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Implementing Dried Blood Spot sampling in transplant patient care

Veenhof, Herman

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Chapter 1

Introduction and outline

Transplantation

In 1954, the first successful kidney transplantation was performed. Since then, there has been an exponential worldwide growth in numbers of solid organ transplantations, which include kidneys, pancreas, lungs, livers, small intestines and hearts, of which kidney transplantation is performed most frequently. In the UMCG for example, 166 kidney transplantations were performed in 2018 and the total number of solid organ transplantations performed was 293. For patients suffering from end stage renal disease, the risk of premature death for kidney transplant recipients is less than half compared to dialysis patients.[1] Apart from reduced risk of premature death, quality of life is drastically improved for kidney transplant patients. Post-transplantation, patients can be free from symptoms like chronic fatigue, the need of multiple hour dialysis sessions 3 times a week and social isolation due to a chronic condition.[2]

One of the major concerns for kidney transplant patients is rejection of the allograft. Since the cells of the donated kidney differ genetically from the cells from the recipient, the recipients' immune system will perceive the donated organ as foreign and this can trigger an immune response.[3] If this response is not controlled, it will usually lead to the destruction of the transplanted organ.

Immunosuppressive drugs

With the introduction of immunosuppressive drugs, a tool to manage this immune response became available, greatly improving clinical outcomes for transplant patients. Treatment protocols including combinations of several immunosuppressants have reduced the first-year incidence of biopsy-proven acute rejections in kidney transplant recipients to 15% or less.[4] The most widely used immunosuppressant in allograft rejection prevention today is tacrolimus. This drug prevents activation and proliferation of T-cells and thereby reduces the immune response.[5] Usually, tacrolimus combined with mycophenolic acid and sometimes prednisolone is the treatment protocol of choice after transplantation.[1] Other immunosuppressants that are used, either in combination with or instead of tacrolimus are cyclosporin A, sirolimus and everolimus.[5] Because rejection of the transplanted organ is always a threat, treatment with tacrolimus and most other immunosuppressants is lifelong or until reinstallation of dialysis treatment.

Immunosuppressive drugs have three effects: (1) a therapeutic effect (suppressing a potential rejection), (2) undesired consequences of immunosuppression (mostly infections and cancer) and (3) non-immune-related toxicity such as nephrotoxicity.[5] Some of these side-effects have detrimental consequences and greatly reduce the

quality of life of transplanted patients. In the past decades, maximizing therapeutic effects while minimizing unwanted side-effects and toxicity has been one of the main focuses in transplant patient care.[1,4]

Therapeutic Drug Monitoring

In basic pharmacology, the effect of a drug is determined by the concentration of the drug at the target site. Ideally, the concentration of the drug in the blood is proportional to the dose of the drug and correlates with the concentration at the target site.[6] If this were true, a fixed dose of a certain drug would result in a predictable effect in every patient. However, this 'one-dose-fits-all' approach has shown to fail in treating transplant patients with immunosuppressants.[1,7] Clinical effects of immunosuppressants are dependent on the pharmacokinetics (PK) and pharmacodynamics (PD) of the drug.[4] PK parameters such as absorption, distribution, metabolism and excretion of the drug can greatly differ between patients and have shown to be of major influence on clinical results.[1,4,6] Many PD parameters for tacrolimus have been described, such as the association of low trough concentrations with increased graft rejection.[6] Currently, the exposure of an individual patient to tacrolimus best predicts clinical outcomes for this patient.[4] This exposure can be measured by obtaining and analyzing multiple blood samples over a period of 12 or 24 hours, depending on the drug formulation. From these mulitiple blood samples, a PK curve can be derived.[6] The Area Under the Curve (AUC) is currently the best method available to describe the exposure. PK studies demonstrated that the trough concentration (C_0 , concentration measured at the lowest point of the PK curve) correlates well with the AUC corresponding to that particular dose.[1] Therefore, in clinical care, dosing of tacrolimus is based on trough concentrations measured in whole blood obtained from a venipuncture.

In addition to varying PK and PD parameters of tacrolimus, target trough concentrations are different depending on time since transplantation. Early after transplantation, higher trough concentrations are targeted. Several months after transplantation, target trough concentrations are tapered. For all these targeted trough concentrations, the therapeutic window is narrow, which means that the difference between the lower and upper level of the window associated with optimal treatment is small. As a consequence, frequent measurement of trough concentrations of tacrolimus and other immunosuppressive drugs have been a cornerstone of transplant patient care for decades, to make sure that the dose results in a concentration in the therapeutic window. This process of repeated measurement of blood-drug concentrations and adjusting the dose accordingly is known as Therapeutic Drug Monitoring (TDM).[1,4]

Dried Blood Spot sampling

To perform TDM, patients frequently travel to the hospital for venous blood sampling. In general, TDM is performed weekly in the first month post-discharge after kidney transplantation. Over a period of approximately one year, the frequency is tapered to a 3-monthly visit which will last a lifetime in most cases. Given the time delay between blood sampling and availability of analytical results, the blood trough concentrations of immunosuppressants are usually not yet available when the physician sees the patient. This requires the patient to sample a few days earlier, or requires the physician to schedule another appointment (usually by telephone) to discuss the TDM results. For both patient and physician, this workflow is suboptimal.

Recently, a Dried Blood Spot (DBS) sampling method was developed that allows patients to sample at home.[8,9] In DBS sampling, 2 droplets of blood from a fingerprick are applied to a sampling card. After drying, the sample can be sent to the laboratory under ambient conditions using regular mail. From these blood spots, immunosuppressant blood drug concentrations can be measured.[10] Implementation of Dried Blood Spot home sampling can potentially lead to an improved workflow for physician and patient since immunosuppressant blood drug concentrations could be available when the patient is at the outpatient clinic. This could lead to improved patient quality of life as well as cost reduction.[11] In addition, the sampling method is minimally invasive and can be performed by patients at home.

The Dried Blood Spot analysis method was first introduced in 1963 by Guthrie to measure phenylalanine in neonates as part of phenylketonuria screening.[12] With the introduction of new, highly sensitive bioanalytical methods, mainly Liquid Chromatography combined with tandem Mass Spectrometry (LC-MS/MS), very small amounts (10-50 µL) of blood are needed to measure immunosuppressant blood drug concentrations.[8-10,13] Therefore, the use of DBS sampling and –analysis has increased in the field of TDM in the past 15 years.[9] Despite this increase, several challenges remain to be solved in the field of DBS sampling and –analysis.

Current challenges in Dried Blood Spot sampling

Analytical validation

Current DBS analytical methods are developed and analytically validated based on guidelines issued by the EMA and the FDA on bioanalytical method validation.[14,15] However, these guidelines are written for traditional matrices such as liquid

blood, plasma or serum and are not always easily translated to analyses of DBS. In addition, DBS specific parameters such as the effect of the hematocrit on spot formation are not discussed. Therefore, there is currently no optimal development and validation strategy for DBS analytical assays.

Clinical validation

Although many analytical DBS assays are described in literature, very few of them are tested in a clinical study.[16] For immunosuppressants, traditional venous whole blood sampling and analysis has been part of routine care for decades.[1,17] All PK/PD research, including establishment of relevant target trough concentrations is based on venous whole blood data. Therefore, results from a new analysis method (DBS) should be interchangeable with the reference method (venous whole blood).[18] Novel DBS methods should be tested in a clinical study comparing paired fingerprick DBS samples with conventional venous whole blood samples.[16,18] Although for some immunosuppressants, such as tacrolimus and cyclosporine A, these studies exist, they often have a small sample size and sometimes do not use fingerprick blood to produce DBS, but rather blood from a venously collected whole blood sample.[19-21] In addition, specific guidelines on sample size, appropriate statistical tests and study design are lacking.[16] Therefore, there is currently no optimal clinical validation strategy for TDM using DBS assays.

Implementation in clinical care

Because very few TDM DBS assays are used in clinical care, there are very limited data about the implementation of TDM DBS assays. Some studies have focused on the feasibility of DBS sampling regarding sample quality of DBS samples produced by patients.[22-26] Only one study focuses on feasibility and implementation of DBS home sampling for tacrolimus TDM, but this study lacked a control group.[22] Although DBS home sampling is perceived as a cost-saving tool, this has never been shown in a clinical study.[9,11] Therefore, there are currently no data on cost saving and implementation of TDM DBS assays.

Aim of this thesis

The aim of this thesis is to evaluate the implementation of Dried Blood Spot home sampling for immunosuppressant TDM in transplant patients. The evaluation consists of the analytical and clinical performance of the immunosuppressant DBS assay. Furthermore, costs, logistics, patient satisfaction and patient sampling performance are evaluated.

Outline of the thesis

In chapter 2, we plan to develop and analytically validate a multi-analyte DBS assay. This assay consist of the 5 small-molecule immunosuppressants that are currently most widely used in transplantation: tacrolimus, everolimus, sirolimus, cyclosporine A and mycophenolic acid.

In chapters 3 and 4, we will perform clinical validation studies, comparing paired fingerprick DBS samples and venous whole blood samples obtained from transplant patients for the drugs tacrolimus, cyclosporine A, everolimus and sirolimus. In addition, creatinine levels measured from DBS samples will be assessed.

In chapters 5 and 6, quality of DBS samples will be evaluated and discussed. In chapter 5 DBS quality criteria will be presented and applied to a large DBS sample set from four different countries. In chapter 6, a web-based application (app) capable of measuring DBS sample quality by means of taking a picture of the sampling card will be developed. The performance of this app will be tested on the DBS sample dataset from chapters 3 and 5.

In chapter 7 the effects, costs and implementation of DBS home sampling for tacrolimus TDM will be evaluated in a randomized controlled trial involving adult kidney transplant patients who will perform DBS sampling during the first 6 months post-transplantation. Patient satisfaction concerning DBS home sampling will be evaluated and discussed.

In chapter 8 a guideline is presented on the development, analytical and clinical validation and quality control of DBS methods for TDM. This guideline will discuss the DBS-specific parameters that are not discussed in general validation guidelines by the EMA and FDA.[14,15]

In chapters 9 and 10, a different micro-sampling device will be evaluated and discussed. The Mitra© tip is a Volumetric Absorptive Micro Sampling (VAMS) device designed to wick up an exact volume of blood (10 or 20 µL).[27] This approach could in theory mitigate hematocrit-related effects to volume as well as improve sample quality and result in an easier sampling procedure compared to DBS. The analytical validation of the VAMS assay will be presented in chapter 9. We will evaluate paired VAMS fingerprick samples, DBS fingerprick samples and conventional venous whole blood samples in a clinical validation study in chapter 10.

In chapter 11, a general discussion and the future perspectives of this thesis will be presented.

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