA assay (Abbott Laboratories) showed a tacrolimus concentration of 9.9 ng/mL. The study with washed erythrocytes suggested that the interference was in the plasma component of whole blood. The authors concluded that the presence of positive ANCA-MPO autoantibodies may be responsible significant positive bias observed with ACMIA assay in comparison to CMIA assay [10].

The major advantage of using immunoassay for routine therapeutic drug monitoring of tacrolimus is rapid turnaround time. In contrast, LC-MS/MS based methods require much longer turnaround time because specimens are usually batched for analysis. Moreover, acquisition cost of LC-MS.MS analyzer is high and due to complexity of the method only highly trained medical technologists are capable of operating such analyzer. In addition, LC-MS.MS methods are laboratory developed tests with many sources of errors such as ion suppression, isobaric ions to name a few. As a result, tacrolimus values obtained by LC-MS/MS may vary widely between different laboratories, a major limitation of LC-MS/MS based methods. Christians et al. reported a wide interlaboratory variability between tacrolimus results obtained by using LC-MS/MS based methods. The authors speculated that such interlaboratory variations in reported tacrolimus values were due to three main factors: lack of standardization of laboratory procedures between laboratories, lack of uniformity in sample collection and handling, and lack of use of appropriate reference with poor compliance with internationally accepted good laboratory practice guidelines [11]. In contrast, commercially available immunoassays are well standardized. As a result, many laboratories are now transitioning from LC-MS/MS methodology toward reliable immunoassays for routine tacrolimus monitoring in clinical laboratories using FDA approved tacrolimus immunoassay. Therefore, in the opinion of this author, immunoassays can be used for routine therapeutic drug monitoring of tacrolimus. However, when a clinician complains about a tacrolimus values, the left-over specimens should be sent to a reputable reference laboratory to validate immunoassay result using a reference LC-MS/MS method. In our laboratory, we send approximately 1.5% tacrolimus specimens to reference laboratory for analysis using LC-MS/MS analysis. Therefore, FDA approved tacrolimus immunoassays are good enough for routine monitoring of tacrolimus with a backup LC-MS/MS method either available in house or in a reference laboratory.

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