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ORIGINAL ARTICLE

Dried blood spot measurement: application in tacrolimus monitoring using limited sampling strategy and abbreviated AUC estimation

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Summary

Dried blood spot (DBS) sampling and high-performance liquid chromatography tandem–mass spectrometry have been developed in monitoring tacrolimus levels. Our center favors the use of limited sampling strategy and abbreviated formula to estimate the area under concentration–time curve ($AUC_{0–12}$). However, it is inconvenient for patients because they have to wait in the center for blood sampling. We investigated the application of DBS method in tacrolimus level monitoring using limited sampling strategy and abbreviated AUC estimation approach. Duplicate venous samples were obtained at each time point (C_0, C_2, and C_4). To determine the stability of blood samples, one venous sample was sent to our laboratory immediately. The other duplicate venous samples, together with simultaneous fingerprick blood samples, were sent to the University of Maastricht in the Netherlands. Thirty six patients were recruited and 108 sets of blood samples were collected. There was a highly significant relationship between $AUC_{0–12}$, estimated from venous blood samples, and fingerprick blood samples ($r^2 = 0.96$, $P < 0.0001$). Moreover, there was an excellent correlation between whole blood venous tacrolimus levels in the two centers ($r^2 = 0.97$; $P < 0.0001$). The blood samples were stable after long-distance transport. DBS sampling can be used in centers using limited sampling and abbreviated $AUC_{0–12}$ strategy as drug monitoring.

Introduction

Tacrolimus has a narrow therapeutic index with wide interpatient and intrapatient variation in pharmacokinetics [1]. As a result, therapeutic drug monitoring (TDM) is essential. TDM is usually performed using ethylenediamine tetraacetic acid anticoagulated blood, obtained by venous sampling by physicians, nurses, or phlebotomists.

Dried blood spot sampling (DBS) is common for screening of metabolic disorders in newborns [2]. Moreover, its use in TDM has been reported for several drugs such as antimalarials and antiretrovirals [3,4]. Recently, a method for measurement of tacrolimus level, based on DBS and high-performance liquid chromatography tandem–mass spectrometry (HPLC–MS), has been developed [5]. Preliminary results showed that DBS is promising for routine tacrolimus monitoring of stable renal transplant recipients [6].

The gold standard of determining the drug exposure is the estimation of the area under concentration–time curve ($AUC_{0–12}$). $AUC_{0–12}$ should be estimated from six or more concentration–time points. However, its routine clinical use is limited by the need for multiple blood sampling. Patients need to stay in centers for long time and it

is also inconvenient to the clinical staff. Majority of the published data use whole blood trough level (C_0) for dose monitoring and titration of tacrolimus therapy. It has been shown that C_0 has a poor correlation with AUC_{0-12} [7,8]. Our center favors the use of limited sampling strategy and abbreviated formula to estimate the AUC_{0-12}, which allows better prediction of drug exposure [9]. However, it is inconvenient for the patients because they have to stay in the center for at least 2-h waiting for blood sampling. Moreover, blood sampling in center may involve a long journey and absence from work duty. The potential advantage of DBS is that the patients can stay at home. They can obtain capillary blood themselves with an automatic lancet and the drop of blood is applied to sampling paper. After drying, the paper with the blood spot sample is sent by mail to the laboratory for analysis.

This is a collaborative study with the Department of Clinical Pharmacy, University of Maastricht in the Netherlands. In this study, we investigated the application of DBS method in tacrolimus level monitoring using limited sampling strategy and abbreviated AUC_{0-12} estimation approach. Moreover, we also studied the stability of blood samples after storage and long-distance transport.

Methods

The study was approved by the ethical committee. Stable kidney transplant recipients who received tacrolimus-based immunosuppressive therapy and had follow-up in Queen Elizabeth Hospital, Hong Kong were recruited in the study. Written consent was obtained from each patient. Blood samples for the measurement of whole blood tacrolimus levels were collected. Limited sampling strategy and abbreviated AUC_{0-12} estimation were used in our center for tacrolimus monitoring. Calculation of tacrolimus AUC_{0-12} was by the formula: $16.2 + C_2 \times 2.4 + C_4 \times 5.9$ (C_2: 2-h postdose tacrolimus level; C_4: 4-h postdose tacrolimus level) [9]. Based on our previous pilot study in stable patients on tacrolimus, AUC_{0-12} value was kept at around 100–150 ng × h/ml in first 3 months and around 80–100 ng × h/ml after 3 months. Some centers also found very high coefficients of correlation between three time-point strategy and the complete AUC_{0-12} [9–13]. The regression equation using three time-point derived in our group was: $13.3 + 1.2 \times C_0 + 2.4 \times C_2 + 5.6 \times C_4$. [9] Thus, C_0 was also obtained in this study.

For each patient, duplicate venous samples were obtained at each time point (C_0, C_2, and C_4). To determine the stability of blood samples after long-distance transport, one venous sample was sent to our laboratory immediately for measurement of whole blood tacrolimus level using HPLC–MS [14]. The other duplicate venous samples, together with simultaneous drawn fingerprick blood samples, were sent to the Department of Clinical Pharmacy, University of Maastricht in the Netherlands for measurement of tacrolimus levels by HPLC–MS [5].

Fingerprick blood samples were collected using spring-loaded lancets and collected from the fingertip. The first drop was discarded and the next drop was collected to fill an 8-mm premarked circle on the sampling paper (No. 10 535 097, obtained from Whatman Schleicher & Schuell, Dassel, Germany). The procedure was performed by the patients. Volume of the blood drops was approximately 30 μl and blood spots of about 9- to 10-mm diameter were produced. The blood spots were allowed to dry at room temperature for 3 h and packed in sealable plastic minibags. The samples were then stored at room temperature and sent to the laboratory in University of Maastricht by airmail. The transit time from Hong Kong to Maastricht was approximately 24 h. On arrival in the laboratory, the blood spots were inspected. Requirements are complete, homogenous, and symmetric filling of the 8-mm circle and dark-red color on both sides of the paper. Paper disks with a diameter of 7.5 mm were punched out with an electromagnetic-driven hole puncher. DBS sampling and assay were compared with venous sampling and our routine assay in venous blood.

Statistical analysis

MEDCALC statistical package (MEDCALC software, Mariakerke, Belgium) was used for data analysis. Data were expressed as mean ± SD or median (range) wherever appropriate. Whole blood tacrolimus levels and estimated abbreviated AUC from different assays were compared using linear regression analyses. Passing–Bablok regression analysis and the Bland–Altman method were also used [15,16]. A P-value of <0.05 was considered to be statistically significant.

Results

Thirty-six patients were recruited in this study and 108 sets of blood samples were collected (C_0, C_2, and C_4 for each patient). The mean age of patients was 46.8 ± 8.6 (range 29.8–62.6) years. All patients had isolated kidney transplantation. The median interval between transplant and blood sampling was 38 months (range: 2 months to 10 years). The duration of storage of DBS samples before analysis was 40 ± 14 (range: 16–78) days.

Fingerprick sampling was well tolerated and accepted by the patients. None of the patient complained of serious discomfort. They found the sampling easy to perform. Of the 108 fingerprick samples collected, only two samples were unsuitable for analysis because of the

incomplete filling of the predrawn circle. No samples were lost during delivery.

Comparison of venous HPLC–MS (Hong Kong) tacrolimus levels with venous HPLC–MS (the Netherlands) tacrolimus levels (n = 106)

Linear regression analysis showed a highly significant relationship between venous blood tacrolimus levels using HPLC–MS (the Netherlands) and venous blood tacrolimus levels using HPLC–MS (Hong Kong) (r^2 = 0.97, $P < 0.0001$).

The Passing–Bablok regression equation was: venous tacrolimus levels (the Netherlands) (μg/l) = 1.06 (95% CI 1.03–1.09) × venous tacrolimus levels (Hong Kong) (μg/l) – 0.33 (95% CI −0.59 to −0.06). There is a small but significant difference from the line of identity. A Bland–Altman analysis showed that venous tacrolimus levels (the Netherlands) tend to be higher than the venous tacrolimus levels (Hong Kong) and the mean difference was 1% of mean tacrolimus levels. The 95% limits of agreement were 19.2% to −17.3%.

Comparison of fingerprick HPLC–MS tacrolimus levels with venous HPLC–MS (the Netherlands) tacrolimus levels (n = 106)

Linear regression analysis showed a highly significant relationship between venous blood tacrolimus levels using HPLC–MS (the Netherlands) and fingerprick blood samples (r^2 = 0.96, $P < 0.0001$) (Fig. 1).

The Passing–Bablok regression equation was: venous tacrolimus levels (the Netherlands) (μg/l) = 0.95 (95% CI 0.89–1.00) × fingerprick tacrolimus levels (μg/l) – 0.39 (95% CI −0.83 to 0.04). There is no significant difference from the line of identity. A Bland–Altman analysis showed that fingerprick tacrolimus levels tend to be slightly higher than the venous tacrolimus levels and the mean difference was 11% of the mean tacrolimus levels. The 95% limits of agreement were 36.1% to −14.1% (Fig. 2).

Comparison of fingerprick HPLC–MS tacrolimus $AUC_{0–12}$ with venous HPLC–MS (the Netherlands) tacrolimus $AUC_{0–12}$: 2 time-point sampling strategy (n = 36)

Linear regression analysis showed a highly significant relationship between $AUC_{0–12}$ estimated from venous blood samples using HPLC–MS (the Netherlands) and fingerprick blood samples (r^2 = 0.96, $P < 0.0001$) (Fig. 3).

The Passing–Bablok regression equation was: venous tacrolimus $AUC_{0–12}$ (the Netherlands) (h × μg/l) = 0.98 (95% CI 0.90–1.08) × fingerprick tacrolimus $AUC_{0–12}$ (h × μg/l) – 5.93 (95% CI −17.33 to 2.98). There is no significant difference from the line of identity. A Bland–Altman analysis showed that fingerprick tacrolimus $AUC_{0–12}$ tends to be higher than the venous tacrolimus $AUC_{0–12}$ and the mean difference was 7.8% of mean tacrolimus $AUC_{0–12}$. The 95% limits of agreement were 25.1% to −9.4% (Fig. 4).

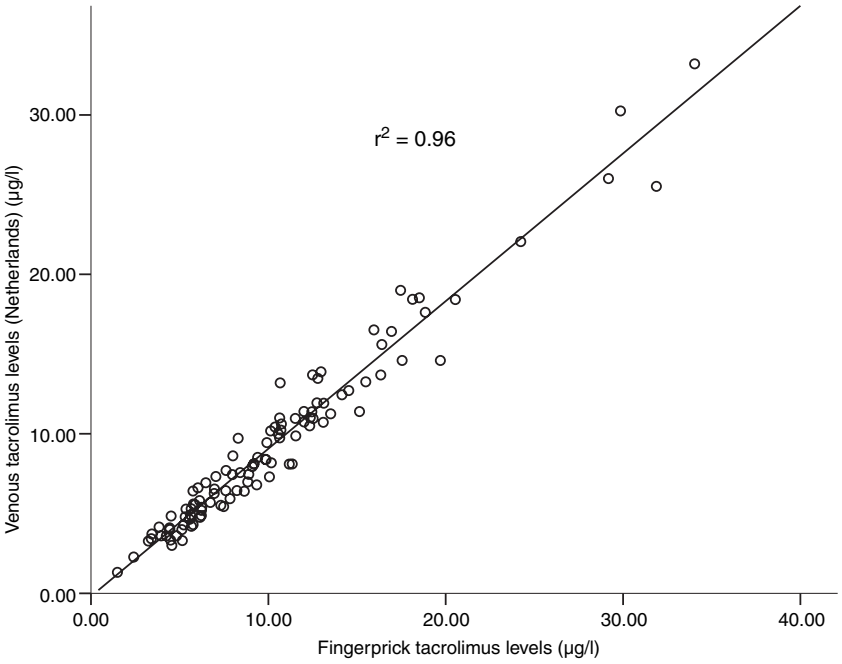

Figure 1 Linear regression analysis: fingerprick tacrolimus level versus venous blood tacrolimus level measured in the same center.

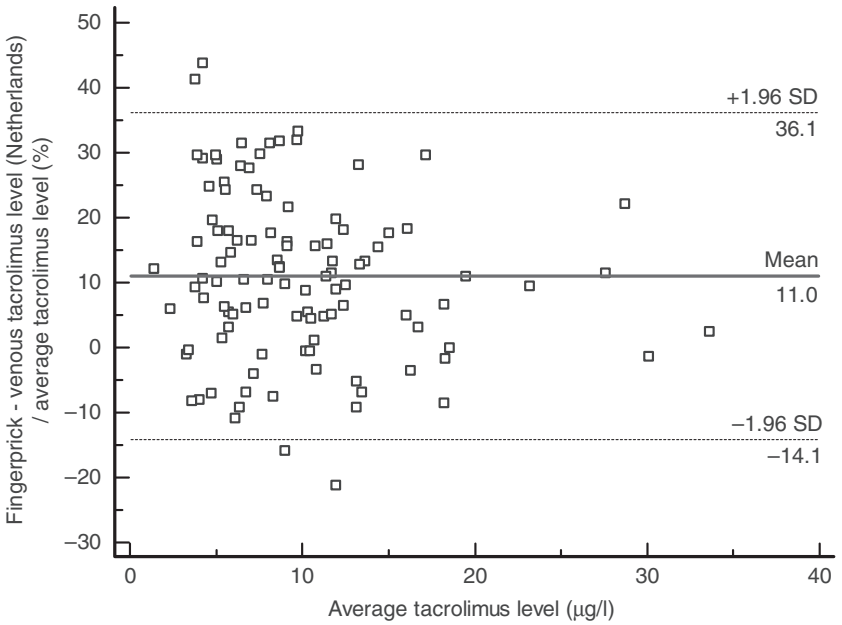

Figure 2 Bland–Altman analysis of the difference (% of average) between fingerprick tacrolimus level and venous blood tacrolimus level measured in the same center.

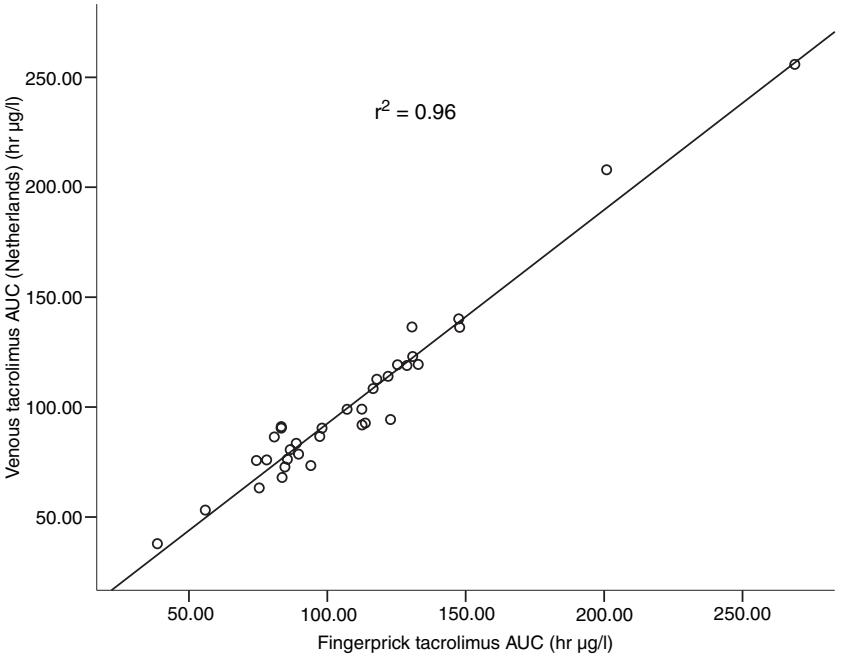

Figure 3 Linear regression analysis: fingerprick tacrolimus AUC_{0-12} versus venous blood tacrolimus AUC_{0-12} using two time-point strategy measured in the same center.

Comparison of fingerprick HPLC–MS tacrolimus AUC_{0-12} with venous HPLC–MS (the Netherlands) tacrolimus AUC_{0-12}: 3 time-point sampling strategy (n = 36)

Linear regression analysis showed a highly significant relationship between AUC_{0-12} estimated from venous blood samples using HPLC–MS (the Netherlands) and fingerprick blood samples ($r^2 = 0.96$, $P < 0.0001$).

The Passing–Bablok regression equation was: venous tacrolimus AUC_{0-12} (the Netherlands) (h × μg/l) = 0.97 (95% CI 0.90–1.06) × fingerprick tacrolimus AUC_{0-12} (h × μg/l) − 5.24 (95% CI −15.99 to 2.97). There is no significant difference from the line of identity. A Bland–Altman analysis showed that fingerprick tacrolimus AUC_{0-12} tends to be higher than the venous tacrolimus AUC_{0-12} and the mean difference was 8.3% of mean tacrolimus AUC_{0-12}. The 95% limits of agreement were 25.7% to −9%.

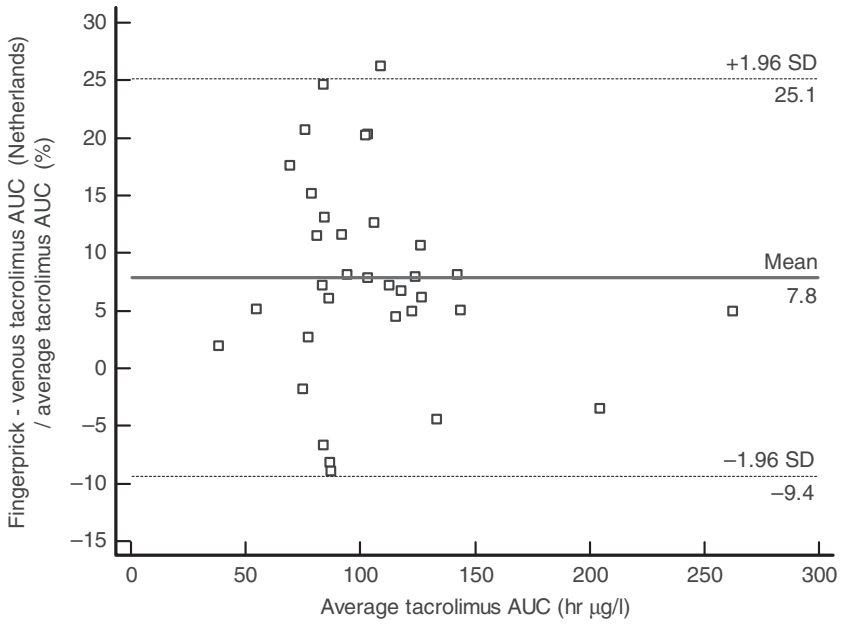

Figure 4 Bland–Altman analysis of the difference (% of average) between fingerprick tacrolimus $AUC_{0–12}$ and venous blood tacrolimus $AUC_{0–12}$ measured in the same center.

Discussion

This study has shown that the tacrolimus levels measured from fingerprick blood samples using HPLC–MS have an excellent correlation with those obtained from venous blood sampling using the same method in the same center ($r^2 = 0.96$; $P < 0.0001$). The difference between them is small (11% of the mean tacrolimus level). It is of limited clinical significance in view of the relatively wide range of target tacrolimus level used in most centers.

Our group has validated the use of limited sampling strategy (C_2 and C_4) and abbreviated formula to estimate the $AUC_{0–12}$, which allows better prediction of drug exposure. In this study, we found that the $AUC_{0–12}$, estimated from fingerprick samples, had a high correlation with those estimated from venous blood sampling using same method in the same center ($r^2 = 0.96$; $P < 0.0001$). Moreover, the mean difference between the two methods was 7.8% of mean tacrolimus levels. This difference is of limited clinical significance. Similar results were also found when three time-point sampling strategy was used for estimating abbreviated $AUC_{0–12}$.

We found that there was an excellent correlation between whole blood venous tacrolimus levels using HPLC–MS in the two centers ($r^2 = 0.97$; $P < 0.0001$). The mean difference between the venous tacrolimus levels, measured in the two centers, was 1% only. The assay was reproducible and the blood samples remained stable after storage and long-distance journey.

Our results demonstrate that it is justifiable to use DBS sampling as an alternative for conventional venous sampling. We have shown that self-administered fingerprick blood sampling for tacrolimus levels is practical to implement. It is highly convenient for patients, especially those who have follow-up in transplant centers where limited sampling strategy is used for drug monitoring.

In conclusion, there was no significant difference between the abbreviated $AUC_{0–12}$ estimated from venous blood samples and fingerprick blood samples. The blood samples were stable after storage and long-distance transport.

Authorship

CYC, JvdH, MC, CSL, JvH and LS designed the research. CYC, YLL, YHC, KSC, CCS, KFC, CSL and LS collected data. CYC, JdvH, Kh, MC, AvdP, JvH and LS analysed data. CYC, JvdH, KH, YLL, YHC, KSC, AvdP, KFC, CSL, JvH and LS performed research. JvdH, KH, AvdP and CCS contributed important reagents.

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