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EDUCATIONAL REVIEW

Therapeutic drug monitoring in pediatric renal transplantation

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Abstract Finding the balance between clinical efficacy and toxicity of immunosuppressive drugs is a challenge in renal transplantation (RTx), but especially in pediatric RTx patients. Due to the expected longer life-span of pediatric transplant patients and the long-term consequences of drug-induced infectious, malignant and cardiovascular adverse effects, protocols which minimize immunosuppressive therapy make conceptual sense. In this context, therapeutic drug monitoring is a tool which provides support for the individualization of therapy. It has, however, limitations, and specific data in the pediatric cohort are comparatively sparse. There is large heterogeneity among the studies conducted to date in terms of methods, follow-up, endpoints, immunosuppressive regimens and patients. In addition, data from adult studies are not readily transferrable to the pediatric situation. This educational review gives a concise overview on aspects of therapeutic drug monitoring in pediatric RTx.

Keywords Pharmacokinetics · Pharmacodynamics · Solid organ transplantation · Immunosuppressive therapy · Individualization of therapy

Definitions

> "Therapeutic drug-monitoring can be defined as the measurement of drug concentrations in biological fluids to assess whether they correlate with the patients' clinical condition and whether the dosage or dosage intervals need to be changed. This is done to optimize the management of patients receiving drug therapy for the alleviation or prevention of disease." [1]

Since the introduction of ciclosporin (CSA) about 30 years ago, monitoring the concentrations of immunosuppressive drugs has been an integral part of post-surgical patient care following organ transplantation and contributes to achieving a good balance in that narrow therapeutic region between efficacy and toxicity (Fig. 1). From this it follows that therapeutic drug monitoring (TDM) is reasonable when:

- effective and toxic concentrations are close together (narrow therapeutic window);
- there is an association between drug concentration and pharmacological effect;
- there are large inter-individual differences in pharmacokinetics (PK);
- there are drug–drug interactions;
- compliance needs to be monitored.

In addition to these general arguments for TDM there are a number of important case-related indications, such as:

- appearance of specific side-effects;
- no or inadequate response to standard dose;
- therapy beyond licensing of the drug (off-license use) and clinical studies.

TDM is of special relevance in any kind of minimization protocol that makes particular conceptual sense in pediatric transplantation [2] to ensure the efficacy of the remaining immunosuppression.

In general, one differentiates between:

- Pharmacokinetic (PK) monitoring, which is most commonly used form and stands for a concentration–time

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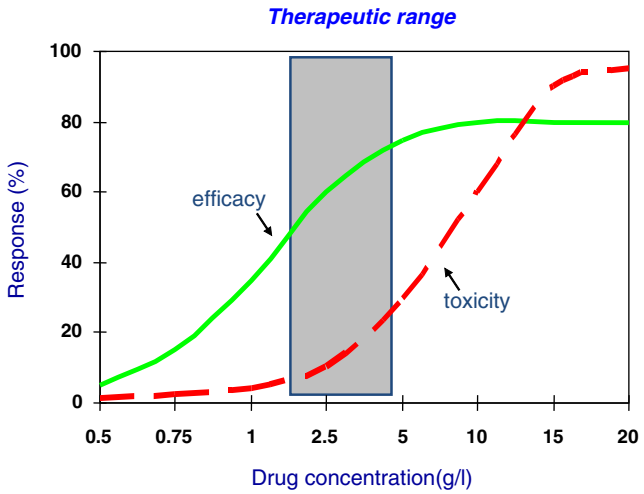

Fig. 1 Balance between efficacy and toxicity showing the narrow therapeutic range. [Courtesy (and in honor of) V.W. Armstrong (†), Göttingen, Germany]

relationship based on the estimation of drug load via blood levels, tissue levels or metabolites. Within a dosing-interval one has to distinguish certain PK parameters (Fig. 2), such as C_{max}, maximum concentration, T_{max}, time to maximum concentration and C_0, predose concentration). The area under the concentration–time curve (AUC) can be calculated by using the linear trapezoidal rule.

- Pharmacodynamic (PD) monitoring, which stands for an effect–time relationship through estimation of the biological effect at the target, i.e. measurement of enzyme activity or gene expression. For example, inosine monophosphate dehydrogenase (IMPDH) for mycophenolic acid (MPA) or residual nuclear factor of activated T-cells (NFAT)-regulated gene expression for CsA.
- Pharmacogenetic monitoring that has the potential advantage to allow monitoring even before treatment begins and is constant over an individual's lifetime [3]. To date, however, this approach has been routinely adopted for only a few drugs (e.g. azathioprine) and is confronted with the problem of an enormous variety of genetic polymorphisms.

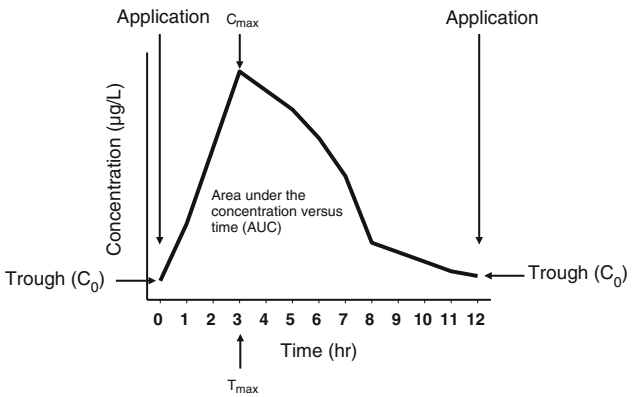

Fig. 2 Pharmacokinetic parameters during a dosing interval. C_{max} Maximum concentration, T_{max} time to maximum drug concentration, C_0 predose concentration

TDM in pediatric renal transplantation

General comments

In pediatric renal transplantation (RTx) there are a number of maintenance drugs that attenuate or suppress the host's immune system (Table 1). In the majority of cases these agents are combined to utilize additive or even synergistic effects [4], thereby offering the possibility to reduce a particular dose with the potential to reduce specific toxicity [5]. Such potential toxicities include parameters that increase cardiovascular morbidity and promote opportunistic infections, thereby considerably increasing the risk of post-transplant lymphoproliferative disease.

The immunosuppressive drugs all share a narrow therapeutic window and a high inter-individual variability [6, 7] (Fig. 3).

TDM in children must take into account developmental changes (ontogeny) in physiological and biochemical parameters causing differences in absorption, distribution, metabolism and clearance of the drug [8]. It is beyond the scope of this article to provide a detailed depiction of the concepts and mechanisms of ontogeny and drug disposition.

The following aspects need to be considered when assessing the value of TDM on the basis of published data:

1) The ontogeny of drug disposition renders results from adult studies unsuitable for pediatric patients (see above).
2) The concepts of drug–drug interactions with glucocorticoids as well as with specific combinations, such as calcineurin inhibitors (CNIs) and mycophenolate mofetil (MMF), and CNIs in combination with mammalian target

Table 1 Maintenance immunosuppressive drugs which require therapeutic drug monitoring

Drug class	Individual drugs
Calcineurin inhibitors	Ciclosporin[a], tacrolimus[b]
Mammalian target of rapamycin (mTOR) inhibitors	Sirolimus, everolimus[c]
Purine antagonist	Azathioprine
Inosine-monophosphate dehydrogenase inhibitor	Mycophenolic acid[d]

[a] Ciclosporin (CsA) is available as corn-oil based, and microemulsified CsA formulations

[b] Available as tacrolimus (Tac) or modified release Tac

[c] Everolimus (EVR) may not be licensed in some countries

[d] Mycophenolic acid (MPA) is available as the prodrug mycophenolate mofetil or as enteric-coated mycophenolate sodium

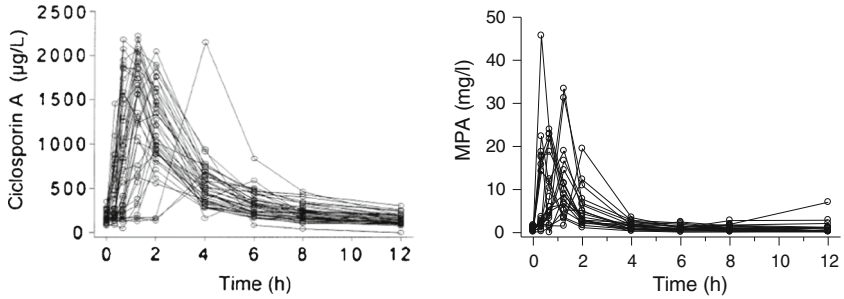

Fig. 3 Individual concentration versus time profiles: high inter-individual variability of ciclosporin A and mycophenolic acid *(MPA)* [6, 7] (used with permission)

of rapamycin (mTOR) inhibitors should be taken into account.

3) The choice of different outcome variables (e.g. graft survival, patient survival, acute rejection episodes, risk of side effects) may affect the interpretation of target concentrations for TDM.
4) The derivation of TDM recommendations have to betaken into consideration:whether they are derived from full pharmacokinetic profiles or from limited sampling strategies which can be restricted to trough or peak levels.
5) The age, gender, race, renal function and liver function of patients have to be considered.
6) Generic formulations may not have identical pharmacokinetics.
7) Measured concentrations may be influenced by preanalytical aspects and vary with the method used for analysis (e.g. enzyme-linked immunosorbent assay, radioimmunoassay, liquid chromatography, mass spectrometry) (see also section "Limitations and perspective").

Ciclosporin

Ciclosporin is a peptide extracted from the fungal *Tolyplocadium inflatum* Gams and has been the mainstay of immunosuppressive therapy in organ transplantation for over 30 years, thereby necessitating a summary of its history in TDM.

Inside the cell CsA forms a complex with cyclophilin, which in turn inhibits calcineurin, a calcium- and calmodulin-dependent phosphatase [9]. By blocking phosphorylation and the translocation of NFAT it inhibits synthesis of interleukin-2 (IL-2) and other cytokines that are mandatory for T-cell activation.

In the mid 1990s a CsA microemulsion was introduced that showed less intra- and inter-individual variability in terms of absorption and clearance, as well as a better correlation of trough levels with AUC, than the former corn-oil based formulation [10–12]. The volume of distribution of CsA is similar in children and adults, but the PK differ in these two patient groups as younger children are characterized by a smaller intestinal surface for adsorption but a higher clearance [13]. The role of p-glycoprotein (P-gp), a product of the human multidrug-resistance protein (MDR)-1 gene, in the absorption of CSA as well as that of the cytochrome P450-3A4/-3A5 system in the metabolism of CsA as explanations for its inter-individual variability remain controversially and a matter of pharmacogenetic monitoring [14–17]. Multiple drug–drug interactions definitely contribute to inter- and intra-individual variability. The most frequent interaction is due to the inhibition or induction of the cytochrome P450 (CYP) system as this is the common metabolic pathway of numerous drugs (Table 2).

Monitoring of the CsA-AUC is considered to be the gold standard to evaluate CsA total body exposure. This procedure is, however, expensive and laborious due to the high number of blood samples that are necessary over a dosing interval of 12 h. In contrast, CsA trough levels are easy to determine but correlate only moderately with CsA exposure [7]. Given that mean CsA concentration does not only correlate positively with the risk of acute rejection and the rate of graft losses within the first year after RTx but also has a predictive value for chronic allograft nephropathy (but often referred to as interstitial fibrosis and tubular atrophy [18]), TDM of CsA is widely accepted.

The superior prediction of transplant outcome by the CsA-AUC compared with trough levels [19] led to development of abbreviated AUCs that enable a more precise estimation of the total CsA-AUC [20]. Studies on the PK/PD relationship over time have shown maximal inhibition of calcineurin and IL-2 production within the first 1–2 h after CsA administration [21]. The hypothesis that the CsA absorption profile (AUC_{0-4}), which is the phase during which most of the variability of CsA exposure takes place, and accordingly the potential CsA peak concentration 2 h after dosing (C_2) might even be superior to total AUC_{0-12} was initially confirmed in adult renal transplant recipients [22, 23]. Both concepts are based on the assumption that associations with efficacy and toxicity may be more accurate when only the pharmacokinetic period within the first 4 hours after administration is examined, as this period covers the absorption and peak concentration of CsA and in turn results in maximal inhibition of IL-2 production.

Our own study [7] on this topic in pediatric renal transplant recipients also demonstrates the value of the absorption profile

Table 2 Potential drug–drug interactions of ciclosporin/tacrolimus/everolimus/sirolimus and mycophenolic acid

Drug	Effect	Kind of interaction
CsA/Tac/ EVR/SIR		
Diltiazem	CsA/Tac/EVR/SIR exposure ↑	Cytochrome P450 system[a] activity decreased by competition with the metabolic pathway (CYP ↓)
Verapamil	CsA/Tac exposure ↑	CYP ↓
Nifedipine	Tac exposure ↑	CYP ↓
Amoxicillin	CsA/Tac exposure ↑	CYP ↓
Clarithromycin	CsA/Tac/EVR/SIR exposure ↑	CYP ↓
Erythromycin	CsA/Tac/EVR/SIR exposure ↑	CYP ↓
Fluconazole	CsA/Tac exposure ↑	CYP ↓
Itraconazole	CsA/Tac exposure ↑	CYP ↓
Midazolam	Tac exposure ↑	CYP ↓
Proton pump inhibitors	EVR/SIR exposure ↑	P-glycoprotein inhibition
CsA	EVR/SIR exposure ↑	CYP ↓
Grapefruit juice	CsA/Tac/EVR/SIR exposure ↑	CYP0 ↓
St John's wort	CsA/Tac/EVR/SIR exposure ↓	CYP activity induced (CYP ↑)
Rifampin	CsA/Tac/EVR/SIR exposure ↓	CYP ↑
Isoniazid	CsA/Tac exposure ↓	CYP ↑
Carbamazepine	CsA/Tac/EVR/SIR exposure ↓	CYP ↑
Phenobarbital	CsA/Tac/EVR/SIR exposure ↓	CYP ↑
Phenytoin	CsA/Tac/EVR/SIR exposure ↓	CYP ↑
Metamizol	Tac exposure ↓	CYP ↑
Sirolimus	Tac exposure ↓	Hepatic first pass effect ↑
MPA		
Proton pump inhibitors	MPA exposure ↓ (MMF only)	Absorption ↓
Cholestyramin	MPA exposure ↓	Absorption ↓
Glucocorticoids	MPA exposure↓	Induction of glucuronidation
CsA	MPA exposure↓	Inhibition of enterohepatic recirculation by multidrug resistance protein 2
Metronidazole	MPA exposure ↓	Inhibition of enterohepatic recirculation, suppression of anaerobic bacterial glucuronidases
Norfloxacin	MPA exposure ↓	Inhibition of enterohepatic recirculation, suppression of anaerobic bacterial glucuronidases
Phosphate binders	MPA exposure ↓	Absorption ↓

SIR, Sirolimus; MMF, mycophenolate mofetil

Note: Azithromycin is the macrolide that altersthe pharmacokinetics of CsA, Tac and EVR/SIR less, and dosage adjustments may therefore not be warranted when used concomitantly

[a] The cytochrome P450 (CYP) superfamily exists of numerous subtypes, with CYP3A4 being the most significant for drug metabolism.

in reducing the risk of acute rejection during the first 3 months post- RTx. A CsA–$AUC_{0–4}$ below the threshold of 4,400 mg h/L increased the risk of acute rejection by 1.7-fold [7]. There was, however, no such association with CsA–C_2 values, probably due to the variability of the patients' absorber status (low/intermediate/high), high intra-patient variability and lack of dose proportionality.

In agreement with these data, subsequent investigations in adult renal transplant recipients showed no association of CsA–C_2 values with either the risk of acute rejection or toxicity within the first 4 weeks after RTx [24]. Finally, data from a prospective comparative study by Kyllönen et al. [25] indicate that there are relevant limitations to CsA–C_2 monitoring in the initial period after RTx, as these authors found that CsA–C_2 monitoring was not superior to trough monitoring in terms of efficacy and tolerability but was, rather, associated with clearly higher CsA doses. The concept of C2 monitoring may, however, provide helpful additional information in the long run since it allows dose reduction in otherwise undetected overexposed patients, resulting in better transplant function and lower blood pressure [23, 26].

Tacrolimus

Tacrolimus (Tac) was first isolated from *Streptomyces tsukubaensis* in 1984. It is an inhibitor of calcineurin following its binding to Tac-binding protein. According to the North American Pediatric Renal Transplant Cooperative Study Registry (NPRTCS; [27]), 47 % of pediatric renal transplant recipients are initially treated with Tac, as is also stated in the Kidney Disease Improving Global Outcomes (KDIGO) guidelines [28]. Trough-level monitoring of Tac has been standard practice since its introduction [29], and there is an association between Tac exposure and both clinical efficacy and toxicity.

Similar to TDM for CsA, TDM of Tac is critical in pediatric renal transplant recipients since Tac does not only create an opportunity for decreasing the risk of acute rejection but it is also contributes to a decline in graft function due to renal CNI toxicity and hypertension [30]. Tac trough levels are considered to be good surrogate parameters for Tac–AUC and hence for Tac exposure. Nevertheless, Tac has a variable bioavailability [31]—for example, due to genetic polymorphisms of transporter proteins and variability of metabolizing enzymes—that may compromise the value of trough level monitoring. Furthermore, the correlation of troughs with AUC seems to be impaired by the use of steroids [30]. In conclusion, the AUC is still considered as the best marker of Tac exposure [32]. Limited sampling strategies have been evaluated to facilitate TDM of Tac [32, 33]; these consist of three to four samples within the first 4 h after Tac administration. Another possibility to facilitating TDM of Tac may be finger-prick blood samples instead of venous samples as the former show a strong significant relationship with Tac levels as measured by high-performance liquid chromatography (HPLC)–tandem mass spectrometry [34].

Tac pharmacokinetic parameters show high inter-individual variability in pediatric renal transplant recipients [35], and the following factors underline the importance of TDM for Tac:

1) Tac clearance depends on age (higher in infants), time after RTx (decreases with time) and liver function (reduced in liver dysfunction).
2) Tac is extensively bound to erythrocytes and serum albumin, resulting in altered metabolism and efficacy in anemia and hypoalbuminemia, and has a large volume of distribution [32, 36].
3) Drug–drug interactions for example steroids increase metabolism [5]; sirolimus (SIR) increases hepatic first-pass effect [37] (Table 2).
4) Persistent diarrhea increases Tac exposure by altered P-gp activity. The underlying mechanism is an inhibition of P-gp activity followed by a restricted drug release back into the intestinal lumen.

Mycophenolic acid

Mycophenolic acid is widely used for maintenance immunosuppressive therapy and is mainly administered as MMF, an ester prodrug of the immunosuppressant MPA. MMF is currently the immunosuppressive drug most frequently prescribed to pediatric renal transplant recipients in the USA and in some European countries. According to the NPRTCS, 63.3 % of pediatric patients are initially treated with MMF [27]. MPA acts as a potent, reversible, uncompetitive inhibitor of IMPDH, the key enzyme in the de novo purine biosynthesis in proliferating T and B lymphocytes, thereby suppressing cell-mediated immune responses and antibody formation. MPA also inhibits glycosylation and expression of adhesion molecule and recruitment of lymphocytes and monocytes [38]. In this context it is important to note that proliferating T and B cells exclusively utilize the de novo pathway of purine synthesis, while brain cells exclusively utilize the so-called salvage pathway that is based on the recycling of purine bases. Other cell types are able to utilize both pathways. This is why MPA has a quite specific effect on proliferating lymphocytes.

The following factors support TDM of MMF:

1) There is a PK/PD relationship between the MPA AUC values and predose levels of MMF, and there is a risk of acute rejection in the initial period after RTx [39, 40].
2) MPA exposure shows high inter-patient variability [41].
3) MPA PK undergo a radical change within the first months after RTx due to improving graft function and serum albumin concentrations [42].
4) There are relevant drug–drug interactions (Table 2).

The following features need to be taken into account in TDM of MMF:

1) An important pharmacokinetic property of MPA is its extinctive and tight protein binding, particularly to serum albumin. The free fraction in individuals with conserved renal function ranges from 1 to 3 %. Decreased renal function increases the plasma concentration of MPA glucuronide (MPAG), which is the main metabolite of MPA. Despite not being pharmacologically active itself, MPAG displaces MPA from its albumin binding sites and thereby increases the amount of free MPA not bound to albumin. Based on in vitro studies, this free fraction is responsible for the pharmacologic activity of the drug and is also an important determinant of MPA clearance [41]. An association between free MPA exposure and hematological and infectious side-effects has been found in pediatric renal transplant recipients [39]. Thus, to make TDM of MMF even more complicated, TDM of free MPA could also be worthwhile in the subset of patients with therapy-associated side-effects.

2) It is important to note the method of MPA measurement because at least one metabolite cross-reacts with the EMIT assay (Enzyme Multiplied Immunoassay Technique), which nevertheless provides comparably adequate data to estimate the risk of acute rejection by HPLC, but requires target values that are about 15 % higher than those measured by HPLC [43].
3) MMF has associated gastrointestinal (GI) side-effects, such as nausea, vomiting, gastritis, abdominal cramps and diarrhea. Enteric-coated mycophenolate sodium salt (EC-MPS) is a compound that delays the release of MPA until it reaches the small intestine in order to reduce GI toxicities. Two pediatric studies have shown that the conversion from MMF to EC-MPS may have the potential to improve GI tolerability [44, 45], albeit neither study was randomized or controlled. EC-MPS differs from MMF in terms of a high variability in the time to maximal concentration of MPA (T_{max}), which is due to the enteric coating. Therefore, limited sampling strategies developed for MMF are useless for this formulation. Data on concentration-controlled dosing of EC-MPS in pediatric patients are not available. However, when MPA exposure is assessed with a full 12-h pharmacokinetic profile, therapeutic ranges for MPA are similar to those for the MMF and the EC-MPS formulations [41].
4) Due to enterohepatic recirculation that causes a secondary peak in mean plasma MPA concentration between 6 and 12 h after oral administration of MMF, as demonstrated in studies with healthy volunteers [46] and children after RTx [6], the term "predose level" should be used instead of "trough level."

Sirolimus

Sirolimus (SIR) is a macrocyclic triene antibiotic that is produced by the actinomycete *Streptomyces hygroscopius* [47]. SIR binds FKBP-12 to form a complex that inhibits mTOR, thereby suppressing T lymphocyte proliferation [48]. Pharmacokinetic studies have demonstrated that SIR has a much shorter half-life in children than in adults [49], thus young children especially may require twice-per-day dosing schedules in order to maintain therapeutic levels—particularly during the early post-transplant period and in CNI-free protocols when the half-life of SIR is shortest [48, 50]. Besides considerable inter-individual variability of pharmacokinetics [49] and age dependency of clearance [49, 51] there are substantial drug-drug interactions that require TDM of sirolimus (Table 2). Since SIR shares the same metabolic pathway, any drug affecting the cytochrome P450 system is able to alter the metabolism of SIR. It is of special interest that Tac exposure decreases significantly when SIR is added [37]. Current suggestions for therapeutic levels of SIR remain speculative and depend on the concomitant immunosuppressive medication. Because of delayed wound healing associated with the use of SIR, early post-transplant use of mTOR inhibitors is avoided.

Everolimus

Everolimus (EVR) is another more recent inhibitor of mTOR that blocks proliferative signals, thereby preventing T cells from entering the S phase of the cell cycle [4]. A limiting factor of EVR is the absence of license in many countries. EVR exerts its effects at a later stage than do CNIs and is not limited to IL-2-dependent proliferation of T cells [52]. Because of the complementary mechanisms of action of EVR and CsA, synergism is possible and may lower the required therapeutic dose of CsA [53].

EVR was developed to improve the PK profile of its antecessor SIR. It has an elimination half-life ranging from 18 to 35 h, which is shorter than that of SIR (60 h) and results in twice-daily dosing. In children its clearance is positively correlated with age, body surface area and weight [54]. African Americans have a 20 % higher clearance [55]. EVR is metabolized extensively in the gut and liver by CYP3A4. Since CsA and EVR are both substrates for CYP3A4 and P-gp, there is potential for drug–drug interactions (Table 2). A strong, positive correlation has been shown between EVR trough concentrations and clinical outcome [56, 57]. Because of the potential for improved efficacy and reduction of adverse effects, TDM has been recommended for EVR [52]. PD monitoring of mTOR inhibition via the phosphorylation status of p70 S6 kinase may further improve TDM.

Table 3 (modified after [58] used with permission) gives an overview of the potential target ranges of TDM of immunosuppressive agents discussed in this review. It also provides a proposed time schedule of when to perform TDM and points out limited sampling strategies and specific characteristics. The reader should keep in mind that the given target ranges may vary subject to the overall immunosuppressive load and the immunosuppressive protocol.

Limitations and perspective

One important precondition for TDM being useful in clinical practice is consistency in terms of drug administration and sampling [29]. The measured drug levels may be quite variable in patients who take their medication with meals sometimes and while fasting at other times, which could lead to under- and overdosing, especially in terms of C_{max} monitoring. For example, C_{max} (and AUC) of CNIs may be decreased by meals [75, 76]. Requesting patients to be consistent in this respect is certainly a challenge in the pediatric transplant population.

Table 3 Overview of potential target ranges, limited sampling strategies and specific characteristics of the immunosuppressive drugs discussed in this review

Agent	Parameter	Target range after RTx	Proposed timing of TDM In general: • After dose alteration • In case of potential drug–drug interaction • In case of suspected toxicity • In case of rejection	Specific characteristics/limited sampling strategies	Reference
Ciclosporin microemulsion (CsA)	CsA trough level (C_0)[a]	Month 0–3: 120–200 ng/mL From month 4: 80–160 mg/mL	• Once daily within the first 3 weeks after RTx • Three times per week in week 4 after RTx • Once weekly months 2–o 3 after RTx • Once every second week months 4– 6 after Rtx • Once every 4 weeks months 6–12 after RTx • At any outpatient visit in the long-term run	[a]EMIT assay, co-medication: Mycophenolatmofetil	
	CsA C_2	Month 1: 800–1,400 ng/mL Month 2–6: 800–1,200 ng/mL Month 7–12: 600–1,000 ng/mL Thereafter: 400–800 ng/mL Additional published target ranges: >1,500 ng/mL (day 5 after RTx in combination with azathioprine); 1,300–1,700 ng/mL (weeks 1–6 after RTx); >750 ng/mL (>1 year after RTx)	• Twice per week within the first 3 weeks after RTx • Once per week 4 after RTx • Once every 2 weeks in months 2–3 after RTx • Once per month in months 3–6 after RTx • Once every 3 months thereafter	C_2 monitoring is not recommended during co-medication with ketokonazole or diltiazem (concentration-curve flattened)	[22, 59–63]
	CsA AUC_{0-4} (C_0, $C_{0.5}$, C_1, C_2, C_4) (absorption-profile)	4,400–5,500 ng h/mL	Days 5–7 after RTx	Calculated CsA AUC_{0-4}=114+1.1 × C_0+2.0 × $C_{0.5}$+2.14 × C_2 Absorber status: low (C_2/C_0<3.5) intermediate (C_2/C_0=3.5–7.5) high (C_2/C_0>7.5)	[7, 22, 64]
	CsA-AUC_{0-12} (C_0, $C_{0.5}$, C_1, C_2, C_4, C_6, C_8, C_{12})	3,500–5,000 ng h/mL (≥6 months after RTx)	Determination of total exposure; individual indication	Calculated CsA AUC_{0-12}=51.1+4.82 × C_0+0.94 × C_1+1.47 × C_2+3.61 × C_4	[33]
Tacrolimus (Tac)	Tac trough level (C_0)	5–10 ng/mL within the first year after RTx 3–8 ng/mL in follow-up TWIST Study (pediatric; + MMF/steroids): Day 0–21: 10–20 ng/mL: Day 22–183: 5–15 ng/mL Symphony-Study (adult, +Daclizumab induction, MMF/steroids): 3–7 ng/mL	• Three times per week within first 4 weeks after RTx • Once weekly months 2–3 after RTx • Once every 2 weeks months 4–6 after RTx • At any outpatient visit in the long-term run	Dose modifications in 25 % steps, not more than twice per week MEIA (Abbott-IMx®), please note: Hematocrit<30 %→10–40 % overestimation of Tac concentration possible (according to up to 20 % underestimation possible in case of elevated hematocrit)	[33, 65–68]
	Tac AUC_{0-12}	150–200 ng h/mL (AUC–TDM not established) ≤1 year after RTx: approx.150 ng h/mL >1 year after RTx: approx.100 ng h/mL	Individual indication	Calculated Tac AUC=4.15+3.17 × C_0+1. 28 × C_1+0.76 × C_2+5.35 × C_4 Calculted Tac AUC=19.422+4.317 × C_0+1.226 × C_1+4.273 × C_3	[32, 33, 69]

Table 3 (continued)

Agent	Parameter	Target range after RTx	Proposed timing of TDM In general: • After dose alteration • In case of potential drug–drug interaction • In case of suspected toxicity • In case of rejection	Specific characteristics/limited sampling strategies	Reference
Mycophenolatemofetil (MMF)	MPA predose level (C_0)	1.0–3.5 mg/L (HPLC) 1.3–4.5 mg/L (EMIT)	• Three times per week within the first 3 weeks after RTx • Once per week in weeks 4–8 after RTx • Once per month >8 weeks after RTx • At any outpatient visit in the long-term run	Reduce daily dose by 50 % in case of leukocytopenia<4,000/μL or neutropenia of <1,600/μL Interrupt therapy in case of leukocytopenia <2,000/μL or neutropenia<1,300/μL In case of distinct diarrhea for >3 days without different cause give daily dose t.i.d. to q.i.d. and/or reduce daily dose by 50 %	[42, 70, 71]
	MPA AUC_{0-12}	30–45–60 mg h/L (HPLC/MS) 35–52–70 mg h/L (EMIT)	• Day 3–7, days 10–14 and 3–9 months after RTx • In case of clinical change (e.g. loss of renal function, hypalbuminemia) • If necessary: TDM of free MPA in case of suspected toxicity	Calculated MPA AUC=18.6+4.3 × C_0+0.54 × $C_{0,5}$+2.15 × C_2 (+ CsA) Calculated MPA AUC=10.0+3.95 × C_0+3.24 × $C_{0,5}$+1.01 × C_2 (+ Tac or without CNI)	[39, 72, 73, 74]
Everolimus (EVR)	EVR trough level (C_0)	3–8 μg/L 2–5 μg/L in follow-up	Adapted to trough level monitoring of the added CNI	Dose modifications in 20 % steps after second deviation upward or downward	[4]
Sirolimus (SIR)	SIR trough level (C_0)	4–12 ng/mL (with CNI) 5–10 ng/mL (CNI-free regimen)	• Once per week during the first month • Every other month thereafter	Pay attention to dyslipidemia! Use not recommended in proteinuria.	[47]

RTx, Renal transplantation; TDM, therapeutic drug monitoring; C_{max}, maximum drug concentration, T_{max}, time to maximum concentration; C_0, predose concentration; C_{1-12}, concentration a 1–12 h after administration; AUC area under the concentration–time curve; CNI, calcineurin inhibitor; HPLC, high-performance liquid chromatography; MS, mass spectrometry

[a] EMIT assay, co-medication: Mycophenolatemofetil

Table 4 Exemplary average deviation of available immunoassays as compared to liquid chromatography/tandem mass spectrometry[a]

Drug	Method	Mean deviation to LC-MS/MS (%)
CsA	TDx FPIA-non-specific	300–500
	TDx FPIA-specific	38
	AxSYM FPIA-specific	17
	RIA Cyclo-Trac-specific	11
	EMIT	9
	Dimension ACMIA	−5
	CEDIA plus	13
Tac	IMx MEIA tacrolimus II	6
	Pro-Trac II	12
	EMIT 2000 tacrolimus	23
MPA	EMIT	15–20
EVR	FPIA	24 %
SIR	MEIA	9 %
	CEDIA	20 %

LC-MS/MS, Liquid chromatography/tandem mass spectrometry; TDx, fully automated techniques; FPIA, Fluorescence polarization immunoassay; RIA, radioimmunoassay; EMIT, enzyme multiplied immunoassay; ACMIA, automated antibody conjugated magnetic immunoassay; CEDIA, cloned enzyme donor immunoassay; MEIA, microparticle enzyme immunoassay

[a] References: [43, 79–82]

Furthermore, blood samples must be collected at the correct time. For monitoring trough level, blood should be drawn 12 h after the last dose, which means immediately before the following dose. Levels that are drawn at other time points, such as 10–14 h after the last dose, may lead to unnecessary dosage adjustments. Correct sampling is even more important in CsA C_2 monitoring, where blood samples should be drawn within a 15-min time frame of the 2-h post-dose time point [77].

A more sophisticated approach to evaluate single time points and limited sampling strategies, such as surrogate markers for AUC, and thereby for total drug exposure, is provided by Bayesian forecasting, which is based on population pharmacokinetic data and takes into account the pharmacokinetic characteristics of a typical population, data collected from individual patients, as well as the variability of the PK parameters in the population studied [32]. The prediction using Bayesian forecasting is therefore more precise and offers higher flexibility in blood sample collection [32, 78].

A discussion of the limitations and perspective of TDM would not be complete without some mention of the analytical methods used to measure drug concentrations. In general, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) can be considered the gold standard. Fully automated immunoassay systems with high throughput are also widely used but have the drawback of being associated with variations in performance in terms of specificity and sensitivity due, for example, to cross-reactivity with metabolites that may or may not be pharmacologically active. Consequently, immunoassays usually overestimate the concentration of immunosuppressants (Table 4). These differences in measurement accuracy do not affect the clinical usefulness of the assays but they do add to the variability of the concentrations reported in the literature and may impact local target ranges [79]. A clinician should therefore be aware of the analytical methods used locally!

Classical TDM monitors a medication by assessing the pharmacokinetic parameters of the drug, which may not always reflect the medication's pharmacodynamic effects. Therefore, TDM also needs to be extended to monitoring the PD aspects. In previous studies, calcineurin inhibition, IL-2 production and cytokine mRNA production were measured as markers of the degree of calcineurin inhibition [21, 83, 84]. In more recent investigations, the expressions of NFAT-regulated genes have been measured as PD biomarkers of CsA, and a relationship with infectious complications and malignancies was observed, leading the authors to conclude that pharmacodynamic aspects of TDM have the potential to identify over-immunosuppressed renal transplant recipients [85, 86].

The determination and monitoring of IMPDH activity has recently been advocated as a pharmacodynamic biomarker of MPA effects [87]. In addition, pre-transplant IMPDH activity has been associated with clinical outcome in adult renal transplant recipients [88]. It is currently being debated whether the determination of pre-transplant IMPDH activity is sufficient to guide MMF dosing for improving outcome or whether pre-dose IMPDH activity [89] or maximal IMPDH inhibition is superior in identifying patients at risk of acute rejection and MMF-related side-effects. Our group has shown that there is a comparable inhibition of IMPDH activity by MPA in children and adolescents after RTx and that, similar to adults, IMPDH activity was inversely correlated with MPA plasma concentration [90].

Future TDM approaches will also consider virus-specific T-cell monitoring as a surrogate for overall immunosuppressive potency (T. Ahlenstiel, personal communication, November 5, 2013). However, to date, no PD method has become widely accepted in clinical practice.

The third approach to TDM is pharmacogenetics which, given its potential to individualize therapy and to improve medical care post-transplant, has generated high expectations. However, despite the abundance of data on genetic associations with either the PK or PD of drugs, these data have only rarely been translated into patient care [91]—but progress can be expected. One major problem of pharmacogenetics is the variety of genetic polymorphisms. The answer may be to combine a number of polymorphisms to predict PK [92].

Future effort is also necessary to validate thresholds and therapeutic ranges several years post-transplant and to provide a basis for various combination therapies with conventional and new immunosuppressive drugs. This is particularly important with respect to the development of minimization protocols.

Last but not least, the utility of TDM should be evaluated for each immunosuppressive agent, especially against the background of cost and effort. A very elegant way to do so is the published nine-step decision-making algorithm that requests answers to the following questions [4, 93]:

1) Is the patient on the best drug for his/her specific subpopulation (disease state) and specific indication?
2) Can the drug readily be measured in the desired biologic matrix?
3) Has a good relationship between drug concentration and pharmacological response been reported in pharmacokinetic studies conducted in humans?
4) Is the drug's pharmacological response readily assessable?
5) Does the relationship between concentration and pharmacological response still apply to the patient's specific subpopulation (disease state) and specific indication?
6) Does the drug have a narrow therapeutic range for the specific subpopulation (disease state) and specific indication?
7) Are the pharmacokinetic parameters unpredictable because of either intrinsic variability or the presence of other confounding factors?
8) Is the duration of drug therapy sufficient for the patient to benefit from clinical pharmacokinetic monitoring?
9) Will the results of the drug assay make a significant difference in the clinical decision-making process (i.e, provide more information than sound clinical judgment alone)?

Summary points

- TDM has the potential to optimize efficacy and to minimize toxicity in pediatric RTx.
- AUC is considered as best marker of drug exposure. Surrogate parameters, such as trough levels or limited sampling strategies, may facilitate TDM.
- Clinicians should be aware of the analytical method used locally due to differences in assay performances.
- Extending TDM to pharmacodynamic and pharmacogenetic approaches will advance individualization of immunosuppressive therapy after pediatric RTx, since these parameters might also reflect the patient's sensitivity to the immunosuppressive medication.

Questions (answers are provided following the reference list)

1) Which answer is wrong?
Therapeutic drug-monitoring is reasonable when:
   A) There is an association of drug concentration and pharmacological effect
   B) There are drug–drug interactions
   C) The therapeutic window is wide
   D) There is no or inadequate response to standard dose
   E) Side-effects appear
2) Which of the following statements is correct?
   A) Pharmacokinetic monitoring stands for a concentration–time relationship
   B) Pharmacodynamic monitoring stands for a concentration–time relationship
   C) Pharmacogenetic monitoring is highly variable over a patient's life
   D) The area under the concentration–time curve (AUC) can be exactly calculated by simply adding trough level and peak concentration
   E) C_0 values are sometimes higher than C_{max} values
3) Which of the following is correct?
   A) Glucocorticoids are well suited for pharmacokinetic drug monitoring
   B) Absorption, distribution, metabolism and clearance of a drug do not change with a child's development
   C) Inter-individual variability of plasma concentrations is not an argument for TDM
   D) Generic formulations of a drug may not have identical PK as the original formulation
   E) Trough levels are not a surrogate marker for AUC
4) Which of the following statements is incorrect?
   A) Clearance of tacrolimus depends on age, time after transplant and liver function
   B) Tacrolimus is extensively bound to erythrocytes
   C) Despite limitations in C_2 monitoring of CsA may be helpful to detect overexposed patients
   D) There is a PK/PD relationship of MPA AUC values and the risk of acute rejection episodes in the initial period after renal transplantation
   E) MPA exposure hardly shows any inter-patient variability
5) Which of the following is incorrect?
   A) Antacids do influence MPA exposure
   B) TDM of everolimus is not recommended because it has no potential to improve efficacy and to reduce toxicity

C) For TDM using pharmacokinetic parameters it is important to know whether the drug has been taken after a meal or in a fasting state

D) When TDM is conducted using pharmacokinetic parameters the drug's concentration is measured in the desired biological matrix

E) Inosine monophosphate dehydrogenase may serve as a biomarker for MPA efficacy

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Answers

1) C
2) A
3) D
4) E
5) B