Implementing Dried Blood Spot sampling in transplant patient care

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Summary

Renal transplantation is currently the best treatment option for patients suffering from end stage kidney disease. Once transplanted, patients receive immunosuppressive drugs to prevent rejection of the graft by the recipient. When immunosuppressants are dosed too low, there is an increased chance of acute rejection. When these drugs are overdosed, major side-effects and toxicity can occur. Because of great intra- and interpatient variation in drug exposure, dosing is based on blood drug concentrations which requires the transplant patients to frequently travel to the hospital for venous blood sampling. This process is called Therapeutic Drug Monitoring (TDM). With the introduction of Dried Blood Spot (DBS) sampling, transplant patients are able to sample at home using a finger prick and applying a few drops of blood on a sampling card that can be send to the laboratory by mail. From these blood spots immunosuppressant drug concentrations and serum creatinine levels can be measured. This potentially reduces patient burden and costs. In this thesis, the implementation of this DBS home sampling method for transplant patients was evaluated regarding analytical and clinical performance of the DBS assay, in addition to costs, logistics, patient sampling performance and patient satisfaction.

In **chapter 2** we have improved the available liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis method for immunosuppressant DBS samples. The method is able to analyze 4 immunosuppressants (tacrolimus, everolimus, sirolimus, cyclosporin A). Mycophenolic acid was added to this method. The aim was to analytically validate this DBS assay on two different LC-MS/MS systems (Thermo® and Agilent®) across a clinically relevant hematocrit range without the need to correct for hematocrit. In addition, this validation was performed on Whatman DMPK-C cards instead of 31-ET-CHR cards. On both LC-MS/MS systems the analytical requirements were met for all immunosuppressants. Bias caused by the hematocrit was within 15% for all immunosuppressants for hematocrit levels between 0.23 (v/v) and 0.48 (v/v) across a relevant range of trough level concentrations, meaning no hematocrit correction is needed. The bias caused by the hematocrit for everolimus and sirolimus was higher compared to the other 3 drugs, particularly at lower concentrations (3 µg/mL). The method employed on the Thermo LC-MS/MS was used in a clinical validation study where analytical results from the finger prick DBS samples were compared to the analytical results from the paired venous whole blood samples. For ciclosporin A and for tacrolimus, the results from DBS were interchangeable with the venous whole blood results showing that this DBS analysis method can be used in patient home sampling.

In **chapter 3** the aim was to show interchangeability between analytical results from fingerprick DBS samples and venous samples for both tacrolimus, cyclosporin A and creatinine. The DBS results from the Agilent method mentioned in **chapter 2** were used. All finger prick DBS and venous whole blood samples were obtained by trained phlebotomists within 10 minutes of each other during routine adult kidney transplant patient visits to the hospital for TDM and nephrologist consultation. After exclusion of several samples because of insufficient quality, a total of 172, 104 and 58 samples were available from 172 different patients for method comparison of creatinine, tacrolimus and cyclosporin A, respectively. In Passing & Bablok regression analysis and Bland-Altman analysis no clinical significant differences between DBS and whole blood were found for tacrolimus and cyclosporin A. For creatinine, a difference between DBS and plasma results was found, as was expected because of the different matrices (venous plasma and finger prick capillary blood). A systemic difference was observed, allowing the conversion of DBS results to plasma creatinine results using the formula (creatinine plasma concentration in µmol/L) = (creatinine concentration in DBS in µmol/L)/0.73. In conclusion, this chapter showed that DBS sampling can replace venous sampling for the monitoring of tacrolimus, cyclosporin A and creatinine.

In **chapter 4** a similar clinical validation study was performed as described in **chapter 3**, but for the immunosuppressants sirolimus and everolimus. Because these drugs are not used as frequently as tacrolimus, the sample size was limited (39 and 44 paired DBS and venous samples respectively for sirolimus and everolimus). In addition to the validation steps described **chapter 3,** two additional validation parameters were investigated; the limits of clinical acceptance and the predictive performance as described by Sheiner and Beal. The limits of clinical acceptance were set in a multidisciplinary team consisting of pharmacists, analysts and transplant physicians at >80% of all paired samples to be within 15% of the mean of both samples. The Passing & Bablok regression analysis and Bland-Altman analysis showed no clinically relevant differences between DBS and whole blood. The predictive performance met the predefined criteria, showing that whole blood values can be predicted from DBS values. However, the limits of clinical acceptance were not met showing values of 77.3% for sirolimus and 61.5% for everolimus. In this chapter we concluded that DBS sampling cannot replace venous sampling at this time for sirolimus and everolimus trough concentration monitoring because the pre-defined limits of clinical acceptance were not met. However, if less strict limits are acceptable for clinical practice, this DBS method will be suitable for clinical use.

In **chapter 5** the quality of 464 individual blood spot cards from 4 different countries (Paraguay, Belarus, Bangladesh, Indonesia) were assessed. These samples were obtained as part of a TDM study for drugs used in the treatment of tuberculosis, by

untrained healthcare workers who only had a written instruction available on how to perform DBS sampling. A checklist was developed consisting of multiple criteria to assess the quality of the obtained DBS samples. Two DBS experts used the checklist to score the samples independently of each other and found that only 54% of the samples complied with present quality standards. In most of the cases, this was due to incorrect sampling. In addition, samples from relatively humid countries (Paraguay, Bangladesh and Indonesia) seemed to be affected by the high air humidity causing light-red rings around the blood spots during drying of the samples. This chapter showed that training of health care workers in DBS sampling is very important for yielding a high amount of sufficient quality DBS samples in clinical research.

In **chapter 6,** the development of a web-based application (app) capable of assessing DBS quality at the time of sampling by means of analyzing a picture of the DBS was described. Regarding DBS sample quality, the judgment of an experienced laboratory technician is, based on the criteria mentioned in **chapter 5,** the golden standard. After development, the app was tested by comparing the results of the app to this golden standard. The performance qualification was set a priori at 95%, meaning that the app should make the same decision as the golden standard in 95% of the cases. The datasets of **chapter 3** and **chapter 5** were used to test the app and were defined as the trained and untrained setting, respectively. In a trained setting the app yields an adequate decision in 90.0% of the cases with 4.1% false negatives (insufficient quality DBS incorrectly not rejected) and 5.9% false positives (sufficient quality DBS incorrectly rejected). In an untrained setting this is 87.4%, with 5.5% false negatives and 7.1% false positives. If the app had been present in the trained and untrained setting, was used properly and resampling would have yielded a sufficient quality DBS sample, the amount of sufficient quality samples would have increased from 80.0% to 95.9% and 42.2% to 87.9%, respectively. In conclusion, the app can be used in both a patient care and research setting to increase the amount of sufficient quality DBS samples.

In **chapter 7**, we have described the first randomized-controlled clinical study assessing the costs and effects of the implementation of DBS home sampling in transplant patient care. In this single-center randomized-controlled clinical trial, 25 patients used DBS home sampling on top of usual care 6 months after renal transplantation while 23 patients received usual care only. The aim was to assess whether DBS home sampling would lead to a reduced amount of outpatient visits, reduced costs from a societal point of view and improved patient satisfaction. Unfortunately, the number of outpatient visits was not significantly lower in the DBS group (11.2, SD: 1.7) compared to the control group (10.9, SD: 1.4) ($p = 0.48$). In addition, costs per visit in the DBS group were not significantly different (€537, SD €179) compared to the control group

(€510, SD €229) (p = 0.66). This is probably due to the fact that only 56% of the expected DBS was sent in and that 20% of the expected DBS was analyzed on time, meaning that the result from the DBS analysis was present in the Electronic Health Records of the patient at time of nephrologist consultation. However, 82.6% of the patient are willing to perform DBS home-sampling if this would reduce the number of outpatient visits. Optimization of logistical processes concerning the sending and analysis of DBS samples is crucial in implementation of DBS home sampling.

In **chapter 8**, a guideline was presented on the development, analytical and clinical validation of Dried Blood Spot based methods used for TDM. Current validation requirements, described in guidelines for traditional matrices (blood, plasma, serum), do not cover all necessary aspects for this. Therefore, this chapter provides parameters required for the validation of quantitative determination of small molecule drugs in DBS using chromatographic methods, and to provide advice on how these can be assessed. In addition, guidance is given on the application of validated methods in a routine context. First, considerations for the method development stage were described. Second, common parameters regarding analytical validation were described in context of DBS analysis with the addition of DBS specific parameters. Third, clinical validation studies were described, including number of clinical samples and patients, comparison of DBS with venous blood, statistical methods and interpretation, spot quality, sampling procedure, duplicates, outliers, automated analysis methods and quality control programs. Lastly, cross-validation was discussed, covering changes made to existing sampling- and analysis methods.

In **chapter 9**, we have described the development and analytical validation of an LC-MS/MS assay for tacrolimus, everolimus, sirolimus, cyclosporin A and mycophenolic acid using Volumetric Absorptive Micro Sampling (VAMS) tips (Mitra®). These tips wick up an exact amount of blood which potentially mitigate volume-related hematocrit effects and potentially make patient sampling easier. Biases caused by hematocrit effects were within 15% for all immunosuppressants between hematocrit levels of 0.20 and 0.60, except for cyclosporin A, which is valid between 0.27 and 0.60 v/v. There was a trend visible where higher analyte concentrations combined with low hemacrit values result in reduced recovery. However, for the relevant clinical ranges this bias was within requirements and the values are lower than reported for DBS **(chapter 2).** This analysis method was tested for tacrolimus in a clinical validation study described in **chapter 10**. A total of 130 paired fingerprick VAMS, fingerprick DBS and venous whole blood samples were obtained from 107 different kidney transplant patients by trained phlebotomists for method comparison using the same validation criteria as was described in **chapter 4**. A multidisciplinary team pre-defined an acceptance limit requiring >80% of all paired samples within 15% of the mean of both samples as was

described in **chapters 4 and 8**. Sampling quality was evaluated for both VAMS and DBS samples: 32.3% of the VAMS samples and 6.2% of the DBS samples were of insufficient quality. Passing & Bablok regression showed a significant difference between VAMS and whole blood, with a slope of 0.88 (95%CI 0.81-0.97) but not for DBS (slope 1.00; 95%CI 0.95-1.04). For VAMS and DBS, the acceptance limit was met for respectively 83.0% and 96.6% of the samples. VAMS sampling can replace whole blood sampling for tacrolimus trough concentration monitoring, but VAMS sampling was inferior to conventional DBS sampling, both regarding sample quality and agreement with whole blood tacrolimus concentrations.

In **chapter 11** the thesis was discussed and future perspectives were given. In this thesis, we have described the steps necessary to implement Dried Blood Spot sampling for immunosuppressant TDM for transplant patients. This is possible if the following criteria are met. (1) The analysis method used for analyzing the DBS samples is fast, robust and meets all general and DBS-specific analytical requirements. (2) DBS assays prove to be valid in a well-designed and executed clinical validation study and are monitored by external quality control programs. (3) Logistics are optimal, and might include Track-and-Trace sending of samples, reminder systems for patients and standardized days of sampling and analysis (4) Patients are trained and re-trained in DBS sampling using a training method that includes practicing the complete sampling procedure under supervision of someone experienced in DBS sampling.