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Validation of a Capillary Dry Blood Sample MITRA-Based Assay for the Quantitative Determination of Systemic Tacrolimus Concentrations in Transplant Recipients

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Background: Tacrolimus is a narrow therapeutic index medication, which requires therapeutic drug monitoring to optimize dosing based on systemic exposure. MITRA microsampling offers a convenient, minimally invasive approach for the collection of capillary blood samples from a finger prick versus conventional venous blood sampling for quantitation of tacrolimus blood concentrations. However, the suitability of MITRA microsampling for the determination of tacrolimus concentrations requires assessment in clinical settings.

Methods: Paired venous (2 mL) and capillary (10 μL) blood samples were collected pre–tacrolimus dose and 1 and 3 hours postdose during routine outpatient visits from stable adult liver or kidney transplant patients receiving prolonged-release tacrolimus. Tacrolimus concentrations were determined by liquid chromatography–tandem mass spectrometry, and the concentrations obtained by the 2 sampling methods were compared by linear regression and Bland–Altman agreement analyses.

Results: Samples were available for 82 transplant recipients (kidney, n = 41; liver, n = 41). A high correlation was observed between tacrolimus concentrations in capillary and venous blood samples (Pearson correlation coefficient, 0.97; Lin concordance coefficient, 0.87; slope of the fitted line, >1.0). Tacrolimus concentrations in capillary samples were 22.5% higher on average than in the corresponding venous blood samples (95% limits of agreement, 0.5%–44.6%). Similar results were observed in both transplant subgroups.

Conclusions: MITRA finger prick sampling provides a convenient alternative to venipuncture for therapeutic drug monitoring in transplant recipients maintained on prolonged-release tacrolimus. When using the finger prick MITRA method, the positive bias in tacrolimus concentrations observed with this technique, when compared with venipuncture, needs to be taken into consideration.

Key Words: LC–MS/MS, MITRA microsampling device, tacrolimus, therapeutic drug monitoring, transplantation

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BACKGROUND

The calcineurin inhibitor tacrolimus is the cornerstone of immunosuppressive therapy after solid organ transplantation. Tacrolimus is a narrow therapeutic index medication, which exhibits large inter- and intrapatient variability in systemic exposure.[1,2] Therapeutic drug monitoring (TDM) is, therefore, necessary to optimize dosing on an individual basis

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to ensure that tacrolimus exposure (area under the concentration–time curve over the dosage time interval, AUC_T) remains within the desired target range.[1–3] Ideally, AUC_T should be estimated from 6 or more concentration–time points. However, the measurement of AUC_T is not a practical option in routine clinical practice because it requires patients to remain at centers for an extended period to provide multiple samples. Instead, the tacrolimus concentration at the end of the dosing interval (trough concentration) is often used as a surrogate marker of AUC. Furthermore, pharmacokinetic models that allow a more precise estimation of AUC using limited sampling have been developed for both immediate-release (IR-T) and prolonged-release (PR-T) tacrolimus formulations.[4–6] For TDM of tacrolimus in kidney and liver transplant recipients receiving PR-T–based therapy, this approach requires taking 3 blood samples to cover the absorption phase post oral intake (eg, predose and at 1 and 3 hours postdose) to estimate the AUC_T for PR-T.

Several assay methods are available for the quantitative determination of tacrolimus concentrations in whole blood, including immunoassays and liquid chromatography–tandem mass spectrometry (LC–MS/MS).[7–9] Currently, whole blood samples obtained through venous sampling by medical practitioners are used for TDM of tacrolimus. However, this can be inconvenient for the patient and may affect the timing of collection. Dried blood spot (DBS) sampling using capillary blood samples from a finger prick has also been used and could be more convenient.[10–13]

The MITRA microsampler (Neoteryx LLC, Torrance, CA) is a Food and Drug Administration Class I, CE marked blood sample collection device that enables collection of a small fixed volume of blood from a finger prick for the subsequent quantitative determination of circulating drug concentrations. Recent studies suggest that it is possible to monitor immunosuppressant drug concentrations in capillary blood samples collected from transplant patients using this device.[14–19]

Recently, a new bioanalytical method for the quantitative determination of tacrolimus concentrations in blood samples obtained using the MITRA sample collection device using liquid–liquid extraction with LC–MS/MS detection has been developed and validated.[20] It is necessary to assess the suitability of this method for the determination of tacrolimus concentrations in clinical settings. This study was undertaken to compare systemic tacrolimus concentrations determined using this bioanalytical method in capillary blood samples collected on MITRA tips with those in corresponding venous blood samples determined using an established LC–MS/MS assay method.

MATERIALS AND METHODS

Study Design

This was a cross-validation study for a bioanalytical assay method, undertaken in adult (≥18 years) liver or kidney transplant recipients. All participants were clinically stable and had been maintained on a stable dose of prolonged-release tacrolimus (Advagraf; Astellas Pharma Europe BV, Netherlands)–based immunosuppressive therapy for at least 3 months. Participants were recruited at 4 investigator sites in France and the United Kingdom between March 20 and September 14, 2018 (NCT03465969).

Paired venous (2 mL) and capillary (10 μL) whole-blood samples were obtained from each participant during routine outpatient visits immediately before and at 1 and 3 hours after tacrolimus dosing. Participants were monitored for procedure-emergent adverse events (AEs) from the time that the first blood sample was collected until 1 hour after the last blood sample was collected.

The study was conducted in accordance with the principles outlined in the Declaration of Helsinki, International Conference on Harmonisation guidelines, and Good Clinical Practice. An independent ethics committee approved the study in each country, and written consent was obtained from all participants. All participants received an honorarium.

Blood Sampling

Samples were collected by a trained nurse/phlebotomist to ensure that paired venous blood and MITRA samples were accurately obtained and with minimum time delay. MITRA samples were collected in accordance with the manufacturer's specifications. All individuals responsible for obtaining samples received training on MITRA sample collection before commencement of the study, which was provided by a nurse with extensive experience in finger-tip capillary blood sample collection.

Venous blood samples (2-mL aliquots) were drawn into polystyrene tubes/vacutainers containing ethylenediamine tetra acetic acid as an anticoagulant. Samples were stored at ambient temperature until shipment. Following a finger prick using a standard lancet, capillary blood samples were collected using 10-μL MITRA tips. The tips were allowed to air dry for a minimum of 2 hours and then stored in sealed polypropylene bags containing desiccant sachets at room temperature. All blood samples were shipped to the bioanalytical laboratory within 48 hours of collection.

Bioanalysis

Tacrolimus concentrations in venous blood samples were determined using a validated LC–MS/MS assay (lower limit of quantification, 0.1 ng/mL).[9] Tacrolimus was extracted after protein precipitation and solid-phase extraction using C18 200 mg/3-mL cartridges, followed by quantification using an AB Sciex 4000 mass spectrometer (AB Sciex LLC, Framingham, MA). For this assay, the internal standard was ascomycin.

Tacrolimus concentrations from MITRA tips were determined using a validated LC–MS/MS assay (lower limit of quantification, 1.0 ng/mL).[20] Tacrolimus was extracted from the MITRA tip using 200-μL acetonitrile:water (50:50) containing [^{13}C]tacrolimus-D_2 as the internal standard. Chromatographic separation was performed using a Kinetex XB-C18 analytical column (Phenomenex Inc.,

Torrance, CA), followed by quantification on an AB Sciex 5500 mass spectrometer (AB Sciex LLC).

All bioanalytical procedures were performed in a Good Laboratory Practice-accredited central laboratory.

Statistical Analysis

The primary analysis included all participants with tacrolimus concentration data for at least one pair of capillary and venous blood samples (full analysis set). The safety analysis set comprised all participants who provided at least one tacrolimus concentration measurement. All analyses were performed in the overall population and both transplant subgroups.

Tacrolimus concentrations in finger prick MITRA and venous blood samples were compared by linear regression analysis. Pearson correlation coefficient (r^2), the slope of the fitted line, and Lin concordance correlation coefficient were calculated.

The comparability of methods was assessed via the percent-of-average difference and the 95% limits of agreement around that difference using Bland–Altman analysis,[21,22] to capture the range within which most of the differences between measurements by the 2 methods would lie. The precision of the 95% confidence interval around the limits of agreement was considered for sample size determination using the Bland–Altman equation.[21,22] The true percent-of-average difference in tacrolimus levels between the 2 methods (venipuncture and MITRA) was assumed to be normally distributed with a mean of 11 and a SD of 13. Therefore, a sample size of 105 paired measurements of tacrolimus levels (eg, 35 participants with 3 paired measurements) was required to demonstrate, with 95% confidence, that the true 95% limits of agreement between measurements methods was no more than 2.22% of the observed value (ie, the 2-sided 95% confidence interval for the mean difference would be within 8.5%–13.5%). Assuming a dropout rate of 12% owing to the unsuitability of samples for analysis, 120 measurements from 40 participants were required to provide the evaluable pairs of tacrolimus level measurements for each organ.

All analyses were performed using SAS statistical software version 9.3 or later (SAS Institute, Inc, Cary, NC).

RESULTS

Study Population

In total, 82 participants were enrolled (41 kidney transplant recipients and 41 liver transplant recipients), all of whom completed the study. Two participants (one in each transplant subgroup) had not been on a stable dose of tacrolimus for the required minimum of 3 months before study entry. Blood sampling time deviations were reported for 9 participants (7 kidney transplant recipients and 2 liver transplant recipients). None of the reported protocol deviations were considered to have implications for the study assessments, and all enrolled participants were included in the full analysis set and safety analysis set. All but one participant provided capillary and venous blood samples at all 3 time points, providing a total of 245 paired blood samples for analysis. No samples were discarded, and all samples received by the laboratory were analyzed; none were declared unfit for analysis.

Baseline characteristics are shown in Table 1. Overall, 65.9% of study participants were male. Mean ± SD age was 51 ± 14.8 years (range, 20–80 years), and the mean ± SD tacrolimus daily dose was 4.6 ± 2.44 mg.

Tacrolimus Blood Concentrations

Linear regression analysis showed a high correlation between tacrolimus concentrations in fingerprick MITRA and venous blood samples (Fig. 1). For the overall population and both transplant subgroups, Pearson correlation coefficient (r^2) was 0.97 (slope of the fitted line, >1.0). Furthermore, a high level of agreement was observed between tacrolimus concentrations determined by the 2 methods; Lin concordance correlation coefficient was 0.87 for the overall population, 0.84 for the kidney transplant subgroup, and 0.88 for the liver transplant subgroup.

Bland–Altman agreement analysis revealed that the mean tacrolimus concentrations in capillary blood samples were 22.5% higher on average than in the corresponding venous blood samples; the 95% limits of agreement were 0.5%–44.6% (Fig. 2). Similar results were seen in both organ transplant subgroups (Table 2).

Safety

There were no safety concerns associated with the use of finger prick MITRA blood sampling. The only reported AE was presyncope in a liver transplant recipient following cannulation for venous blood sampling, which was considered to be procedure related and unrelated to immunosuppressive therapy. No treatment was required and this participant completed the study.

DISCUSSION

In the present study, the results showed a high correlation between tacrolimus levels measured by LC–MS/MS in whole blood samples obtained using the finger prick MITRA-based assay method and those obtained using the established and validated venous blood method, irrespective of organ transplant type. Mean tacrolimus concentrations in capillary blood samples obtained by the finger prick MITRA method were 22.5% higher on average than in the corresponding venous blood samples across a range of tacrolimus concentrations. Although the uncertainty of this average was not quantified (eg, via 95% confidence intervals), it is likely to be relatively narrow owing to the sample size.

The results using the MITRA technique are consistent with those of a previous study, which also found a high correlation (Pearson $r^2 = 0.96$) between tacrolimus concentrations in finger prick DBS samples, obtained using a standard technique, and venous blood samples in stable kidney transplant recipients.[10] As in the present study, Bland–Altman analysis revealed a positive bias toward higher tacrolimus concentrations in DBS samples than in the corresponding venous blood samples; the difference was approximately 11%, with 95% limits of agreement ranging from −14.1% to

TABLE 1. Summary of Baseline Characteristics (FAS)

	All Participants (n = 82)	Transplant Type	
		Kidney (n = 41)	Liver (n = 41)
Age (yr), mean ± SD	51 ± 14.8	51 ± 15.7	52 ± 14.0
Sex, %			
Male	65.9	73.2	58.5
Female	34.1	26.8	41.5
Country, %			
United Kingdom	51.2	51.2	51.2
France	48.8	48.8	48.8
Tacrolimus daily dose (mg), mean ± SD	4.6 ± 2.44	5.4 ± 2.63	3.7 ± 1.95

FAS, full analysis set.

36.1%.[10] Additionally, wide 95% limits of agreement following Bland–Altman analysis have been reported in another recent study comparing tacrolimus concentrations in capillary blood samples collected by MITRA sampling with those in corresponding venous blood samples (−38.6% to 27.4%).[19] Collectively, these findings suggest that, as is the case with the DBS assay,[10] the limits of agreement and positive bias in tacrolimus concentrations observed with the finger prick MITRA method when compared with venipuncture need to be considered when employed for TDM of tacrolimus in transplant patients or for measuring pharmacokinetic parameters of tacrolimus.

Notably, the MITRA method may reduce or eliminate the volumetric blood hematocrit assay bias associated with DBS sampling.[23] Indeed, hematocrit was not found to affect the quantitation of tacrolimus in whole blood samples obtained by MITRA sampling over the range of hematocrit levels likely to be observed in clinical settings in the recent assay validation study.[20] Specifically, accuracy and precision acceptance criteria were met for all samples at all hematocrit levels tested (20%–50%).

Capillary blood sampling using the MITRA method seems to offer a convenient alternative to venous blood sampling for the TDM of tacrolimus, including easier collection, transport, and storage of samples. Moreover, the MITRA-based assay has the potential to enable transplant patients to be monitored remotely, by self-collection of blood samples at home for subsequent bioanalysis. The need to visit an outpatient clinic for venous blood sampling for quantitation of tacrolimus blood concentration is time consuming and can be inconvenient for patients. Furthermore, the timing of blood sample collection for accurate determination of tacrolimus trough concentrations can be difficult in busy outpatient settings. Capillary blood sampling is also less invasive than venipuncture, making it particularly attractive for use in pediatric or elderly patients, in whom venous blood sampling may be challenging. Our study showed no safety concerns associated with the use of finger prick MITRA blood sampling. In another recent study, 82% of transplant patients expressed a preference for monitoring tacrolimus

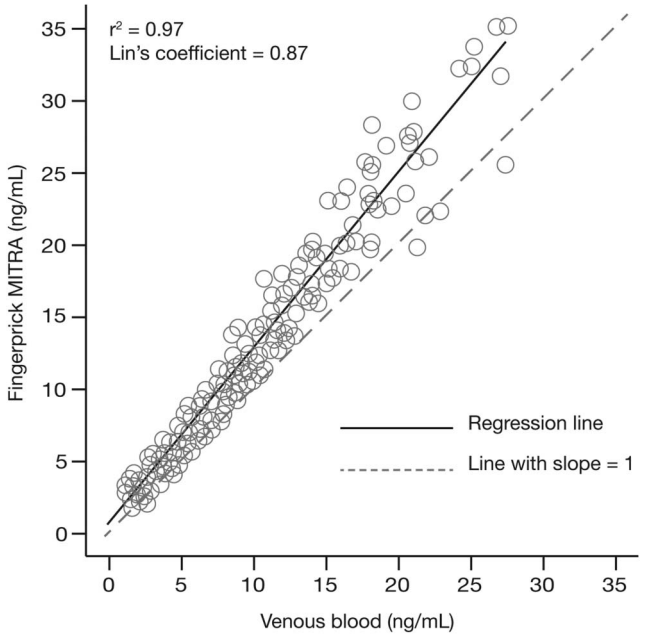

FIGURE 1. Linear regression analysis: scatter plot of tacrolimus concentrations in finger prick MITRA samples versus venous blood samples. FAS, full analysis set; r^2, Pearson correlation coefficient.

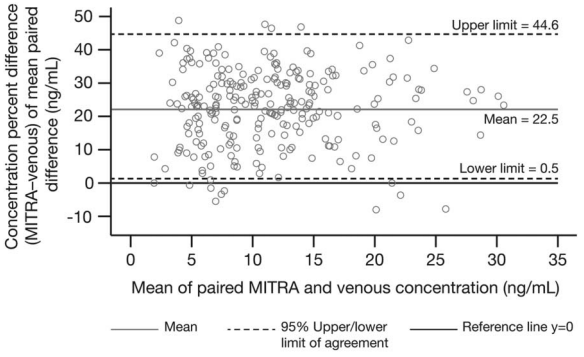

FIGURE 2. Bland–Altman analysis of the difference between tacrolimus concentrations in finger prick MITRA samples versus venous blood samples (FAS). FAS, full analysis set.

TABLE 2. Bland–Altman Agreement Analysis of Finger Prick MITRA Versus Venous Blood Tacrolimus Log Concentrations (FAS)

Transplant Type	MITRA Geometric Mean (ng/mL)	Venous Geometric Mean (ng/mL)	Concentration Ratio (MITRA:Venous)	
			Mean	95% Limit of Agreement
Overall	11.0	8.73	1.25	1.07–1.47
Kidney	12.5	10.1	1.24	1.07–1.45
Liver	9.58	7.56	1.27	1.07–1.50

Mixed model with fixed effect time point and random effect patient. Blood samples were obtained predose and 1 and 3 hour postdose.
FAS, full analysis set.

concentrations by MITRA capillary blood sampling at home over venous blood sampling.[18] For the success of remote TDM, it is essential that patients receive appropriate training on how to collect adequate samples using MITRA for the collection of capillary blood samples at home.

The strengths of this study include that the LC–MS/MS assay used has been validated in accordance with current Food and Drug Administration and European Medicines Agency guidelines for bioanalytical methods[24,25] over the range of tacrolimus blood concentrations expected during TDM in transplant patients.[20] Furthermore, although this study analyzed blood samples from kidney and liver transplant recipients, it is reasonable to assume that these findings are applicable to other patient populations. A potential limitation is that, should the assay fail, then further samples would be required for analysis. This is not the case with venous blood samples, as additional aliquots for analysis can generally be obtained from the original sample. However, it should be noted that all MITRA samples received by the laboratory were analyzed in this study and none were declared unfit for analysis.

CONCLUSIONS

In summary, MITRA finger prick sampling would appear to provide a convenient alternative to venipuncture for TDM in organ transplant recipients maintained on prolonged-release tacrolimus. When using the finger prick MITRA method, the limits of agreement and the positive bias in tacrolimus concentrations observed with this technique when compared with venipuncture need to be taken into consideration.

REFERENCES

1. Venkataramanan R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet.* 1995;29:404–430.
2. Tanzi MG, Undre N, Keirns J, et al. Pharmacokinetics of prolonged-release tacrolimus and implications for use in solid organ transplant recipients. *Clin Transpl.* 2016;30:901–911.
3. Laskow D, Vincenti F, Neylan J, et al. An open-label, concentration-ranging trial of FK506 in primary kidney transplantation: a report of the United States Multicenter FK506 Kidney Transplant Group. *Transplantation.* 1996;62:900–905.
4. Wong K, Shek C, Chau K, et al. Abbreviated tacrolimus area-under-the-curve monitoring for renal transplant. *Am J Kidney Dis.* 2000;35:660–666.
5. Saint-Marcoux F, Debord J, Undre N, et al. Pharmacokinetic modeling and development of Bayesian estimators in kidney transplant patients receiving the tacrolimus once-daily formulation. *Ther Drug Monit.* 2010; 32:129–135.
6. Woillard J, de Winter BCM, Kamar N, et al. Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations—twice daily Prograf and once daily Advagraf. *Br J Clin Pharmacol.* 2011;71: 391–402.
7. Seger C, Shipkova M, Christians U, et al. Assuring the proper analytical performance of measurement procedures for immunosuppressive drug concentrations in clinical practice. *Ther Drug Monit.* 2016;38:170–189.
8. Kalt DA. Tacrolimus: a review of laboratory detection methods and indications for use. *Lab Med.* 2017;48:e62–e65.
9. Alak AM, Moy S, Cook M, et al. An HPLC/MS/MS assay for tacrolimus in patient blood samples. Correlation with results of an ELISA assay. *J Pharm Biomed Anal.* 1997;16:7–13.
10. Cheung CY, van der Heijden J, Hoogtanders K, et al. Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation. *Transpl Int.* 2008;21: 140–145.
11. Shokati T, Bodenberger N, Gadpaille H, et al. Quantification of the immunosuppressant tacrolimus on dried blood spots using LC–MS/MS. *J Vis Exp.* 2015;105:e52424.
12. Martial LC, Hoogtanders KEJ, Schreuder MF, et al. Dried blood spot sampling for tacrolimus and mycophenolic acid in children: analytical and clinical validation. *Ther Drug Monit.* 2017;39:412–421.
13. Gallant J, Wichart J, Kiang T. Predictability of capillary blood spot toward venous whole blood sampling for therapeutic drug monitoring of tacrolimus in solid organ transplant recipients. *Eur J Drug Metab Pharmacokinet.* 2019;44:729–741.
14. Vethe N, Gustavsen M, Midtvedt K, et al. Tacrolimus can be reliably measured with volumetric absorptive capillary microsampling throughout the dose interval in renal transplant recipients. *Ther Drug Monit.* 2019;41:607–614.
15. Gruzdys V, Merrigan SD, Johnson-Davis KL. Feasibility of immunosuppressant drug monitoring by a microsampling device. *J Appl Lab Med.* 2019;4:241–246.
16. Gustavsen M, Midtvedt K, Lønning K, et al. Tacrolimus area under the concentration versus time curve monitoring, using home-based volumetric absorptive capillary microsampling. *Ther Drug Monit.* 2019;42:407–414.
17. Paniagua-González L, Díaz-Louzao C, Lendoiro E, et al. Volumetric Absorptive Microsampling (VAMS) for assaying immunosuppressants from venous whole blood by LC–MS/MS using a novel atmospheric pressure ionization probe (UniSpray™). *J Pharm Biomed Anal.* 2020; 189:113422.
18. Mbughuni MM, Stevens MA, Langman LJ, et al. Volumetric microsampling of capillary blood spot vs whole blood sampling for therapeutic drug monitoring of tacrolimus and cyclosporin A: accuracy and patient satisfaction. *J Appl Lab Med.* 2020;5:516–530.
19. Marshall DJ, Kim JJ, Byrne C, et al. Assessment of tacrolimus and creatinine concentration collected using Mitra microsampling devices. *Ann Clin Biochem.* 2020;57:389–396.
20. Undre N, Hussain I, Meijer J, et al. Quantitation of tacrolimus in human whole blood samples obtained using the MITRA microsampling device. *Ther Drug Monit.* 2021;43:364–370.

21. Bland J, Altman D. Measuring agreement in method comparison studies. *Stat Methods Med Res.* 1999;8:135–160.
22. Bland JM, Altman DG. Agreement between methods of measurement with multiple observations per individual. *J Biopharm Stat.* 2007;17: 571–582.
23. Wilhelm AJ, den Burger JC, Swart EL. Therapeutic drug monitoring by dried blood spot: progress to date and future directions. *Clin Pharmacokinet.* 2014;53:961–973.
24. US Food and Drug Administration. *Bioanalytical Method Validation. Guidance for Industry*. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed November 14, 2020.
25. European Medicines Agency. *Guideline on Bioanalytical Method Validation*. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed November 14, 2020.