Implementing Dried Blood Spot sampling in transplant patient care

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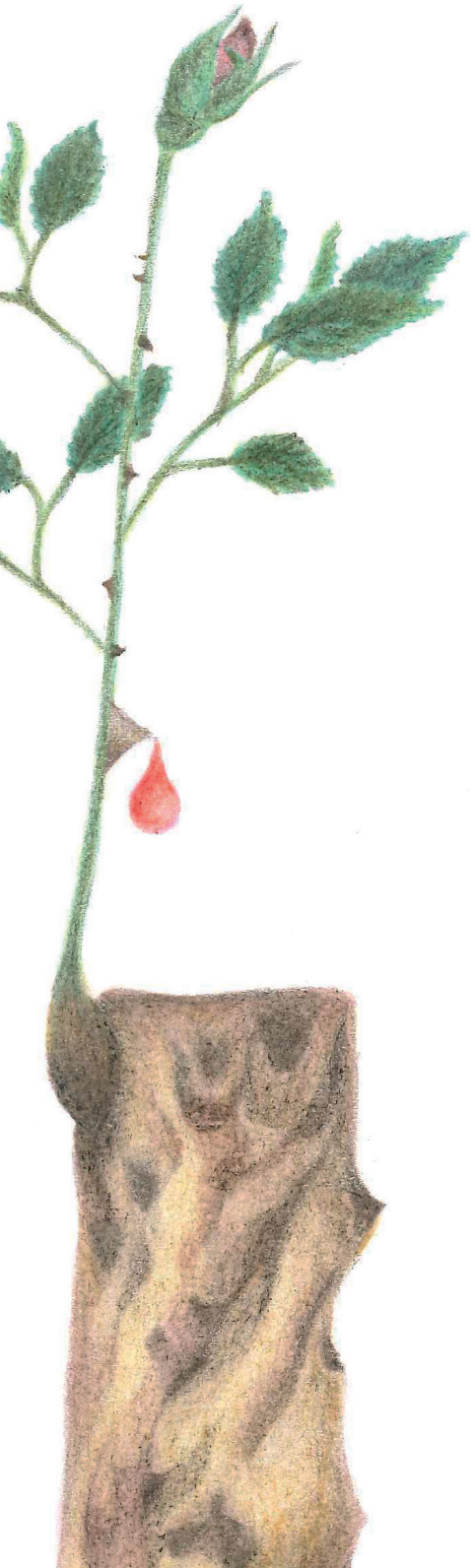

Chapter 11

General discussion and future perspectives

Kidney transplantation is currently the best treatment option for patients suffering from end stage kidney disease.[1] To prevent rejection of the transplanted organ, chronic use of immunosuppressive drugs is required. When these immunosuppressants are inadequately used or when they are dosed to low, there is an increased chance of acute rejection. When these drugs are overdosed, major side-effects and toxicity can occur.[2] Therefore, dosing is based on frequent assessment of blood drug levels. This requires the transplant patients to frequently travel to the hospital for venous blood sampling. With the introduction of Dried Blood Spot (DBS) sampling, transplant patients are enabled to sample at home, potentially reducing patient burden and costs.[3] In this thesis, the implementation of this method was evaluated regarding analytical and clinical performance of the DBS assay, in addition to costs, logistics, patient sampling performance and patient satisfaction.

Analytical performance

Implementation of a DBS method in clinical practice for the purpose of Therapeutic Drug Monitoring (TDM) is only feasible if the method used for analyzing the DBS samples is fast, robust and meets all bio-analytical requirements.[4-6] The analytical method used in our hospital is able to simultaneously assess levels of tacrolimus, sirolimus, everolimus and cyclosporin A.[6] In **chapter 2** we describe an improvement of our multi-analyte assay, including the addition of mycophenolic acid.

Currently, analysis of immunosuppressants in whole blood (tacrolimus, cyclosporin A, sirolimus, everolimus) or plasma (mycophenolic acid) is the standard.[7] These analyses are performed on highly sensitive LC-MS/MS systems. These methods are robust, fast, have been used for over a decade, have external quality control programs and do not suffer from DBS-related problems such as the effect of the hematocrit.[8] A novel DBS method should be in line with these standards. This means that sample preparation should be straight-forward, fast and without difficult and time-consuming steps like solid phase extraction.[9] The assay described in **chapter 2** is slightly more labor-intensive for lab technicians compared to the venous whole blood assay.[7] This is mainly due to the fact that DBS analysis requires manual punching of the blood spots and some additional steps like vortexing, sonication and a freeze step to improve protein precipitation. However, the additional time needed for DBS analysis is limited and analysis of DBS samples can be performed within a day, which is similar to whole blood analysis.

Although DBS assays can meet the quality criteria put forward in relevant guidelines of the EMA and FDA, additional aspects specific to DBS assays need to be addressed.[4,5] One of the most challenging aspects is the influence of hematocrit on analytical results.[8]

The influence of the hematocrit can be interpreted as the influence of hematocrit on spot formation only.[10] This can wrongfully lead to the conclusion that, if whole spots with a known volume are analyzed, hematocrit is not of influence. However, an effect of hematocrit on extraction recovery is always present, irrespective of the sampling device or sampling paper used for the micro sampling method.[11] Therefore, a potential influence of hematocrit should always be taken into consideration during analytical validation. In **chapter 8,** specific steps to investigate and interpret the effect of hematocrit are described. In **chapter 2,** we describe how both the hematocrit and the concentration of the drug of interest are of influence on analytical results. However, only cyclosporine concentrations outside of the target trough concentration range (>200 µg/L cyclosporin A) in combination with extreme values of hematocrit (e.g. 0.20 v/v), resulted in a bias which was higher than the predefined criterion of 15%. Therefore, it was concluded that for application in clinical practice, the assay is independent of hematocrit effects.

In circumstances where hematocrit would be of influence on recovery, several strategies have been suggested to overcome this problem. These are all based on the incorporation of the patients' individual hematocrit values in calculating DBS values.[12] To make this possible, the hematocrit should be known for individual samples. This lead to the development of several strategies of measuring hematocrit in DBS samples, including measurement of potassium, use of near-infrared spectroscopy, use of sulfolyser reagent and use of noncontact diffuse reflectance spectroscopy.[13-17] However, if the hematocrit has such a major impact on analytical result that this becomes necessary, one might argue that the used extraction method is not optimal. For everolimus, a major impact of hematocrit on analytical performance was observed in Volumetric Absorptive Micro Sampling (VAMS) tips.[11] In our VAMS analytical validation, which was described in **chapter 9**, this was not the case. This is best explained by a difference in extraction methods between our analytical method and earlier methods. It should be noted that in literature there is a great variety in extraction methods for immunosuppressants in micro sampling devices.[6,9,11,18-32] Future research should focus on the most optimal extraction procedure which should be independent of hematocrit and the sampling device.

Another advantage of DBS is the possibility of automated analysis. Several strategies to automate punching, extraction and analysis of DBS samples have been described.[33-35] The further development and clinical validation of these methods might greatly contribute to the implementation of DBS in routine care. In future, the most ideal laboratory procedure for DBS analysis is the insertion of a freshly arrived DBS sample into a fully automated LC-MS/MS setup, which can produce an analytical result within a few hours without the need of sample preparation by the lab technician.

Clinical performance

In 2016, a review was published showing a list of 90 drugs that could be determined from DBS.[36] This number has undoubtedly increased in the past years. However, the number of clinical validation studies published is probably just a fraction of this number. In a clinical validation study, a candidate analytical method (DBS or other micro-sampling device) is tested against the standard (usually analysis in whole blood, serum or plasma). The purpose of these studies is to investigate whether there is sufficient agreement between the DBS method and reference plasma, serum or whole blood method. To perform these studies, paired fingerprick DBS samples and venous liquid blood samples are obtained, analyzed and compared using appropriate statistical tests. We describe such studies in **chapters 3,4 and 10**. In **chapter 8**, a guideline on how to perform such studies is presented. In the previous paragraph it was stated that the DBS assay should meet the analytical standards as set by the whole blood method. This is also true regarding the clinical standard.

There can be several reasons why clinical validation studies are not published in literature. A potential reason is that these studies can be labor- and cost intensive and require ethical clearance before they can be conducted. In addition, patients who use the drug of interest need to be included in the study. To realize this, a multidisciplinary approach is needed and the treating physicians, pharmacists, analysts and (sometimes) patients, should be part of the research team. For labs, outside of (academic) hospitals, this can be a challenge, which might be too hard to overcome. Another reason for the lack of published clinical validation studies might be publication bias. There is a possibility that clinical validation studies are performed, but that they show insufficient agreement between the novel DBS method and the reference method, and are therefore not published. Although one of the first clinical validation studies was published in 2005, it took until 2018 for the first 'negative' study to be published by Kloosterboer et al.[24,37] In their study, Kloosterboer et al. describe a clinical validation study for antipsychotics where all drugs investigated did not meet the predefined criteria set for the Bland-Altman analyses. This was interesting, because the DBS analysis method had already been analytically validated in an earlier publication.[38] This underlines the need for clinical validation studies – and independent replication thereof – as a standard part of the development, validation and implementation of DBS assays. In **chapters 3 and 10,** we have shown that tacrolimus, cyclosporin A and creatinine can be reliably measured from DBS. In addition, tacrolimus can also be measured in VAMS, as is described in **chapter 10**. Unfortunately, for everolimus and sirolimus the clinical validation was unsuccessful according to our predefined criteria as is described and discussed in **chapter 4.**

The predefined criteria for acceptance of the method as is described in **chapter 8** and applied in **chapters 4 and 10,** are very important in clinical validation studies. Analytical

results from DBS assays can have direct clinical consequences, such as tacrolimus dose adjustment based on a trough concentration measured in a DBS sample. This clinical decision making should be taken into account in a clinical validation study. Therefore, before starting a study, limits for clinical acceptance should be defined. Ideally, these limits should be defined in such a way that results assessed with DBS sampling will translate in making the same clinical decision as would have been made if results came from a whole blood sample. However, analytical factors such as bias and precision, clinical factors such as target trough concentration range and patient factors such as patient-specific pharmacokinetic and pharmacodynamic parameters are all of influence. Therefore, the limits of acceptance should be set by a multi-disciplinary team which include pharmacists, physicians and lab technicians. Some clinical validation studies are designed in such a way that the clinical interpretation of a DBS sample is done separately from the whole blood sample.[29] This provides the opportunity to assess whether results from a DBS sample and a whole blood sample will result in the same clinical decision. In future clinical validation studies, this approach is highly recommended and should include setting of pre-defined limits for acceptance.

In this thesis, a multi-analyte assay is presented, which is able to determine blood concentrations from 5 immunosuppressants. Unfortunately, only 4 out of 5 of these immunosuppressants are tested in a clinical study. Mycopohenolic acid remains to be tested in a clinical validation study. Although monitoring of mycophenolic acid trough concentrations is done less frequently than tacrolimus, it could prove to be useful. This could be particularly true because it is part of the DBS analysis method, but not of the whole blood analysis method. This means that analyzing mycophenolic acid in DBS requires no additional work from lab technicians.

To date, only a few hospitals use DBS sampling as part of routine transplant patient care for tacrolimus TDM. This might be a reason why no external quality control program, such as proficiency testing exists. The International Organization for Standardization (ISO) states that all medical laboratories are required to participate in inter-laboratory comparison or proficiency testing to ensure quality, comparability and acceptability of analytical results.[39] Therefore, there is an urgent need for proficiency testing programs for DBS. Ideally, this program should contain patient samples as well as spiked samples. In addition, the spiked blood that is used to prepare DBS samples can be used as a sample itself. These samples can be analyzed by participating labs on the routine whole blood analysis method and can serve as a quality control.

If DBS assays prove to be valid in a well-designed and executed clinical validation study and are monitored by external quality control programs in clinical practice, transplant patient treatment can be based on results from DBS samples.

Implementation in clinical care

In this thesis, we present a tacrolimus DBS assay that meets analytical and clinical standards. However, having a high quality analysis method is only the beginning of a trajectory of implementing DBS sampling in standard transplant patient care. As is demonstrated in **chapters 5,6 and 7**, logistical challenges and sample quality are of major concern in implementing DBS in routine care.

Costs, effects and patient satisfaction

In **chapter 7** we have described a study in which the results do not show a cost reduction when transplant patients use DBS home sampling for tacrolimus TDM and creatinine monitoring. Main reasons for this negative finding are logistical issues concerning the sending and analysis of the samples. When it comes to logistics, the standard is set (again) by the whole blood method used for TDM. If a doctor asks a patient to donate a venous blood sample in the hospital, this will result in availability of a tacrolimus trough concentration in the patients' Electronic Health Records (EHR) by the end of the same day in >99% of the cases. Even if a DBS home sampling method results in 80% of the DBS results available in the patient's EHR prior to the outpatient visit to the physician, this still can be perceived as insufficient by both patient and physician. Because of this, the logistical challenges of DBS sampling are as important as the analytical and clinical performance of DBS assays. In **chapter 7**, we have shown a number of important leads for the further improvement of the implementation of DBS home sampling. First of all, adult kidney transplant patients are enthusiastic about the prospect of the possibility of reduction of frequency of outpatient visits. Therefore, if DBS leads to reduced outpatient visits, patients will be highly motivated to correctly perform DBS sampling. In **chapter 7**, we also have described the societal costs involved in one outpatient visit. From this, cost-reduction can easily be calculated for DBS after improved implementation. Although the logistical challenges concerning DBS home sampling are serious, they can be regarded as teething problems. In the future, the logistics can be improved by automatically sending the patient the sampling kit a few days prior to scheduled sampling accompanied with an automated reminder system by e-mail or phone. This will greatly reduce the chance of the patient forgetting to sample. After sampling, a pick-up service could collect the samples at home (or work) and send them with track-and-trace to the laboratory. If there are standardized days of sampling and analysis, the chance that no results will be available during the outpatient visit will be minimized. Disadvantages are the increased costs of such a service, but they will most likely be very small compared to the costs of one saved outpatient visit. Another disadvantage of this system would be that the DBS method will not be feasible for patients who visit the outpatient clinic every week, in the first month after transplantation.

This proposed way of improvement of implementation of DBS should be studied. Inclusion of implementation specialists from the emerging field of implementation science in such a study is recommended.[40] One of the main aspects will be management of expectations from patients, pharmacists and physicians, since it will be likely that a >99% success rate cannot be achieved.

Sampling quality

Even if logistics can be organized perfectly, incorrect sampling by the patient will still result in no tacrolimus trough concentration available during the next outpatient visit. Sample quality and sampling procedures are therefore an important factor in DBS implementation.

Various studies have been performed on sampling performance by patients and researchers.[25,41,42] For patients using DBS for home sampling, rejection rates of samples because of insufficient sample quality of up to 20% are described. However, in **chapter 7**, the rejection rate of patient home-sampled DBS is only 4.9% which is comparable to the rejection rate of DBS samples obtained by trained phlebotomists. The patients that we included were all instructed by one experienced study coordinator, and the instruction protocol included practicing the DBS method by the patient while they were supervised by the study coordinator. In a research setting, a similar training method yielded a 0% rejection rate when trained phlebotomists were asked to perform the DBS sampling.[42] In **chapter 6**, we have shown that total absence of training results into rejection rates of up to 58%. This shows that training is the key factor in achieving a high rate of sample quality.

Various novel sampling devices have been introduced in the past years, which claim improved analytical performance and easier sampling by the patient. Examples include the Mitra© tip, The HemaXis DB device, Capitainer-B and HemaPEN.[8] However, they have rarely been tested in direct comparison to conventional DBS. In **chapter 10**, we have described such a comparison and we demonstrate that the Mitra© tip is inferior to conventional DBS sampling regarding both analytical performance and sampling quality.

Regardless of the sampling device, the person handling the device needs training as described earlier. If this is the case, the kind of sampling device becomes of lesser importance. Even for conventional DBS sampling, it is possible to achieve very low sample rejection rates, even when patients perform sampling at home. We developed an app to aid in judging the quality of a DBS. This app is described in **chapter 6**. The app can indeed contribute to improved sample quality. The benefits of the app are most prominent in a setting where training of people who obtain the samples is not

possible or not feasible. In situations where (repeated) training is possible, the app can serve as a way to identify patients who repeatedly fail to adequately perform DBS sampling. These patients can receive additional training, which will help improve their sampling performance.

Conclusive remarks

In this thesis, we described the steps necessary to implement Dried Blood Spot sampling of immunosuppressant TDM for transplant patients. This thesis shows that this is possible if:

1. The analysis method used for analyzing the DBS samples is fast, robust and meets all general and DBS-specific bio-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 in clinical practice.

3. It is likely that logistics can be optimized including 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.

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