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Tacrolimus Measured in Capillary Volumetric Microsamples in Pediatric Patients—A Cross-Validation Study

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Background: Therapeutic drug monitoring of tacrolimus (Tac) is mandatory in solid organ transplant (SOT) recipients. Finger-prick microsampling is more flexible and tolerable during the therapeutic drug monitoring of tacrolimus and has been shown to be applicable in adult SOT recipients. In this study, a previously validated method applying volumetric absorptive microsampling (VAMS) to measure Tac in adults was cross-validated in a pediatric population.

Methods: Patients with SOT scheduled for standard posttransplant follow-up visits were recruited. Blood samples were obtained by trained phlebotomists using standard venipuncture and capillary microsampling, before the morning dose of Tac as well as 2 and 5 hours after dosing. Tac concentrations were quantified using liquid chromatography–tandem mass spectrometry. Concordance between Tac concentrations obtained with venipuncture and VAMS was evaluated using Passing–Bablok regression, calculation of absolute and relative differences, and percentage of samples within $\pm 20\%$ and $\pm 30\%$ difference.

Results: A total of 39 SOT patients aged 4–18 years (22 male) were included. The median (range) predose venous blood concentration was 4.8 (2.6–13.6) mcg/L, with a difference between VAMS and venous blood samples of -0.2 ± 0.7 mcg/L. The relative mean difference was -1.3% [95% confidence interval (CI), -5.9% to 3.4%]. Ninety-two percent and 97% of the sample pairs demonstrated differences within $\pm 20\%$ and $\pm 30\%$, respectively. Postdose (2 hours and/or 5 hours, $n = 17$) median concentration in venous blood was 7.9 (4.8–19.2) mcg/L. The difference between VAMS and venous blood samples was 0.1 ± 1.0 mcg/L, with a relative mean difference of -2.5% (95% confidence interval, -8.8% to 3.8%). Eighty-eight percent of

the postdose sample pairs were within $\pm 20\%$ difference, and all were within $\pm 30\%$ difference.

Conclusions: Tac concentrations can be accurately measured using VAMS technology in pediatric SOT recipients. This makes home-based Tac monitoring feasible in the pediatric population.

Key Words: tacrolimus, microsampling, therapeutic drug monitoring, transplantation, pediatric

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INTRODUCTION

Solid organ transplantation (SOT) is the preferred treatment for patients with end-stage organ diseases. Transplanted patients undergo lifelong immunosuppressive treatment accompanied by therapeutic drug monitoring (TDM). Currently, a combination of tacrolimus (Tac), mycophenolate, and steroids is the preferred regimen for maintenance of immunosuppression to prevent allograft rejection in adults and pediatric transplant patients at most transplant centers around the world.

Tac has a narrow therapeutic window.¹ In general, the trough concentration aimed for is between 3 and 15 mcg/L, depending on individual patient factors. Excessive exposure is associated with an elevated risk of hypertension, posttransplant diabetes mellitus, neurotoxicity, and nephrotoxicity, whereas too low levels can lead to acute graft rejection and development of donor-specific antibodies.² TDM during the entire life span of SOT recipients is mandatory to individualize immunosuppressive treatment.³ In the early posttransplant phase in particular, frequent blood sampling, followed by dose adjustments, is required to ensure graft function and minimize potential adverse effects. In addition to the time-dependent changes in Tac pharmacokinetics during the first posttransplant year, Tac dose requirements may be affected by growth and development in the pediatric population,⁴ prolonging the intensive monitoring phase in this population. TDM is therefore indicated at least every 3 months in pediatric patients and in many situations more often. In addition, TDM is an important tool for adherence assessment in this age group.

Currently, blood is collected for Tac TDM in children and adolescents using standard venipuncture or capillary sampling (usually 0.5 mL) to measure trough concentration. A venipuncture procedure is perceived as a stressful event associated with pain in many pediatric patients. At our center, we recently validated a novel capillary technique to measure

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The authors declare no conflict of interest.

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Tac concentrations in adults using the Mitra sampling device.⁵ Mitra uses volumetric absorptive microsampling technique and absorbs an accurate volume (eg, 10 μ L) onto a hydrophilic polymer tip in which the blood is dried. This microsampling can be performed at home by the patient or caregiver, timed according to dose administration, and sent to the hospital before their next prescheduled appointment where the remaining blood test can be performed if required. Tac in the dried microsample is stable in this device for at least 1 month, and it may be shipped with standard mail service to the laboratory.⁵ This can minimize the time spent at the hospital for clinical follow-up visits and is probably more cost-effective for patients, families, and health care providers.⁶ An additional benefit of home-based microsampling is that area under the curve (AUC)-based Tac TDM is clinically applicable because extended stays at the hospital are not needed to collect the necessary samples for proper AUC estimation.² With just a blood drop needed, microsampling is more convenient for children and adolescents, especially when it can be performed in safe and known environments of their own homes. Considering the importance of accurate determination of Tac concentrations for TDM, it is important to validate this novel methodology in pediatric patients before application in the clinic.

Our study aimed to validate Tac concentrations obtained with capillary absorptive microsampling using the Mitra device in pediatric SOT recipients (kidney and liver) to determine possible differences in children and adolescents as compared to adults. The concentration of Tac obtained using this novel capillary microsampling technique was compared with that of samples collected through standard venipuncture. Capillary sampling is commonly preferred in the pediatric population because it is minimally invasive and requires small blood volumes. Validation was performed using predose (trough concentrations) and postdose samples.

MATERIALS AND METHODS

Study Population

From November 2019 to June 2020, we recruited pediatric transplant recipients (kidney or liver) who came for prescheduled follow-up visits at our outpatient transplant center at Oslo University Hospital, Rikshospitalet, Norway. Patients aged 2–18 years, with tacrolimus as part of their immunosuppressive regimen, were invited to participate. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and Good Clinical Practice. This study was approved by the Regional Committee for Medical Research Ethics in Norway (REK number: 2019/101). Written informed consent was obtained from participants of age 16 years and older, whereas assent was obtained from parents/guardians of participants younger than 16 years before being subjected to any study-specific procedure.

Sample Collection

Blood samples to compare Tac concentrations were collected in the morning, before the patient had taken the morning dose of Tac, and 2 and 5 hours after dose administration. The predose sample (C0) was obtained from

all participants, and the 2 hours (C2) and 5 hours (C5) postdose samples were obtained when possible according to the participant's preferences. At all 3 time points, both capillary 10 μ L volumetric absorptive microsampling tips included in the Mitra cartridge device (Neoteryx, Torrance, CA) were used. In addition to Mitra, a peripheral venous EDTA blood sample was collected simultaneously. Approximately 10–12 phlebotomists, experienced in capillary blood collection in children, conducted capillary microsampling and corresponding venous sampling. Before initiation of the study, the phlebotomists were given a short instruction on how to use Mitra according to the manufacturer's instructions. The sequence of capillary microsampling and venous sampling was prerandomized and specified in written instructions attached to each microsampling kit. All samples from the same patient were collected by the same phlebotomist, and the exact time was recorded.

The fingertip was disinfected with chlorhexidine 0.5%, left to dry for 1 minute, and punctured with a lancet (Medlance 1.5 mm; HTL-STREFA, Marietta, GA). The first blood drop was wiped off with a nonwoven compress, followed by dipping of the micro sampler tip into the blood surface; absorption of blood continued until both tips were completely filled. The cartridge containing the 2 tips was closed and sealed within the specimen bag still containing a desiccant. Peripheral venous blood was collected in EDTA tubes (BD vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) that were slowly inverted to ensure thorough mixing with the anticoagulant. Both the capillary microsamples and venous samples were kept at ambient temperature (18–22°C) until analysis.⁷

Tacrolimus Concentrations Analysis

Venous blood sampling was part of routine care, and blood samples were analyzed on the same day. The microsamples were kept overnight before sample preparation. The laboratory technicians inspected the quality of the tips to ensure adequately filled tips, and any deviation was noted. Quantification of Tac followed respective sample preparation, as previously described,⁵ and performed with liquid chromatography–tandem mass spectrometry using a Transcend II LX-2 system coupled to a TSQ Quantiva mass spectrometer (Thermo Fisher Scientific, Waltham, MA), as described in the validation studies performed in the adult population at our center in 2018 and 2019.^{5,8}

Data Analyses

The C2 and C5 samples were pooled for data analysis as postdose samples. Data analyses using Passing–Bablok regression and difference plots were performed using the Analyse-It add-in in Microsoft Excel (Analyse-It Software, Leeds, United Kingdom). Venous Tac concentrations were used as a comparator when calculating the absolute and relative differences between venous blood samples and capillary microsamples. The differences are presented as mean \pm SD unless otherwise stated. Demographic data were analyzed using SPSS Statistics for Macintosh, version 26.0 (IBM, Armonk, NY) and Stata Statistical Software 2019, Release 16 (StataCorp, College Station, TX).

RESULTS

Thirty-nine patients (age 4–18 years, 26 kidney and 13 liver transplant recipients, 22 males) fulfilled the protocol requirements. Fourteen (36%) patients were in the children group (2–12 years old) and 25 (64%) were in the adolescent group (13–18 years old). The median time since engraftment was 5.1 (range, 0.0–15.1) years for both kidney and liver transplant patients (Table 1).

In total, there were 55 capillary and venous sample pairs eligible for analysis; 38 predose (C0) and 17 postdose (4 C2 and 13 C5) sample pairs. Three patients provided the C0, C2, and C5 sample pairs. One capillary trough sample did not pass the visual inspection because of overfilling, explaining why this patient only provided a single C5 sample. Patients who refused venous sampling postdose did so to avoid extra venipuncture. One C0 specimen bag was without a desiccant, and one C5 specimen bag was not sealed on arrival in the laboratory. The latter 2 were still included in the analysis, showing deviations of –4.8% and –21% compared with the respective venous samples.

The time differences between capillary microsampling and venous sampling were within –10 to +7 minutes, with a mean difference of 1 ± 4 minutes (exact time differences were missing for 12 of the C0 sample pairs). Microsamples were analyzed within 2 weeks of sampling.

The median C0 concentration in venous samples was 4.8 mcg/L (range: 2.6–13.6 mcg/L). The mean difference in Tac concentrations between dried capillary microsamples and liquid venous samples was –0.2 ± 0.7 mcg/L, leading to a relative mean difference of –1.3 ± 14.2% [95% confidence interval (CI), –5.9% to 3.4%]. In 92% of the C0 sample pairs, the differences in the concentration measures were within ±20%. Only one sample pair had a difference exceeding ±30% (ie, +49% at venous blood concentration 2.6 mcg/L and at 3.9 mcg/L microsampling concentration). Thus, 97% of the sample pairs showed differences within ±30%.

In the postdose samples (C2 and C5, n = 17), median blood concentration in the venous samples was 7.9 mcg/L (range: 4.8–19.2 mcg/L) for C2 and C5. The mean difference between capillary microsamples and venous samples, both C2

and C5, was 0.0 ± 1.5 mcg/L, with a relative mean difference of –2.5 ± 12.3% (95% CI, –8.8% to +3.8%). Eighty-eight percent of the postdose sample pairs were within ±20%, and all were within ±30% with respect to relative differences. The largest postdose difference was +27% at venous blood concentration 19.2 mcg/L and at 24.5 mcg/L microsampling concentration.

Altogether, the predose and postdose sample pairs (n = 55) had a mean difference of 0.1 ± 1.0 mcg/L. The relative mean difference was –1.7 ± 13.5% (95% CI, –5.3% to +2.0%). Overall, 91% of the sample pairs had differences within ±20% and 98% were within ±30%. The difference plot is shown in Figure 1. The Passing–Bablok regression analysis provided estimates of intercepts of 0.047 (95% CI, –0.37% to +0.66%) and slope of 0.97 (95% CI, 0.85%–1.05%) (Fig. 2).

Data on corresponding hemoglobin levels in venous samples were obtained for 97% of the participants (n = 38 C0 sample pairs), and the median was 12.7 g/dL (range 8.7–15.6 g/dL). The correlation coefficient for the relationship between relative differences in Tac measurements (dried capillary/liquid venous blood) and the corresponding hemoglobin levels showed no significant correlation (Spearman rho was 0.17, 95% CI, –0.48% to +0.16%, n = 38 C0 sample pairs).

DISCUSSION

The results from our cross-validation study demonstrate good concordance between Tac concentrations measured in capillary volumetric microsamples and liquid venous blood sampling in pediatric solid organ transplant recipients. This was true for both trough and postdose measurements.

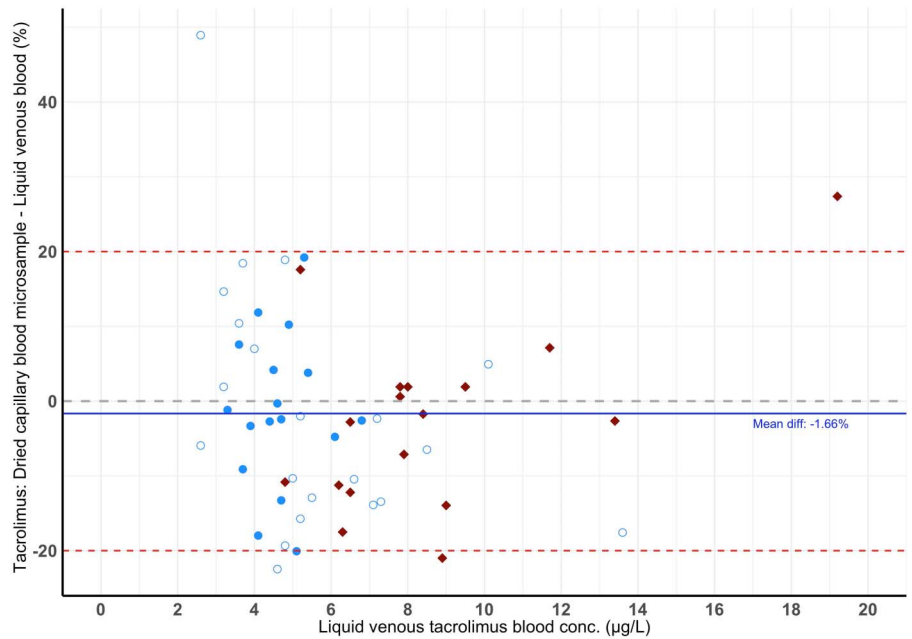
The narrow therapeutic window of Tac requires repetitive measurements throughout the lifetime of SOT patients. The capillary microsampling technique is convenient for home sampling and is particularly beneficial in the pediatric transplanted population. For some patients, it may be more convenient to take blood samples at home at *true* trough time, instead of timing the dose to the opening hours at the laboratory and appointments with the physician. With the results

TABLE 1. Demographic Data of Pediatric Solid Organ Transplant Recipients at Time of Blood Sampling for Comparison of Tacrolimus Blood Concentrations Based on Capillary Microsamples and Venous Blood samples

	2–12 years, n = 15 (38%)	13–18 years, n = 24 (62%)	Total, n = 39
	Median (Range)	Median (Range)	Median (Range)
Age (yr)	10.2 (3.7–12.8)	15.8 (13.3–18.0)	14.1 (3.7–18.0)
Male sex, n (%)	9 (60%)	13 (54%)	22 (56%)
Organ			
Kidney, n (%)	9 (23%)	17 (44%)	26 (67%)
Liver, n (%)	5 (13%)	6 (15%)	11 (28%)
Multiorgan, n (%)	1 (3%)	1 (3%)	2 (5%)
Time since Tx (yr)	3.0 (0.0–12.0)	7.1 (0.0–15.1)	5.9 (0–15.1)
Creatinine (μmol/L)	42 (32–178)	82 (42–164)	75 (32–178)
Hemoglobin (g/dL)	12.3 (8.7–15.0)	12.9 (9.3–15.6)	12.7 (8.7–15.6)

Data are presented as median (range) or number (%).

FIGURE 1. Difference plot of tacrolimus concentrations measured in dried capillary blood microsamples and liquid venous blood samples for predose (C0, blue circles) and postdose (C2 or C5, red diamonds) samples (n = 55). Filled blue circles indicate both samples within respective patients' therapeutic range, and open symbols indicate at least one of the measurements outside the range. Horizontal blue solid line is mean difference (-1.66%), dashed red lines indicate ±20% difference, and dashed gray line zero difference.

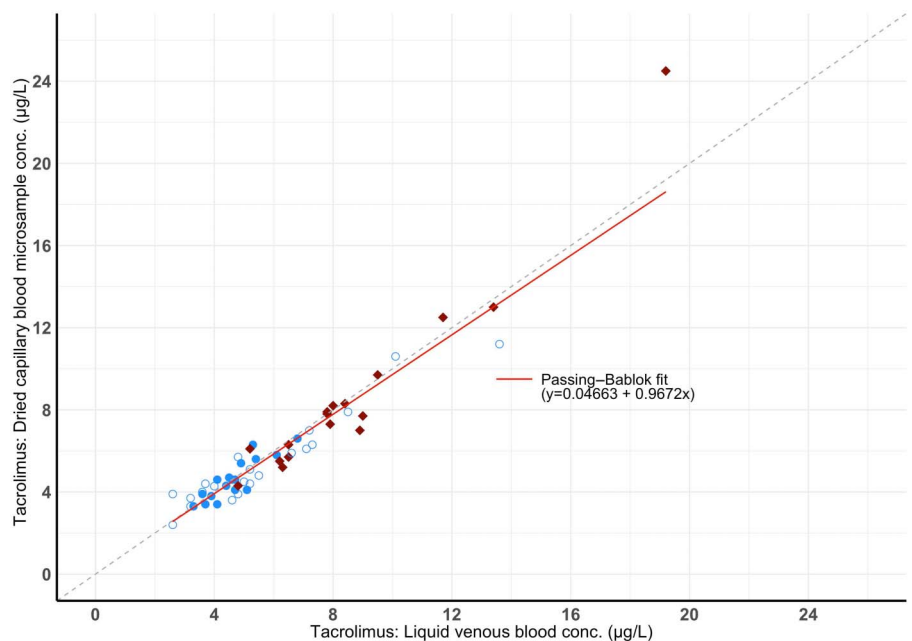


from our capillary volumetric microsamples (Mitra 10 µL) validation, this methodology may be included in the clinical follow-up of pediatric patients. Because blood volumes in children are related to weight, small-volume sampling extends the advantage of maintaining in vivo blood volume. In addition, the technique from our experience seems to be more tolerated in children who are reluctant to undergo venipuncture.

A strength of this study is that the study population is representative of transplanted pediatric patients who visited our clinic for follow-up visits. The samples were taken by the

phlebotomists on call that day (ie, not called in for study purposes). They were experienced in capillary blood collection from pediatric patients and received a brief Mitra sampling training before initiating the study. In a previous study, one-third of the Mitra samples were rejected because of their poor quality and attributed it to the high number of phlebotomists (n = 75).⁹ Although our study did not involve as many phlebotomists (n = 10–12), the clinical applicability of this novel method was still substantiated because it was part of a daily routine with several phlebotomists involved. The quality of the capillary microsampling seemed to be high.

FIGURE 2. Passing–Bablok regression plot of tacrolimus concentrations measured in dried capillary blood microsamples and liquid venous blood samples for predose (C0, blue circles) and postdose (C2 or C5, red diamonds) samples (n = 55). Filled blue circles indicate both samples within respective patients' therapeutic range, and open symbols indicate at least one of the measurements outside the range. Gray dashed line indicates unity, and solid red line indicate the regression line ($y = 0.04663 + 0.9672x$).



We rejected one of the 56 samples because of erroneous filling (2 tips in a single cartridge). Two samples had irregularities with respect to the specimen bag, although they were still included in the analyses.

Our primary aim was to cross-validate predose Tac measurements in dried capillary volumetric microsamples against standard liquid venous samples. Second, we aimed to evaluate the quality of microsamples in a subgroup that underwent simultaneous venous sampling at 2 and/or 5 hours postdose. Postdose sampling was based on the preferences of each child, and majority (64%) preferred to avoid additional venipuncture; hence, fewer postdose sample pairs were available. Nevertheless, this indicates that the pediatric population is positive toward finger-prick sample methods and may prefer capillary microsampling while venipuncture is believed to be difficult or painful. Approximately 90% of the capillary/venous sample pairs demonstrated Tac concentration differences within $\pm 20\%$. A single predose sample pair had a relative difference outside $\pm 30\%$ (+49%), observed in the low concentration range, both values outside the patient's therapeutic range. On the other hand, the sample pair with the second highest deviation (+27%) was a postdose sample in the higher concentration range. No explanation for these deviations could be identified. The time difference between the capillary and venous blood sampling was low, and a potential time-dependent bias in the analysis was minimized by randomizing the sequence in collection of capillary and venous samples. The Passing–Bablok regression analysis indicated no systematic bias and no concentration-dependent bias because 0 was included in the CI of the intercept and 1 was included in the CI of the slope. We found no significant correlation between relative difference in Tac measurements and hemoglobin suggesting that the hemoglobin range investigated (8.7–15.6 g/dL) did not affect Tac measurements in the capillary microsamples. Overall, the results fulfilled the cross-validation method criteria as given in the European Medicines Agency Guideline on bioanalytical method validation.¹⁰

Because the quality of capillary volumetric microsamples in the pediatric population is comparable with that of standard liquid venous samples, not only for predose but also for postdose measurements, this may pave the way for AUC-targeted Tac TDM. A limited sampling strategy at home can be used and may be more informative compared with single measurements performed before consultations and has been hypothesized to improve outcomes in transplanted patients.³ Capillary microsampling also allows for repetitive measures at home, making it ideal for closer follow-up and monitoring in an age group with a high nonadherence rate.¹¹

CONCLUSION

Tac monitoring in a pediatric population of solid organ transplant recipients using capillary volumetric microsampling

with the Mitra 10 μL device, demonstrated good concordance with standard venous sampling procedures. With these cross-validation results, transplanted children and adolescents are one step closer to a more convenient home-based blood sampling method for Tac monitoring.

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REFERENCES

- de Jonge H, Naesens M, Kuypers DR. New insights into the pharmacokinetics and pharmacodynamics of the calcineurin inhibitors and mycophenolic acid: possible consequences for therapeutic drug monitoring in solid organ transplantation. *Ther Drug Monit.* 2009;31:416–435.
- Wallemacq P, Armstrong VW, Brunet M, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. *Ther Drug Monit.* 2009;31:139–152.
- Brunet M, van Gelder T, Åsberg A, et al. Therapeutic drug monitoring of tacrolimus-personalized therapy: second consensus report. *Ther Drug Monit.* 2019;41:261–307.
- Knops N, Herman J, van Dyck M, et al. Tacrolimus dose requirements in paediatric renal allograft recipients are characterized by a biphasic course determined by age and bone maturation. *Br J Clin Pharmacol.* 2017;83:863–874.
- Vethe NT, Gustavsen MT, Midtvedt K, et al. Tacrolimus can be reliably measured with volumetric absorptive capillary microsampling throughout the dose interval in renal transplant recipients. *Ther Drug Monit.* 2019;41:607–614.
- Martial LC, Aarnoutse RE, Schreuder MF, et al. Cost evaluation of dried blood spot home sampling as compared to conventional sampling for therapeutic drug monitoring in children. *PLoS One.* 2016;11:e0167433.
- Marshall DJ, Kim JJ, Brand S, et al. Assessment of tacrolimus and creatinine concentration collected using Mitra microsampling devices. *Ann Clin Biochem.* 2020;57:389–396.
- Klaasen RA, Bergan S, Bremer S, et al. Longitudinal study of tacrolimus in lymphocytes during the first year after kidney transplantation. *Ther Drug Monit.* 2018;40:558–566.
- Veenhof H, Koster RA, Junier LAT, et al. Volumetric absorptive microsampling and dried blood spot microsampling vs. conventional venous sampling for tacrolimus trough concentration monitoring. *Clin Chem Lab Med.* 2020;58:1687–1695.
- Guideline on Bioanalytical Method Validation.* European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). 2020. Available at: www.ema.europa.eu/en/bioanalytical-method-validation. Accessed November 16, 2020.
- Dobbels F, Ruppert T, De Geest S, et al. Adherence to the immunosuppressive regimen in pediatric kidney transplant recipients: a systematic review. *Pediatr Transpl.* 2010;14:603–613.