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Dried Blood Spot Sampling for Monitoring Children With Immune-Mediated Glomerulopathies and After Kidney Transplantation

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Introduction: Monitoring kidney function and immunosuppressant levels in children post–kidney transplantation or those with glomerulopathies is challenging due to frequent venipunctures and clinic visits. Capillary dried blood spot sampling (DBS) offers a potential alternative.

Methods: In this prospective single-center study, 89 children (38% female and 62% male) requiring therapeutic drug monitoring (TDM) and kidney function assessment were enrolled. Of the patients, 79% were kidney transplant recipients, and 21% had immune-mediated glomerulopathies. The mean age was 13.4 (range, 5.7–18.0) years. DBS and standard venous serum samples were collected simultaneously for tacrolimus (TAC), cyclosporine A (CsA), everolimus (EVR), and creatinine levels. Furthermore, patient feedback on pain perception and feasibility was collected via questionnaire.

Results: No significant differences in parameter values between DBS and standard methods were observed (creatinine, -1.7 ± 14.5 μmol/l; EVR, 0.1 ± 1.2 μg/l; TAC, 0.3 ± 1.1 μg/l; CsA, 2.8 ± 9.8 μg/l). DBS demonstrated sufficient accuracy compared with standard methods. Patients favored DBS and telehealth consultations, especially due to less travel and school absences. Patients preferred finger pricking over ear pricking.

Conclusion: Capillary DBS proves reliable for TDM and kidney function assessment in pediatric kidney disease. It reduces patient and family burden compared with venous blood collection and enables telehealth consultations.

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The monitoring of kidney function and levels of immunosuppressants is an essential component in the care of children after kidney transplantation or with immune-mediated glomerulopathies. The aim is to detect deterioration of kidney or allograft function early and to avoid either under- or over-immunosuppression. The former may promote allograft rejections and relapses of glomerular disease, whereas the latter may result in severe medication-associated side effects such as calcineurin toxicity in patients on TAC or CsA, leukopenia and diarrhea in patients on mycophenolate mofetil, stomatitis or dyslipidemia in patients on m-TOR inhibitors (EVR and sirolimus [SIR]), and infections or malignancies resulting in significant morbidity and mortality.[1–4] Regular, valid, and precise patient monitoring by a team of experts is the key to long-lasting graft or kidney function and the absence of relapses, especially in children.[1] Alongside immunosuppressant levels, serum creatinine is currently considered a key parameter for monitoring kidney and graft function. Deviations from baseline may indicate graft dysfunction and are thus highly relevant. Likewise, regular TDM is essential in the care of

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children with glomerulopathies on treatment with calcineurin inhibitors or mycophenolic acid (MPA), including those with frequent relapsing or steroid-resistant nephrotic syndrome.[5]

In everyday clinical practice, the standard procedure for monitoring the above-mentioned blood parameters in children post–kidney transplantation and children with glomerulopathies is conventional venipuncture. Commonly, patients' blood is taken from veins on the back of the hand, thus sparing deeper veins as long as possible for potential dialysis access.

This procedure contributes to increased psychological stress for children and can cause pathological changes in endothelial denudation.[6–8] Post–kidney transplantation, pediatric patients are usually scheduled for repeating laboratory and clinical check-ups at the outpatient clinic.[9] During the first year post operation, the check-ups are performed weekly. Later, they continue at monthly intervals.

The collection of predose samples of the immunosuppressant is typically performed in the mornings (before medication intake) at pediatric renal clinics. This is a burden to the patients and their families, because traveling to the check-ups is time-consuming. The children either miss school or kindergarten and their parents or accompanying guardians must take leave from work.

Considering the disadvantages of current TDM, a new standard method is urgently needed that is less time-consuming for patients and their families, less traumatizing for children, and less harmful for the veins of potential future dialysis patients.

Capillary DBS sampling, one of the most popular micro sampling techniques, is a potential alternative because it is easy to perform and inexpensive. First used in the early 1960s, DBS became known as a screening method for phenylketonuria and was a major milestone in the diagnosis of inborn errors of metabolism in newborns.[10] DBS can be collected by finger or ear pricks. Both methods are less invasive compared with venous punctures and require much smaller blood quantities (2–3 drops [∼25–30 μl] vs. 0.5–5 ml).

Previous studies on the use of capillary DBS for TDM in children are hampered by the small sample sizes, are limited to kidney allograft recipients, and/or lack the assessment of kidney function such as creatinine levels. This might partly explain why this method has not yet been implemented in the clinical setting or home environment.[11,12] Creatinine by means of liquid chromatography with tandem mass spectrometry determination has the advantage that only very small amounts of material can be used. Furthermore, this can be done in the same measurement together with the immunosuppressants. The classic determination requires more material and a separate method (Jaffe end point determination or kinetic). In addition, the method is more accurate, because it minimizes or excludes the influences of other parameters (bilirubin, hemoglobin, and others), as aptly shown in the candidate reference method.[13]

To address these issues, we performed a prospective single-center observational study on capillary DBS sampling as a method for monitoring of kidney or allograft function and TDM of common immunosuppressants in children after kidney transplantation and with immune-mediated glomerulopathies. The aim was to compare DBS sampling to the gold standard venous puncture EDTA-anticoagulated blood sampling in terms of feasibility, validity, and patient experiences.

METHODS

Study Population and Design

The patients participating in this study were enrolled at the outpatient clinic of the Department of Pediatric Kidney, Liver and Metabolic Diseases of Hannover Medical School, Germany. The inclusion criteria were age (1–18 years) and regular TDM of immunosuppressants (TAC, CsA, EVR and/or MPA) due to kidney transplantation or glomerulopathies, for example, frequent relapsing or steroid-resistant nephrotic syndrome.

In total, 89 out of 120 patients fulfilling the inclusion criteria could be enrolled between January 2022 and November 2022. All subjects were evaluated once.

The study received appropriate ethics committee approval from the Institutional Review Board of Hannover Medical School (No. 938_BO_S_2021) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all parents or guardians, with consent or assent from patients deemed appropriate for their age.

Laboratory Validation of DBS Compared With Venous Blood Samples for the Determination of Immunosuppressants and Creatinine Concentrations

Blood was drawn via phlebotomy and collected in an EDTA tube. Defined amounts of immunosuppressants and labeled d_5-creatinine were added. The latter was used to measure defined low creatinine concentrations, which is not possible with native creatinine due to unlabeled creatinine found in human blood. Half of the mixture was pipetted on a DBS card and dried in the dark for 3 hours at room temperature. Afterward, the DBS were stored at −20 °C until analysis. To create reference samples for creatinine and MPA in plasma, the remaining blood was centrifuged. The generated plasma was stored at −20 °C until analysis as well.

Validation measurements were performed by analyzing measurements consisting of 5 replicate sample injections into the mass spectrometer of each concentration level on 4 consecutive days.

Patient DBS Sample Collection and Storage

Patients' blood was collected before the morning dose of the immunosuppressive drug as a predose sample. The prick technique was first demonstrated by the doctor in the outpatient unit and then implemented by the parents or patients if they felt confident enough to do so. The CsA, TAC or EVR, and creatinine values were determined from capillary blood from the fingertip or earlobe via a DBS. For this purpose, the ear lobe or fingertip was cleaned with disinfection wipes. The skin was then punctured using a sterile surgical lancet (Terumo/Medisafe FINETOUCH). The first blood drop was wiped off and 3 drops of capillary blood were placed on the DBS card. To avoid contamination with tablet residues, attention was paid not to touch the card. Within 5 minutes after the finger prick, a venous EDTA-anticoagulated blood sample was taken to serve as a standard control. DBS samples were stored for a minimum of 3 hours in a dry and sunlight-protected place, collected, and mailed in a paper envelope to the laboratory (Screening-Labor Hannover) once a week.

Preparation and Technical Procedures

We modified the Chromsystems MassTox kit to detect and quantify immunosuppressants and creatinine in dried blood spots by liquid chromatography with tandem mass spectrometry. Validation measurements were carried out by measuring blood spiked with known immunosuppressant concentrations, as well as by comparing measurement results of dried blood spot analyses to serum analyses of the same blood sample. Further details on the preparation of DBS samples and controls, preparation of calibrators, plasma samples, and plasma controls, chromatography and MS/MS analysis, as well as the applied reagents and chemicals are displayed in the Supplementary Material S2.

Online Survey

All patients enrolled in this study were requested to take part in an online survey. The survey contained 13 questions evaluating the clinical feasibility and patient satisfaction with the new DBS method (Online Questionnaire, Supplementary Material S3).

Statistical Analysis

Validation data were evaluated, and lower limits of quantification (LLOQs) were determined with Abacus (version 3.0, LABanalytics, Jena, Germany). Clinical data were recorded in an Excel 2016 spreadsheet (Microsoft Corporation, Redmond, WA) and analyzed and plotted with SPSS Statistics Version 28.0.1.1 (IBM, Armonk, North Castle, NY) or R v4.3.2 (R Core Team, Foundation for Statistical Computing, 2022). Mean differences of measurements via capillary DBS analysis and a standard sample via venous access were compared according to Bland-Altman (mean bias, limits of agreement, precision [SD of the bias]).

RESULTS

Validation of the Method Using Spiked Dried Blood Samples

To validate our method, we spiked DBS samples with defined concentrations of immunosuppressants (CsA, EVR, MPA, TAC, and SIR) and isotopically labeled d_5-creatinine, and injected every concentration level 5 times on 4 consecutive days. The results of DBS samples were compared with expected target concentrations (Figure 1 and Supplementary Table S1). Concentrations measured in DBS were in good accordance with the immunosuppressants we spiked into the samples. Only SIR showed larger deviations of up to ± 30% from the expected target values at the lowest concentration. We calculated the LLOQ values for each drug using Abacus Method Validation Software. Each compound was measured with at least 4 replicates at different low concentrations. The LLOQs defined as the lowest concentration with a relative squared deviation from the target value <20% were as follows: creatinine, 7.9 µmol/l; CsA, 37.3 µg/l; EVR, 1.1 µg/l; MPA, 0.9 mg/l; SIR, 2.9 µg/l; TAC, 1.2 µg/l. All LLOQs were in good accordance with previous studies assessing LLOQs for these variables. Taken together, the data indicate that our method allows the determination of immunosuppressant drugs and creatinine in dried blood spots with high precision, even at low concentrations.

Patient Characteristics

The clinical characteristics of 89 patients (35 female and 54 male) are shown in Table 1. The mean age was 13.1 (range, 3.8–18.2; IQR, 5–58) years. Of those, 69 patients were kidney transplant recipients, and 20 patients had immune-mediated glomerulopathies.

Comparison of DBS Measurements With the Standard Method of Venous Blood Samples

In total, we measured 214 predose pairs of capillary and venous samples of immunosuppressants and creatinine. Twenty-five samples were excluded due to a lack of proper material. Creatinine ($n = 81$ pairs), TAC ($n = 29$ pairs), EVR ($n = 36$ pairs), and CsA ($n = 43$ pairs) remained for analysis. SIR was

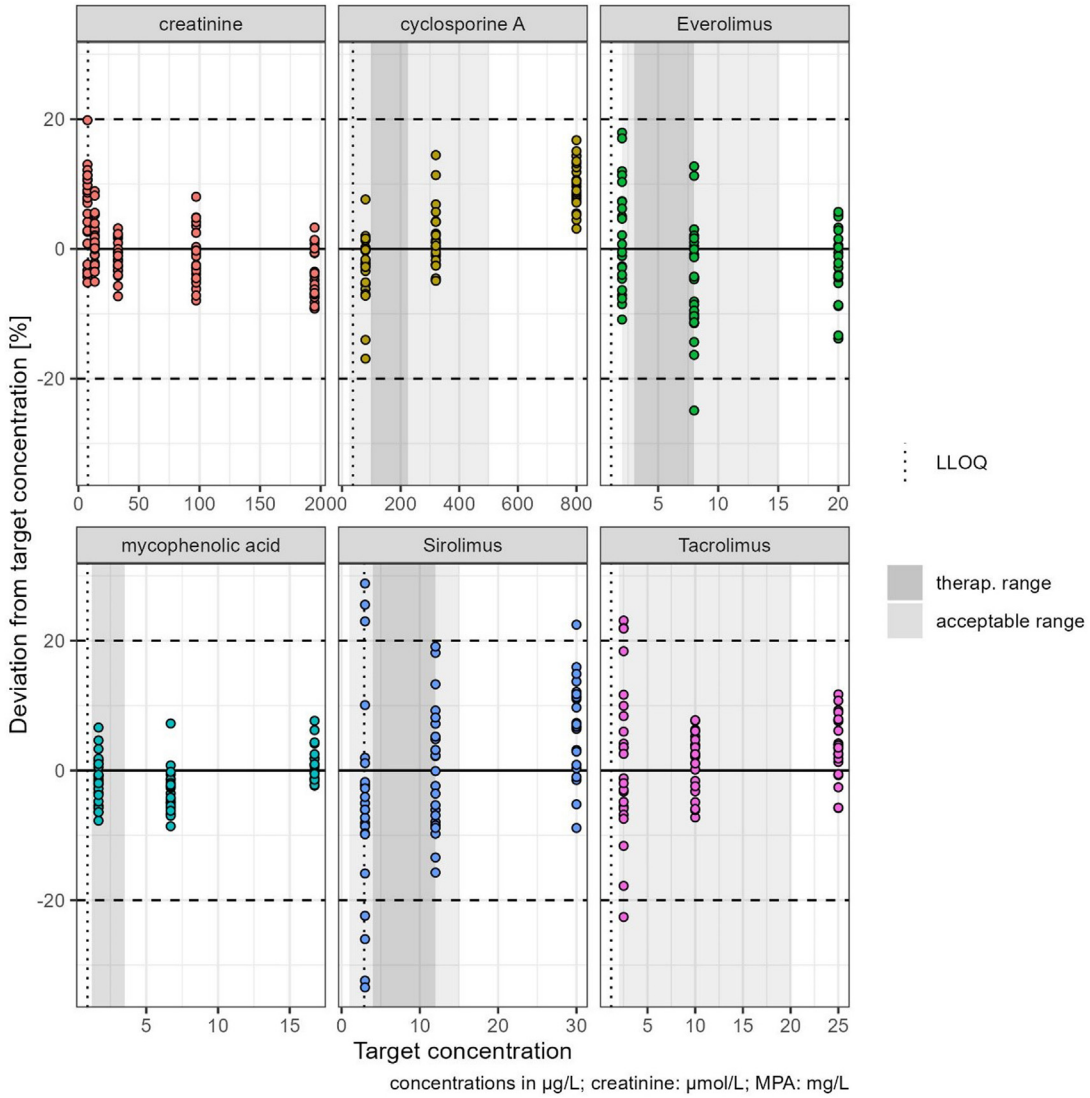

Figure 1. Comparison of DBS analyses with expected target concentrations. DBS, dried blood spot sampling; LLOQ, lower limits of quantification; MPA, mycophenolic acid.

measured only twice in patients and thus not eligible for further analysis. The venous material for creatinine was plasma and not EDTA blood, as for the immunosuppressant drugs.

Table 1. Patient characteristics

Baseline characteristics	Data
Participants	$N = 89$
Age, yr	13.1 ± 4.1
Sex (female, male, unknown)	Female: $n = 35$ Male: $n = 54$
Number of samples	Creatinine: $n = 81$ TAC: $n = 29$
	EVR: $n = 36$
	CsA: $n = 43$
Reasons for the use of immunosuppression	KTx: $n = 69$
	Glomerulopathy: $n = 20$
Hemoglobin	11.9 ± 1.3 g/dl
Hematocrit	35.7% ± 3.9%

CsA, cyclosporine A; EVR, everolimus; KTx, kidney transplant; TAC, tacrolimus.
Data are given as mean ±SD or total numbers.

The mean difference in creatinine concentrations between the dried capillary microsample and the liquid venous sample was −1.69 ± 14.56 µmol/l (Figure 2). A Bland-Altman plot (plot of difference) revealed that the mean difference in capillary blood samples was 1.22% lower (± 16.73%) on average than in the corresponding blood samples (95% confidence interval [CI], −4.92% to 2.48%) (Figure 3). A Passing-Bablok regression analysis provided estimates of intercepts of 0.45 (95% CI, −0.3% to 4.18%) and slope of 0.97 (95% CI, 0.92%–1.03%) (Figure 4).

The mean difference in TAC concentrations between the dried capillary microsample and liquid venous sample was 0.3 ± 1.06 µg/l (Figure 5). The Bland-Altman plot (plot of difference, Figure 6) also revealed that the mean (±SD) difference in capillary blood samples was lower by 4.3% (± 19.6%) on average than in the corresponding blood samples (95% CI, −3.11% to 11.79%). The Passing-Bablok regression

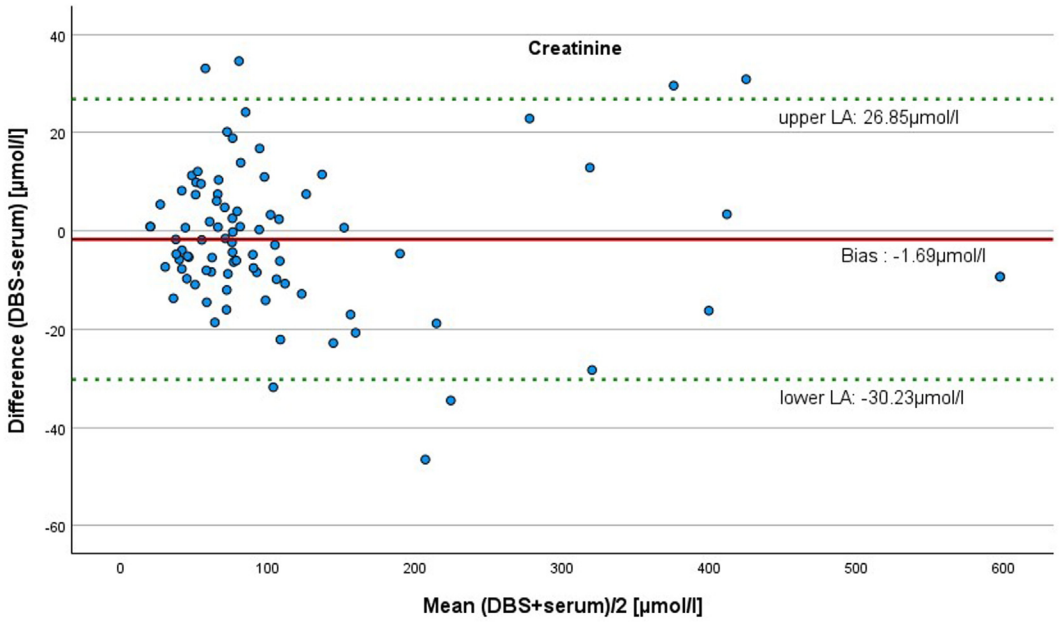

Figure 2. Comparison of creatinine concentrations. DBS, dried blood spot sampling; LA, limits of agreement (−30.69 to 26.85 µmol/l).

analysis provided estimates of intercepts of 0.54 (95% CI, −1.62% to 0.31%) and slope of 1.19 (95% CI, 0.99–1.44%) (Figure 7).

Between the dried capillary microsample and the liquid venous sample, the mean (±SD) difference in EVR concentrations was 0.01 ± 1.22 µg/l (Figure 8). The Bland-Altman plot (plot of difference) revealed that the mean difference in capillary blood samples was 1.1 % (± 25.16%) lower than in the corresponding blood samples (95% CI, −9.59% to 7.44%). The difference plot is shown in Figure 9. The Passing-Bablok regression analysis provided estimates of intercepts of 0.05 (95% CI, −1.38% to 0.87%) and slope of 0.94 (95% CI, 0.72–1.28%) (Figure 10).

The mean (±SD) difference in CsA concentrations between the dried capillary microsample and the liquid venous sample was 2.75 ± 9.83 µg/l (Figure 11). In addition, the Bland-Altman plot (plot of difference) revealed that the mean difference in capillary blood samples was higher by 4.9% (±14.71%) compared with the corresponding blood samples (95% CI, 0.4%–9.45%) (Figure 12). As shown in Figure 13, the Passing-Bablok regression analysis provided estimates of intercepts of 1.29 (95% CI, −11.18 to 11.6 %) and slope of 1.03 (95% CI, 0.85% to 1.23%) (Figure 13).

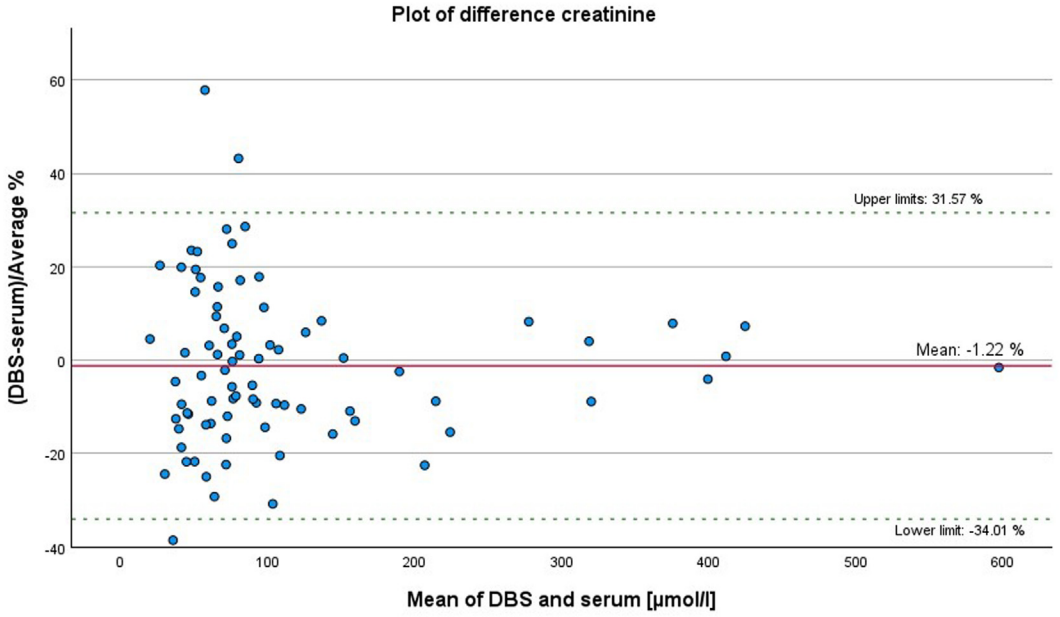

Figure 3. Plot of difference creatinine. DBS, dried blood spot sampling; LA, limits of agreement (−34.01 to 31.57%).

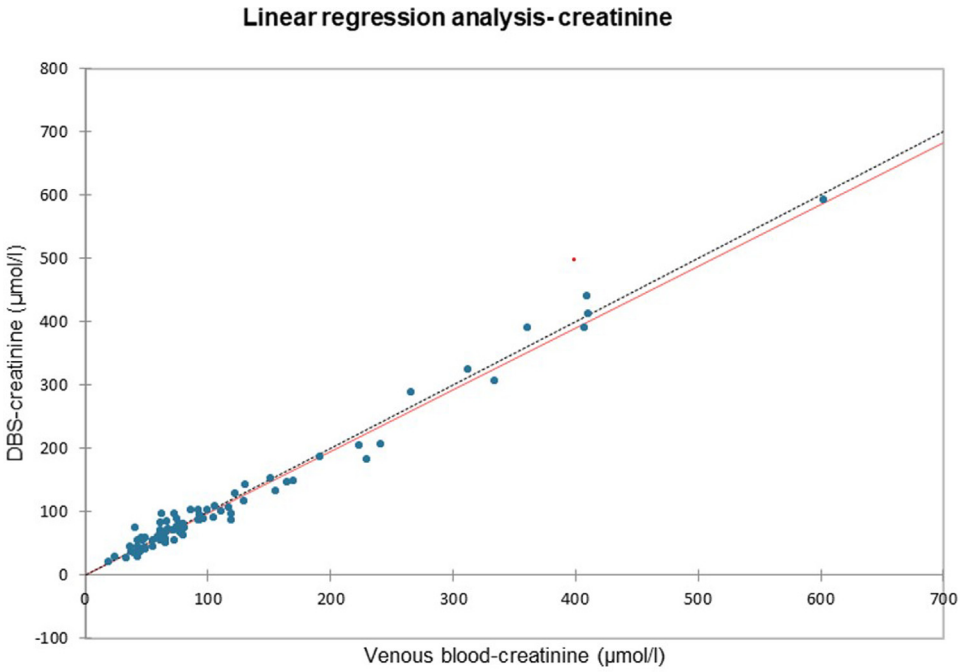

Figure 4. Linear regression analysis creatinine. DBS, dried blood spot sampling.

Online Survey

The online survey revealed that 27 out of 29 patients and parents or guardians (93%) felt confident in taking a DBS at home. Most patients (79%) stated they would prefer being pricked by a parent. In comparison to the earlobe prick, 70% of patients preferred the finger prick. However, most patients only tested the finger prick. The most frequently mentioned benefits of using capillary DBS versus regular outpatient visits were regular school attendance (50%), more time for playing with friends (23%), and getting more sleep (11%) (Figure 14).

The level of experienced pain caused by the prick was assessed by the participants via the Visual Analogue Scale with pain levels reaching from 1 to 10. Of the patients, 32% reported no pain (level 1 of 10), the majority reported mild pain levels (32% reported level 2/10, 24% reported level 3/10, 12% reported level 4/10). No patient reported a pain level above 4/10. (Figure 15).

DISCUSSION

In this single-center prospective study, we evaluated the feasibility and validity of capillary DBS

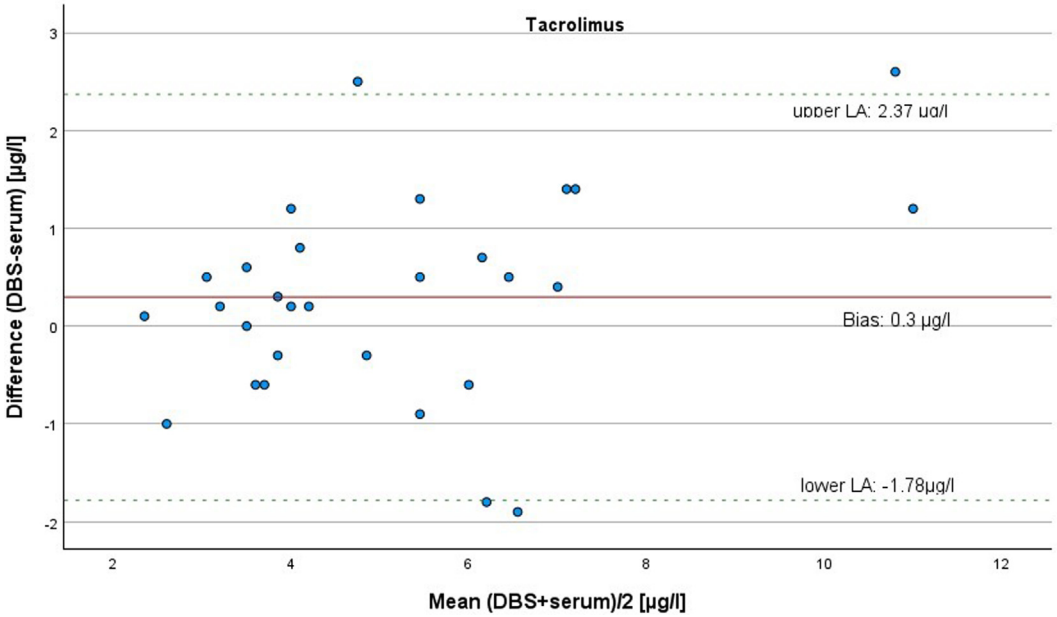

Figure 5. Comparison of TAC concentrations. DBS, dried blood spot sampling; LA, limits of agreement (−1.78 to 2.37 µg/l); TAC, tacrolimus.

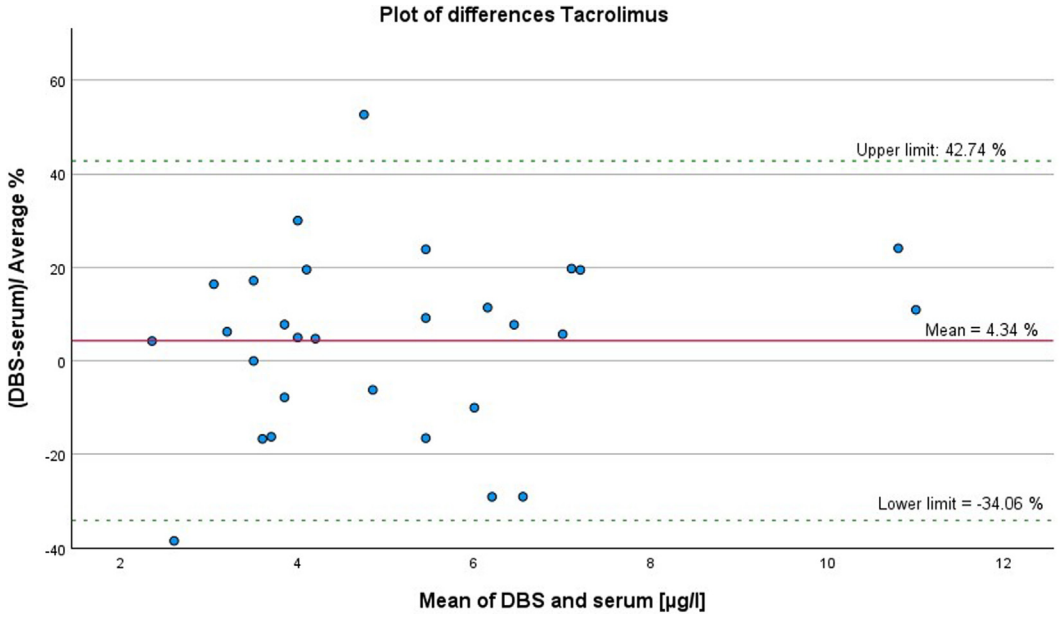

Figure 6. Plot of differences TAC. DBS, dried blood spot sampling; TAC, tacrolimus.

measurements to monitor kidney function and the levels of common immunosuppressants in a cohort of children after kidney transplantation and with glomerulopathies. We demonstrated that DBS measurements provide reliable results compared with standard venous blood sampling measurements. We further evaluated patient experience and found that the DBS method enhances the patients' quality of life.

Previous Research on DBS

Various studies and reviews have been published with encouragingly consistent results comparing capillary DBS with venous blood collection.[14–21] These include the monitoring of immunosuppressant medication after solid organ transplantation.[11,12,22–34] Nevertheless, the method has not yet been implemented in everyday clinical practice.

Among the documented advantages are the small amount of required material; the simplicity of handling, storage, and shipment of the DBS samples; and the reduced costs and efforts for patients and the health care system.[11,18,22,35] Moreover, capillary blood collection is particularly attractive for patients with impaired kidney function who are continuously dependent on their vascular health status, that is, for the placement of a fistula for dialysis.[6–8]

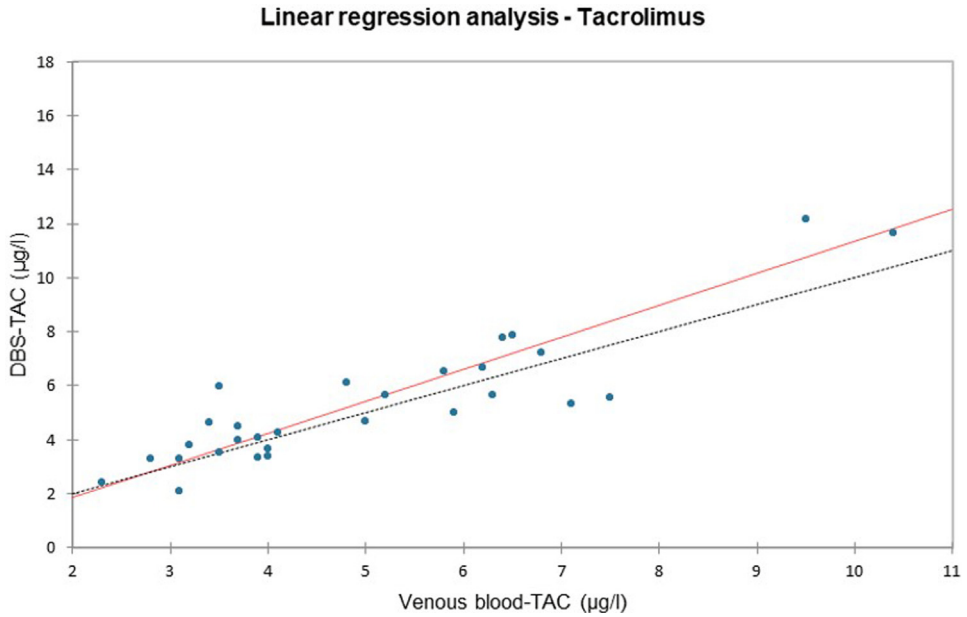

Figure 7. Linear regression analysis TAC. DBS, dried blood spot sampling; TAC, tacrolimus.

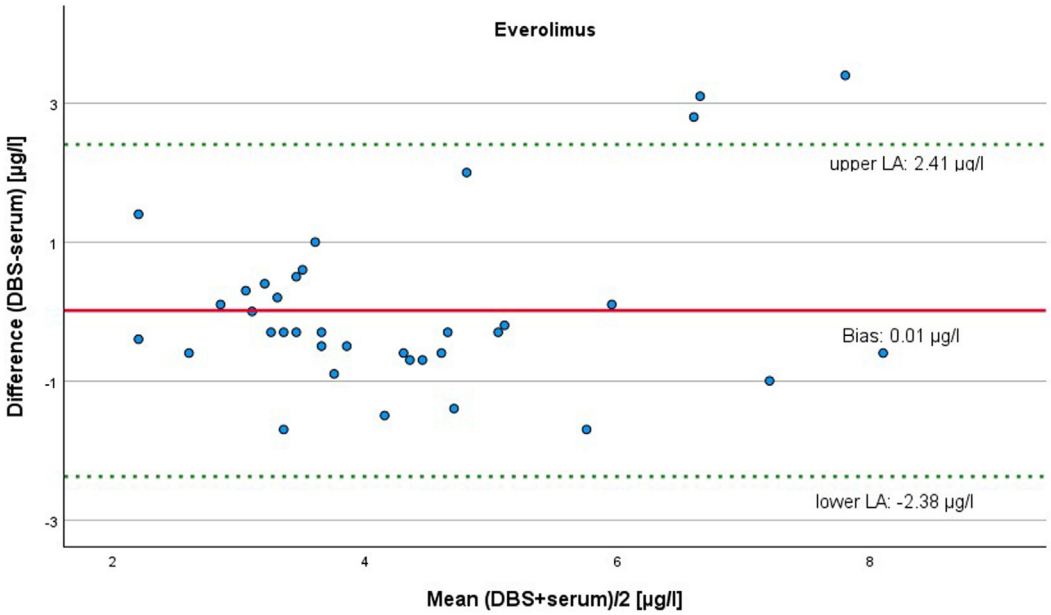

Figure 8. Comparison of EVR concentrations. DBS, dried blood spot sampling; EVR, everolimus; LA, limits of agreement (−2.38 to 2.41 µg/l).

The reported disadvantages of DBS are mainly pre-analytical problems and sampling issues. Performing a finger prick correctly to generate evenly distributed blood spots with sufficient material can be challenging, especially in pediatric patients. Potential challenges discussed in the literature are differences regarding volume and hematocrit (Hct) due to divergent performance of the finger prick. Further, accuracy can be an issue because patients might not accurately report the time of collection and shipment issues could lead to unforeseen delays of the samples.[15,19,36,37]

In previous studies, DBS cards were not only compared with blood samples but also with different variations of analysis and collection methods (MITRA, etc).[14,26,36,38,39] In almost all studies, statistical agreement and high patient satisfaction with the DBS outweigh the disadvantages.[11]

Study Design and Clinical Implications

Our research goals were to test the reliability of the individual measurements, compare the DBS card with the standard blood sample, and investigate the practicability in the clinic and patients' experiences. Accordingly, we addressed these goals with 3 different research settings.

First, multiple artificially enriched samples of the same blood sample collected by DBS in the laboratory

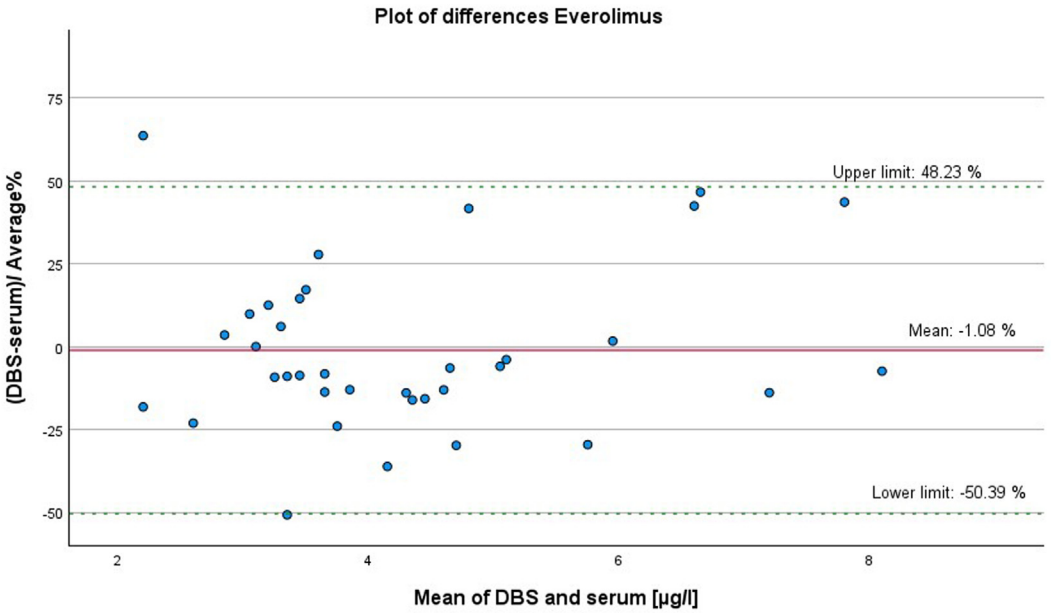

Figure 9. Plot of differences EVR. DBS, dried blood spot sampling; EVR, everolimus.

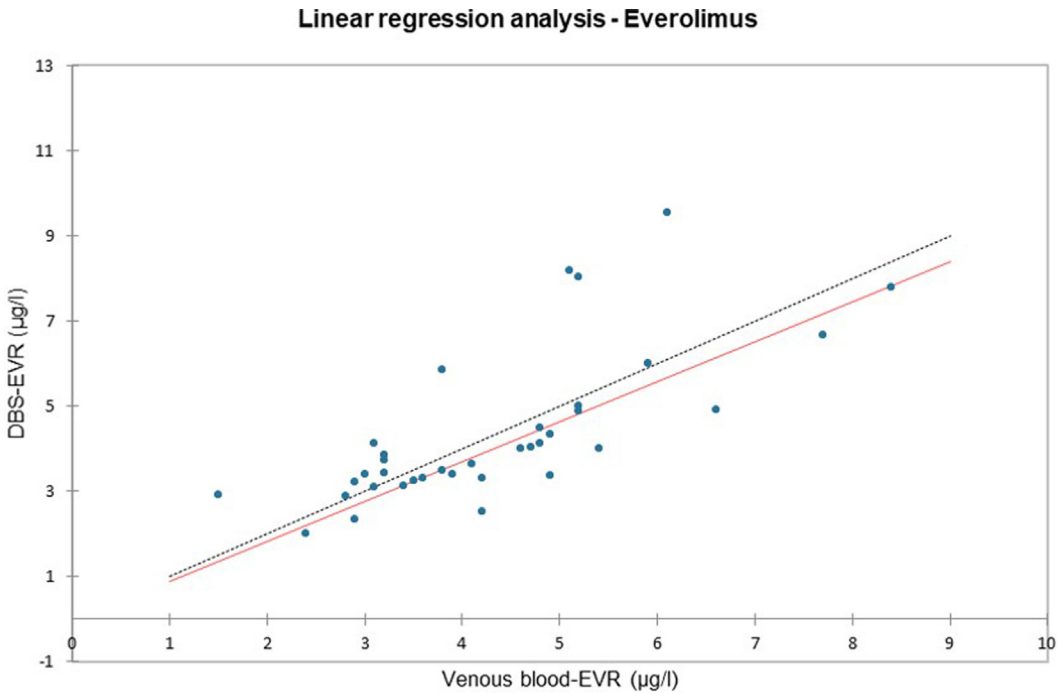

Figure 10. Linear regression analysis EVR. DBS, dried blood spot sampling; EVR, everolimus.

were tested and compared with known target concentrations. The results showed that DBS sampling was highly accurate and precise, with low variation even at low concentrations and small lower levels of quantification, which are well within the acceptable range for clinical laboratory testing (Figure 1).

Second, we compared DBS sampling to blood sampling in a clinical setting with 89 pediatric patients with kidney disease. We were able to confirm a strong correlation for TAC, EVR, and creatinine; and a moderate correlation for CsA between DBS (liquid chromatography with tandem mass spectrometry) and standard venous samples. Mean creatinine concentrations in DBS were 1.2% lower (mean of difference) on average compared with blood samples (95% CI, 31.6% to −34%). Regarding the immunosuppressive predose levels, the mean concentration differences were 4.3% higher for TAC (95% CI, 42.7% to −34%), 1.1% lower for EVR (95% CI, 48% to −50%), and 4.9% higher for CsA (95% CI, 33.8% to −23.9%) (Figures 3, 6, 9, and 13). Various studies support these results.[27,40,41]

Despite difficulties in obtaining standardized blood samples due to the children's age and understanding,

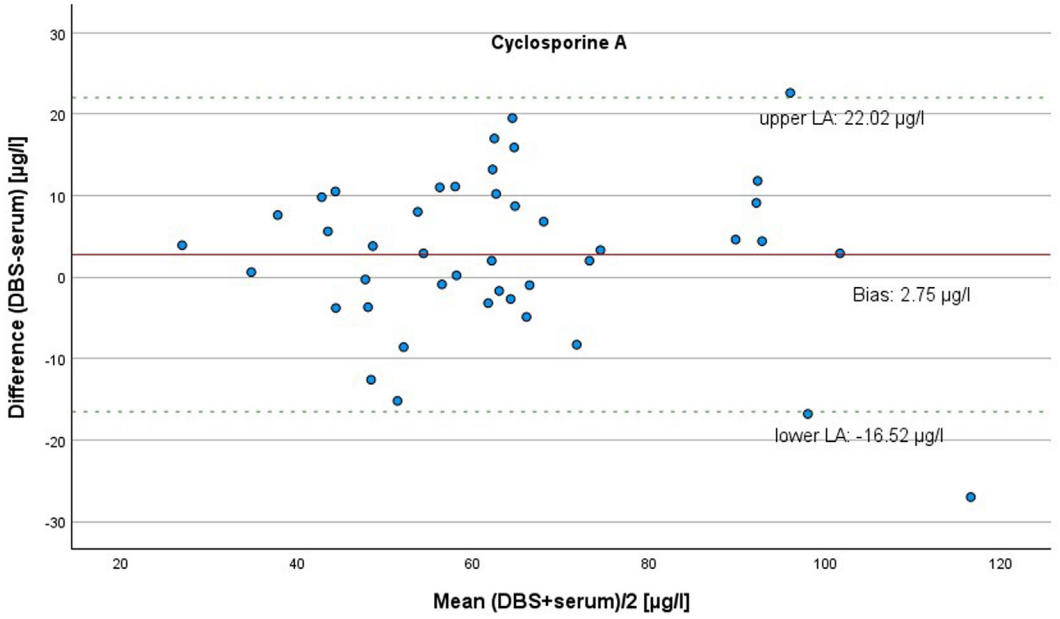

Figure 11. Comparison of CsA concentrations. DBS, dried blood spot sampling; CsA, cyclosporine A; LA, limits of agreement (−16.52 to 22.02 µg/l).

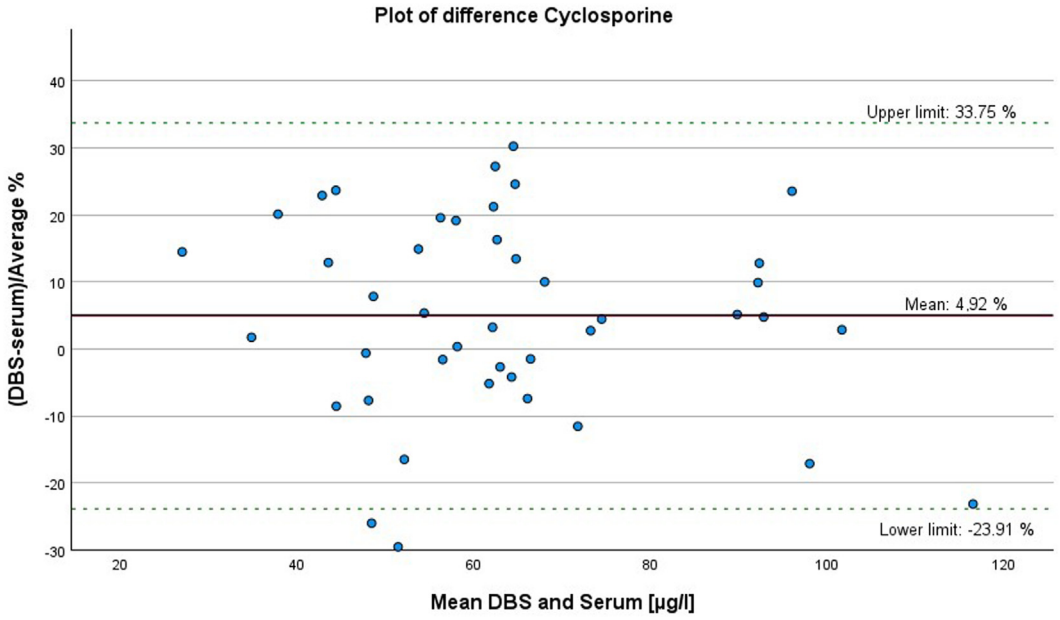

Figure 12. Plot of differences CsA. CsA, cyclosporine A; DBS, dried blood spot sampling.

we encountered no major preanalytical challenges due to the quality of samples. Potential quality issues could have been caused for example by too few drops of blood on the card, uncleaned fingertips, contamination with other drugs, or high levels of sunlight during the drying time. However, we did not encounter quality issues with the stability of analytics, the volume and homogeneity of blood spots, or the influence of the Hct.[19,36,42] This has also been confirmed in other studies.[23,29]

As published previously, the influence of Hct on immunosuppressant concentrations is negligible at physiological Hct concentrations of 20% to 60%.[23] Hct concentrations were measured at 3-month intervals to ensure that the concentrations were within the range of 20% to 60%. For feasibility control, the plasma samples were sent for analysis to a different laboratory than the DBS cards. Veenhof *et al.*[43] showed a great interlaboratory variation for current micro sampling methods compared with whole blood methods.[43] However, in our case, there was no significant difference between the results of the venous samples in the 2 different laboratories.

Third, we conducted an online survey to investigate the patients' experiences. The survey confirmed the expected benefits of the DBS method for patients and

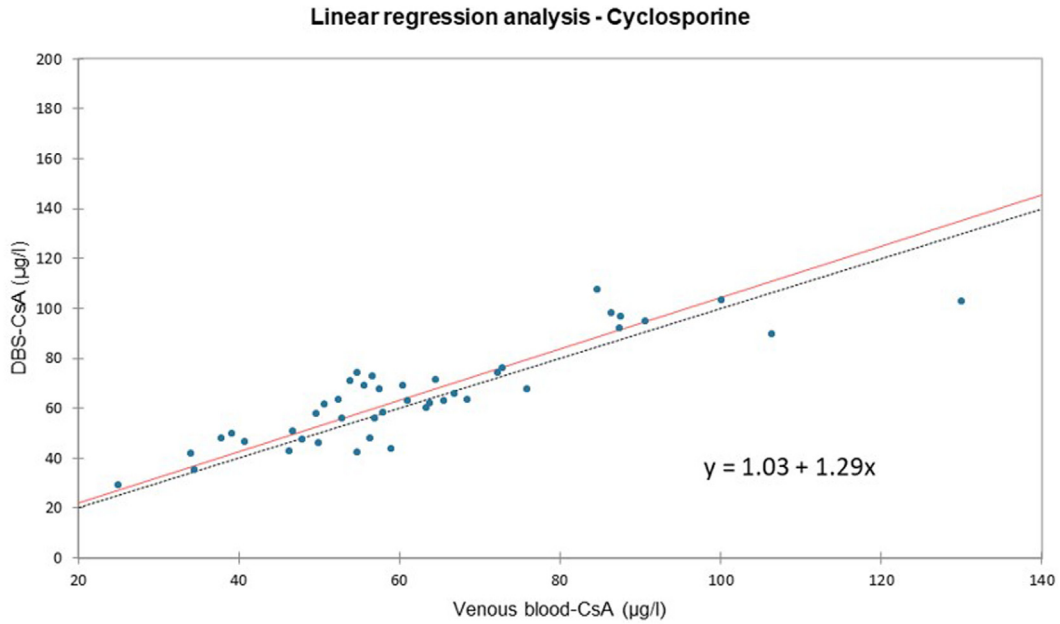

Figure 13. Linear regression analysis CsA. CsA, cyclosporine A; DBS, dried blood spot sampling.

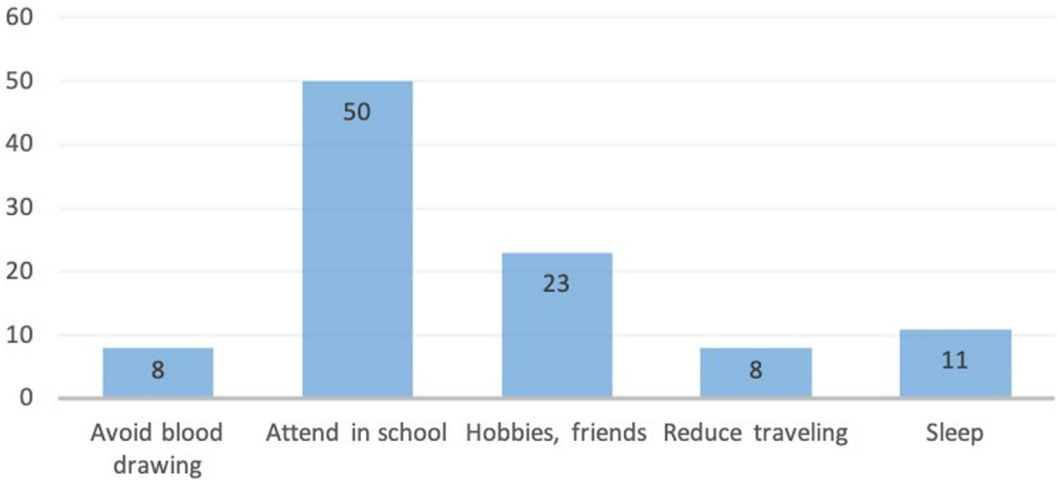

Figure 14. Reasons for DBS. DBS, dried blood spot sampling.

their families. Half of the patients reported that the main advantage of home monitoring was saving school days; a quarter mentioned meeting with friends (25%). According to our survey, the home monitoring reduced stress, pain, time, and costs for patients, which aligns well with similar studies.[27,35]

Limitations and Further Research

This study focuses on the feasibility of DBS in the context of pediatric patients. Due to its scope and research interest, several shortcomings can be identified that should be addressed in further research.

First, the scope of analysis could be extended. It was planned to include MPA in the laboratory analysis and in patient control as well. However, due to patient dropouts, the data were too limited; thus, it was excluded from the patient controls. A larger study with more patient data should include MPA as well.

Second, training for patients, both in clinical settings and the home environment, need to be elaborated and standardized. It would then be possible to observe whether patient training has a relevant impact on the sample quality (e.g., accurate timing), or whether other processes that might diminish the quality (e.g., transport issues) could be optimized.

Third, the impact of telemedicine on home monitoring needs to be further researched. The patients included in our survey all mentioned to be confident to carry out in-home monitoring. However, the setup of continuous home monitoring also poses challenges and the implementation of tele visits has not yet been integrated into everyday clinical practice.[11,44]

Finally, our survey indicates more patient satisfaction with DBS. It has been argued that the DBS method could lead to a normalization of children's everyday lives due to fewer travels and fewer school absences.[38] We propose that a method which is more comfortable and less time-consuming for patients could also lead to better adherence, which would help to reduce the risk of graft loss. This would be a major benefit of DBS,

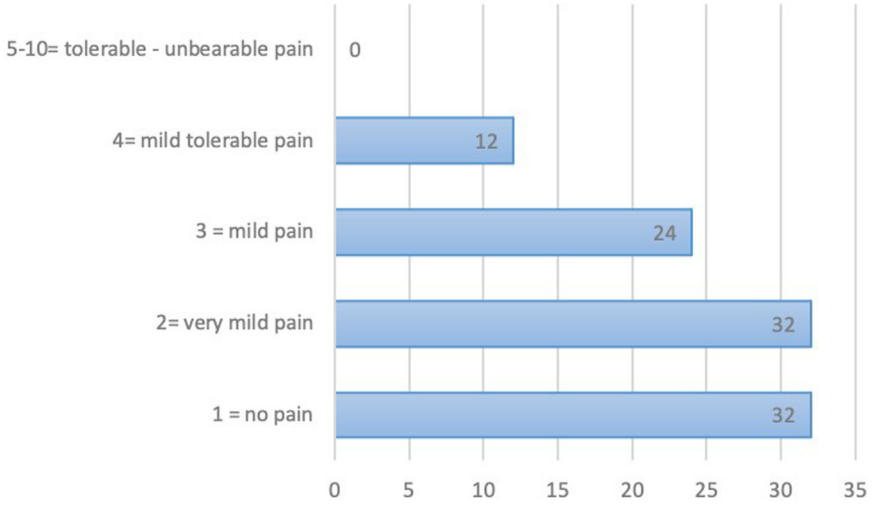

Figure 15. Patients' pain levels.

because nonadherence is a well-described problem in pediatric patients, especially during adolescence, due to family-, socio-economic-, condition-, healthcare- and therapy-related factors.[45,46] It thus needs to be observed in longitudinal studies whether DBS actually improves adherence.

To address further potential practical issues, we propose a trial run on initially stable patients without major adherence problems. The patients should be thoroughly trained beforehand. A protocol for carrying out and sending the dry blood card is required.[42,47] Potential further challenges that might appear in the home setting concerning family issues or telehealth should be closely observed via questionnaires or patient interviews.

Conclusions

Capillary finger-prick monitoring of creatinine and immunosuppressant levels via DBS is a simple, practical, and accurate method. It can precisely measure concentrations in artificially created samples and shows a good correlation with standard venous blood sampling in a pediatric cohort. Interval home monitoring could be the next step to reduce school and work absences for children and their families and help to reduce time, costs, and the psychological stress of outpatient visits. The method promotes self-management and social integration and could thus lead to better adherence. This would be a major advantage, because poor adherence is one of the main reasons for graft loss in adolescent patients. Further, the children's anxieties and trauma caused by venous blood sampling could be avoided. In addition, damage to the vessel walls can be reduced, which is important for patients needing future dialysis access.

Although statistically the methods show good agreement in terms of reproducibility and comparability, the range of variation must be assessed in clinical use.

However, the advantages outlined above outweigh the difficulties.

As further steps, we recommend the implementation of home monitoring in a group of stable patients with good adherence. They should be provided with comprehensive training regarding blood collection and shipment to limit preanalytical measurement failures. Proper protocols and quality control measures must be in place to ensure accurate and reliable results before offering DBS sampling to all patients.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

The study was designed by NK and NJ. MG and LB collected samples and data. BB and MT carried out laboratory measurements and helped with the initial manuscript. LB made the statistical analysis and drafted the initial manuscript. NK, NJ, and DH supervised data collection, laboratory measurements, and statistical analyses; and critically reviewed and revised the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Table S1. Multiple reaction monitoring (MRM) transitions for each compound.

Supplementary Material S2. Technical procedures.

Supplementary Material S3. Online Questionnaire.

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