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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Abstract

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 fingerprick 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 h 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 two 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’s correlation coefficient, 0.97; Lin’s 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% to 44.6%).
Similar results were observed in both transplant subgroups.

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

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

**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 intra-patient variability in systemic exposure.¹² Therapeutic drug monitoring (TDM)
is, therefore, necessary to optimize dosing on an individual basis to ensure that tacrolimus
exposure (area under the concentration-time curve over the dosage time interval, \(\text{AUC}_T\)) remains
within the desired target range.¹³ Ideally, \(\text{AUC}_T\) should be estimated from six or more
concentration-time points. However, the measurement of \(\text{AUC}_T\) is not a practical option in
routine clinical practice, as 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.\textsuperscript{4-6} For TDM of tacrolimus in kidney and liver transplant recipients receiving PR-T based therapy, this approach requires taking three blood samples to cover the absorption phase post oral intake (e.g., predose and at 1 h and 3 h postdose) to estimate the AUC\textsubscript{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).\textsuperscript{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 fingerprick has also been used and could be more convenient.\textsuperscript{10-13}

The MITRA microsampler (Neoteryx LLC, Torrance, CA, USA) is a Food and Drug Administration (FDA) Class I, CE marked blood sample collection device that enables collection of a small fixed volume of blood from a fingerprick 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.\textsuperscript{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.\textsuperscript{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 four investigator sites in France and the UK 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 h and 3 h after tacrolimus dosing. Participants were monitored for procedure-emergent adverse events (AEs) from the time that the first blood sample was collected until 1 h 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 prior to 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 anti-coagulant. Samples were stored at ambient temperature until shipment. Following a fingerprick using a standard lancet, capillary blood samples were collected using 10 \( \mu \)L MITRA tips. The tips were allowed to air dry for a minimum of 2 h and then stored in sealed polypropylene bags containing desiccant sachets at room temperature. All blood samples were shipped to the bioanalytical laboratory within 48 h 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). 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, USA). 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 [13C]tacrolimus-D₂ 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, Framingham, MA, USA).

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 fingerprick MITRA and venous blood samples were compared by linear regression analysis. Pearson’s correlation coefficient ($r^2$), the slope of the fitted line, and Lin’s 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,\textsuperscript{21,22} to capture the range within which most of the differences between measurements by the two 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.\textsuperscript{21,22} The true percent-of-average difference in tacrolimus levels between the two methods (venipuncture and MITRA) was assumed to be normally distributed with a mean of 11 and a standard deviation (SD) of 13. Therefore, a sample size of 105 paired measurements of tacrolimus levels (e.g., 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 (i.e., the two-sided 95% confidence interval for the mean difference would be within 8.5% to 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, USA).
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 prior to 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 three 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 (Figure 1). For the overall population and both transplant subgroups, Pearson’s 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 two methods; Lin’s 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% to 44.6% (Figure 2). Similar results were seen in both organ transplant subgroups (Table 2).

SAFETY

There were no safety concerns associated with the use of fingerprick MITRA blood sampling. The only reported adverse event 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 fingerprick 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 fingerprick 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 (e.g., 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’s $r^2 = 0.96$) between tacrolimus concentrations in fingerprick DBS samples, obtained using a standard technique, and venous blood samples in stable kidney transplant recipients.\(^\text{10}\) 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 36.1%.\(^\text{10}\) 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%).\(^\text{19}\) Collectively, these findings suggest that, as is the case with the DBS assay,\(^\text{10}\) the limits of agreement and positive bias in tacrolimus concentrations observed with the fingerprick 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.\(^\text{23}\) 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.\(^\text{20}\) 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 appears 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 fingerprick MITRA blood sampling. In another recent study, 82% of transplant patients expressed a preference for monitoring tacrolimus concentrations by MITRA capillary blood sampling at home over venous blood sampling. For the success of remote TDM, it is essential 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 FDA and European Medicines Agency guidelines for bioanalytical methods over the range of tacrolimus blood concentrations expected during TDM in transplant patients. 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 analysed in this study and none were declared unfit for analysis.

Conclusions

In summary, MITRA fingerprick sampling would appear to provide a convenient alternative to venipuncture for TDM in organ transplant recipients maintained on prolonged-release tacrolimus. When using the fingerprick 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.

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Data Statement

Researchers may request access to anonymized participant-level data, trial-level data, and protocols from Astellas sponsored clinical trials at www.clinicalstudydatarequest.com. For the Astellas criteria on data sharing see: https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Astellas.aspx

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FIGURE LEGENDS

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

FIGURE 2. Bland–Altman analysis of the difference between tacrolimus concentrations in fingerprick MITRA samples versus venous blood samples (FAS)
FAS, full analysis set
**TABLE 1.** Summary of baseline characteristics (FAS)

|                              | Transplant type | Transplant type | Transplant type |
|------------------------------|-----------------|-----------------|-----------------|
|                              | All participants (n=82) | Kidney (n=41) | Liver (n=41) |
| Age (years), 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, %                   |                 |                 |                 |
| UK                           | 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 |

SD, standard deviation; FAS, full analysis set
TABLE 2. Bland–Altman agreement analysis of fingerprick 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                              |
| Overall         | 11.0                         | 8.73                          | 1.25                              |
| Kidney          | 12.5                         | 10.1                          | 1.24                              |
| Liver           | 9.58                         | 7.56                          | 1.27                              |

Mixed model with fixed effect time point and random effect patient. Blood samples were obtained predose and 1 h and 3 h postdose. FAS, full analysis set.
\[ r^2 = 0.97 \]
Lin’s coefficient = 0.87
Concentration percent difference (MITRA-venous) of mean paired difference (ng/mL)

Mean of paired MITRA and venous concentration (ng/mL)

Mean = 22.5
Lower limit = 0.5
Upper limit = 44.6

95% Upper/lower limit of agreement
Reference line y=0