Transplant International ISSN 0934-0874

REVIEW

Immunosuppressive drug monitoring – what to use in clinical practice today to improve renal graft outcome

Dirk R. J. Kuypers

Department of Nephrology and Renal Transplantation, University Hospitals Leuven, University of Leuven, Leuven, Belgium

Keywords
calcineurin inhibitors, immunosuppressive drug monitoring, Mammalian Target of Rapaurycin (mTOR) inhibitors, mycophenolate mofetil, renal transplantation, therapeutic drug monitoring.

Correspondence
Dirk R. J. Kuypers MD PhD, Department of Nephrology and Renal Transplantation, University Hospitals Leuven, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium. Tel.: 32-16-34-45-80; fax: 32-16-34-45-99; e-mail: dirk.kuypers@uz.kuleuven.ac.be

Received: 8 April 2004
Revised: 2 August 2004
Accepted: 9 September 2004

doi:10.1111/j.1432-2277.2004.00041.x

Summary

Therapeutic drug monitoring (TDM) of immunosuppressive therapy is becoming an increasingly complex matter as the number of compounds and their respective combinations are continuously expanding. Unfortunately, in clinical practice, monitoring predose trough blood concentrations is often not sufficient for guiding optimal long-term dosing of these drugs. The excellent short-term results obtained nowadays in renal transplantation confer a misleading feeling of safety despite the fact that long-term results have not substantially improved, definitely not to a point where longer graft survival could counteract the increasing need for transplant organs and less toxicity and side-effects could ameliorate patient survival. It is therefore a challenging task to try to tailor immunosuppressive drug therapy to the individual patient profile and this in a time-dependent manner. For the majority of currently used immunosuppressive drugs, measurement of total drug exposure by determination of the dose-interval area under the concentration curve (AUC) seems to provide more useful information for clinicians in terms of concentration–exposure and exposure–response as well as reproducibility. To simplify this laborious way of measuring drug exposure, several validated abbreviated AUC profiles, accurately predicting the dose-interval AUC, have been put forward. Together with an increasing knowledge of the time-related pharmacokinetic behaviour of immunosuppressive drug and their metabolites, studies are focusing on how to apply abbreviated AUC sampling methods in clinical transplantation, taking into account the numerous factors affecting drug pharmacokinetics. Eventually, TDM using abbreviated AUC profiles has to be prospectively tested against classic methods of drug monitoring in terms of cost-effectiveness, feasibility and clinical relevance with the ultimate goal of improving patient and graft survival.

Introduction

Combination of powerful immunosuppressive drugs in renal transplantation has resulted in excellent short-term patient and graft survival together with very low acute rejection rates [1–5]. However, all these immunosuppressive compounds are characterized by specific side-effect profiles and manifestations of toxicity that can ultimately limit their long-term use [6]. Drug-induced toxicity (e.g. anaemia, leukopenia, diarrhoea, osteoporosis) may compromise the use of particular drugs as maintenance therapy after successful transplantation while others can cause serious morbidity in recipients [infection, arterial hypertension, hyperlipidaemia, post-transplantation diabetes mellitus (PTDM)] or reduce allograft survival (calcineurin-inhibitor induced nephrotoxicity, PTDM). Tailoring the dose of immunosuppressive drugs according to specific requirements dictated by the individual patient profile, in order to minimize side-effects while maintaining adequate immunosuppression, is a challenging goal

in clinical transplantation practice [6]. The currently available immunosuppressive armament, allowing better control of acute rejection, enables clinicians to focus on long-term transplantation-related problems. The objective of prolonging long-term patient and graft survival has become even more urgent because transplant waiting lists are expanding and recipient age is increasing while a growing demand for donor organs is more often counterbalanced by the use of marginal donors [7,8]. On the contrary, the principal reason for renal allograft loss is still death with a functioning graft, mainly as a result of cardiovascular disease, infections and malignancies [9]. It is clear that the negative impact the immunosuppressive drugs have on the latter, could potentially be ameliorated by optimizing their dosing [10,11].

A prerequisite for individualizing and optimizing immunosuppressive therapy is a reliable methodology of therapeutic drug monitoring (TDM) that enables clinicians to recognize the time-dependent variability in a drug's 'dose–concentration-effect' relationship. The latter is not a simple matter as exposure–response relations are always superimposed on a constant individual genetic profile, characterized by different polymorphisms of key molecules (CYP3A4, CYP3A5, MDR1, cMOAT, UGT1A) involved in the metabolism of immunosuppressive drugs [12–14] and patient susceptibility for adverse effects [15]. The phenotypic expression and activity of these drug metabolizing molecules are influenced by a large variety of clinical variables such as, for example age, gender and concomitant medication [16–18]. Analysing long-term drug pharmacokinetics and studying the importance of clinical variables that can influence the latter will help to gain insight into the complex relationship between drug dosing and clinical efficacy and toxicity. These processes ideally result in the definition of a therapeutic window, which is clinically used as basis for a target concentration dosing strategy [19–23]. An additional source of complexity is added by the fact that in clinical practice immunosuppressive drug therapy is a combination therapy usually consisting of two, three or more compounds, each characterized by a unique pharmacokinetic profile and capable of exercising potentially important influences on each other [24,25]. Finally, the identification of immunosuppressive drug metabolites that influence the pharmacokinetics of the parent molecule [26,27] and vice versa [28,29] and their role in terms of clinical efficacy and toxicity [30,31] constitutes another interesting field of recent research.

In this brief overview an attempt is made to provide a critical analysis of the current developments in immunosuppressive drug monitoring in renal transplantation and the implications for clinicians. The aim is to deduce from clinical research new monitoring concepts in order to help optimizing long-term patient and graft survival.

Calcineurin inhibitors

Tacrolimus

There is a growing consensus that predose trough blood tacrolimus concentrations (C_0) do not accurately reflect total drug exposure as measured by the 12-h dose interval area under the concentration curve ($AUC_{0-12\ h}$), both in renal and other solid organ transplantation [32–37]. Especially when C_0 is evaluated for its clinically relevant predictive accuracy, by calculating the prediction error and bias [32,35], the usefulness of tacrolimus trough concentrations is questionable. Other clinical studies that repeatedly showed an excellent correlation between tacrolimus C_0 and $AUC_{0-12\ h}$ were often in nonrenal transplantation [38,39] and failed to examine predictive performance of C_0 as suggested [40]. It seems not surprising that every single blood concentration sampling time point is prone to a substantial bias, caused by practical factors such as the exact method [41] and timing of sampling [23], the intra- and interassay variability employed for determination of the blood concentration [21,42], influence of biochemical variables (e.g. haematocrit) [43], hepatitis C virus infection [44], (black) race [45], concomitant medication [18], diurnal variation [33,46] and food intake [47,48]. Studies prospectively exploring the clinical usefulness of alternative single sampling time points [49,50] failed to ameliorate this inaccuracy. One strategy to improve predictive performance is to add a second fixed concentration sampling point, preferably in the distributive phase of the AUC [32,51] or using a Bayesian forecasting model for predicting tacrolimus concentration based on population pharmacokinetics [52,53]. The former method implies a second blood sample, taken usually within the first 4 h postdosing, in order to calculate a limited sampling model for predicting the 12-h AUC. With a Bayesian estimation the initial drug concentration can be predicted using pharmacokinetic parameters derived from large patient populations, and compared with the actually measured concentrations. Combining Bayesian fitting with an established compartmental drug model, enables the estimation of the dose-interval AUC using variable instead of fixed sampling times as was recently demonstrated for cyclosporin [54]. However the latter method and other alternatives like the use of neural networks [55], presumes knowledge about (multivariate) pharmacokinetic modelling and its intrinsic limitations and pitfalls, as well as access to a computer-based pharmacokinetic programme. Taking into account the time required to perform the clinical sampling and the subsequent calculation, one has to weigh the advantages of

using variable sampling time points against the relative simplicity of obtaining (limited sampling) abbreviated AUC profiles and calculating the corresponding 12-h AUC using a simple noncompartmental model. Investigators have tested various abbreviated tacrolimus AUC sampling strategies, obtaining the best predictive performance using a 4-h profile [32,49,56]. A tacrolimus 4-h abbreviated concentration curve theoretically seems to provide an acceptable compromise between what is clinically desired and practically feasible. However, particularly the practical execution of abbreviated AUC measurements is rightfully questioned in clinical practice as being cumbersome, impractical and costly [23,57,58]. The added benefit of precise information on drug exposure has to result in clear clinical advantages for the patient in terms of graft survival, side-effects and morbidity before one can justify the additional costs and work involved.

Today, few prospective studies can actually demonstrate a clear relationship between tacrolimus exposure – measured as predose trough concentration – and clinical efficacy and toxicity [59,60]. Data from clinical studies are usually derived retrospectively [61] or are based on heterogeneous patient populations [62] or incorporate surrogate markers for clinical endpoint that are not always reliable [59,62]. For example, studies using a nonspecific decrease in renal allograft function, early after transplantation, as a surrogate marker for calcineurin-inhibitor related nephrotoxicity, without histological prove, are prone to error because many confounding factors immediately after transplantation can influence initial graft function. In one such trial a target range of whole blood tacrolimus levels between 5 and 15 ng/ml was determined as the best compromise between efficacy and toxicity [59] while Undre *et al.* retrospectively determined a minimum predose trough tacrolimus concentration of 10 ng/ml in order to prevent rejection [61]. It is clear at present, that dosing of tacrolimus according to C_0 target trough levels, derived through trial-and-error experience from consecutive clinical trials [63], has resulted in a superior clinical efficacy in the prevention of acute rejection [3] and a somewhat more favourable cardiovascular profile compared with cyclosporin A [64,65]. With the current method of TDM for tacrolimus, based on predose trough concentrations, clinicians have not been able to improve patient or graft survival and are still confronted with a high incidence of calcineurin-inhibitor related nephrotoxicity, not different from cyclosporin A [66], and other side-effects such as PTDM [67]. Although the alternative of obtaining abbreviated AUC concentration profiles constitutes a strenuous, cumbersome and costly activity which at first sight seems to be incompatible with clinical follow-up of renal recipients, prospective comparative studies are necessary to evaluate the cost-effectiveness, the feasibility and the clinical relevance of this intensive method of drug monitoring. For example, we were able to demonstrate in a prospective study involving 100 *de novo* recipients that patients who simultaneously obtained a calculated tacrolimus $AUC_{0-12\ h}$ of 150 ng h/ml and an mycophenolic acid (MPA) AUC_{0-12} of 45 mg h/ml by day 7 post-transplantation, had an incidence of biopsy-proven acute rejection of 7.7% as opposed to 26.3% for recipients who did not attain these $AUC_{0-12\ h}$ targets by day 7 [68]. At the same time patients who suffered from infectious complications early after transplantation, had a significantly higher tacrolimus AUC_{0-12} compared with those who were free of infection. It is therefore practically quite feasible to determine – in analogy with trough concentrations – a target concentration window for tacrolimus based on abbreviated AUC measurements, that could be used clinically in order to better differentiate between efficacy and avoidance of toxicity and with the advantage of a more reliable reproducibility compared with trough levels. However, the practical ease of classic trough concentration monitoring and the excellent short-term results obtained in renal transplantation nowadays make it difficult to convince clinicians that a more laborious and extensive method of drug monitoring might prove necessary in order to improve long-term patient and graft survival. One way to simplify the use of target concentration windows based on abbreviated AUC profiles, is to determine a universally accepted standardized shortened AUC profile for every immunosuppressive drug in order to make the comparison easier. Alternatively, calculating the corresponding average steady-state concentration (C_{ss}) from the AUC would even further facilitate communication across clinicians [69].

Another difficulty with defining useful tacrolimus target concentration ranges is the time dependency of the latter and the changing influence of clinical and biological factors [70]. It is obvious that early after grafting higher immunosuppressive drug concentrations are required in order to prevent rejection while later on during follow-up, chronic drug toxicity becomes an important issue [63,66,68]. As a result, early after transplantation, serious side-effects are caused by overimmunosuppression (e.g. infections, PTDM, neurotoxicity). Prior knowledge of exact individual dose requirements would help to ameliorate these side-effects but are difficult to obtain by routine trough concentrations. In recipients awaiting renal transplantation, even measuring full or abbreviated AUC profiles prior to surgery did not enable clinicians to better predict early dose requirements after transplantation [71,72], probably because of the high dose corticosteroids used early after grafting. In cardiac transplant recipients the use of a 1 mg tacrolimus test dose immediately after

surgery was helpful in predicting subsequent (twice) daily dose requirements necessary to avoid acute rejection and nephrotoxicity [73]. It remains to be determined which clinically applicable methodology in renal transplantation can be further developed in order to solve this problem of identifying early dose requirements and avoiding initial overimmunosuppression. The establishment of the genetic profile of the patient with respect to polymorphisms of key metabolizing molecules (see above) and evaluation of their respective functional status by using drug probes [74] will at least in part help to further unravel this puzzle.

Cyclosporin A

For cyclosporin A part of the questions stated above have already been answered. Mahalati *et al.* was the first to show prospectively that abbreviated 4-h AUC concentration profiles were superior to C_0 with respect to predicting clinical efficacy (prevention of acute rejection) and nephrotoxicity [75]. Abundant other limited sampling strategies for cyclosporin were proposed of which several adequately predicted the full dose-interval AUC [76–79] and were recently summarized by David and Johnston [80]. The conclusion of the latter review of 38 studies was clear: there is no 'best' algorithm for estimating cyclosporin AUC from sparse data. The clinician has a choice of several equations, involving the use of two or three time points which produce results with similar accuracy and precision.

Mainly for reasons of practical simplicity a new single cyclosporin concentration sampling point was derived form Mahalati's AUC_{0-4} data and gave rise to the new concept of C_2 monitoring. Although Mahalati *et al.* found C_3 as best predictor of the 12-h dose-interval AUC that correlated with acute rejection and nephrotoxicity during the first week after transplantation [81], C_2 better reflected the time point of maximal pharmacodynamic effect of cyclosporin measured as percentage inhibition of calcineurin [82] and suppression of interleukin-2 release from T cells [83]. The cyclosporin blood concentration 2 h post-dosing was relatively easy and accurate to determine with different assays [42] and was the best predictor for the 4-h cyclosporin AUC in subsequent studies [84–86]. Studies in kidney and liver transplantation validated the use of cyclosporin C_2 monitoring for prevention of acute rejection and to a lesser extent for reducing calcineurin-inhibitor-induced nephrotoxicity [87–89]. A C_2 level ≥1700 ng/ml on day 3 post-transplantation had a 92% negative predictive value for acute kidney rejection in the first 6 months for recipients without delayed graft function [85]. This minimal C_2 threshold was subsequently refined to 1500 ng/ml (on day 7) by the findings of two other studies in renal recipients, one protocol with and the other without induction with monoclonal antibodies against the interleukin-2 receptor [84,86]. Currently the 'MO2ART' study, a first prospective multicentre trial examining the clinical usefulness of C_2 monitoring in *de novo* renal recipients, has shown low acute rejection rates (11.5%) and excellent patient and graft survival at 12 months [90]. More importantly, this study is a first effort in providing guidelines for decreasing target C_2 concentrations during the first year post-transplantation to as low as 700–900 ng/ml in the second half of the first post-transplant year. If this strategy is beneficial with respect to adverse events compared with classic predose trough (C_0) (or AUC) monitoring, needs to be determined in prospective comparative trials. Studies examining the benefit of cyclosporin C_2 monitoring in chronic maintenance therapy are lacking. In a large retrospective study in 1032 recipients, C_2 concentrations between 700 and 800 ng/ml were associated with significantly better graft function compared with C_2 levels above 950 ng/ml [91]. Whether use of a 2-h single concentration sampling point will help to reduce long-term drug-related nephrotoxicity remains to be determined in a comparative prospective study, focusing at the same time on other calcineurin-inhibitor related side-effects like arterial hypertension, hyperlipidaemia and ultimately patient and graft survival. In a recent trial, 127 long-term renal recipients were monitored by cyclosporin C_2 concentrations in addition to conventional trough levels and followed over a period of 13.6 ± 3.1 months [92]. This observational study revealed that cyclosporin C_2 concentrations between 500 and 600 ng/ml (corresponding to C_0 concentrations of ±100 ng/ml) were safe and well tolerated as maintenance therapy in stable chronic recipients but did not, similar to C_0 levels, allow to differentiate for acute rejection, cyclosporin toxicity or infectious complications [92]. In fact, both single concentration time points exhibited a relevant intra-individual variability, albeit marginally better for C_2 (coefficient of variation 15.3% vs. 17.2%). Again, in analogy with tacrolimus, the potential limitations of a single concentration sampling strategy have to be weighted against the laborious and time-costly measurement of abbreviated cyclosporin (4-h) profiles. However, despite the ability of C_2 concentrations to accurately predict acute renal allograft rejection, the predictive performance of the 4-h AUC remains superior with reference to clinical efficacy and toxicity endpoints [75,84,85]. Kaplan *et al.* was able to show in a small group of recipients of simultaneous kidney–pancreas grafts that one formal cyclosporin $AUC_{0-12\,h}$, performed 1 month post-transplantation, was sufficient to identify patients with poor absorption and recurrent rejection [93]. This small study illustrates that the measurement of abbreviated

AUC profiles, even early after transplantation, can be limited in number without the sacrifice of clinically relevant information. Based on long-term clinical observational data, the time-related pharmacokinetic behaviour of calcineurin inhibitors can be identified [70,84,86] in order to strategically and prospectively plan the optimal time points post-transplantation on which abbreviated AUC profiles would provide the most useful and critical information.

Mycophenolate mofetil

The current issues involving TDM for MPA are distinct from that of calcineurin inhibitors. It is clear in renal transplantation that predose trough plasma concentrations of MPA do not predict total drug exposure measured as 12-h AUC and that abbreviated 2-h AUC profiles, usually obtained by three concentration sampling points (C_0, C_{30} or C_{40} and C_2), are accurate in predicting total exposure [94–96]. The free (pharmacologically active) fraction of the drug is determined by allograft (dys)function [97–100] through alterations in albumin binding [101,102] and secondary to increased levels of the glucuronide MPA metabolites that are excreted by the kidney: the inactive MPAG-glucuronide and the pharmacologically active Acyl-glucuronide [27,103,104]. The fact that the latter is also implicated in the occurrence of clinically relevant side-effects like diarrhoea [105] and anaemia [27], further complicates the situation. Finally, the pharmacokinetics of MPA are characterized by a specific postoperative evolution that is mainly determined by the daily dose of the drug [106] and the concomitant calcineurin inhibitor [25,28].

In one prospective concentration-controlled clinical trial a significant relationship was demonstrated between MPA AUC as well as predose MPA trough concentration and biopsy-proven acute rejection whereas the daily dose of MMF was related to side-effects [107]. Patients in the latter trial received MMF combined with cyclosporin A. Predefined target MPA AUC values associated with significant less rejection varied between 32.2 mg h/l ('intermediate' group) and 60.6 mg h/l ('high' group) in the latter trial and the actually obtained MPA AUC values at week 20 postoperative even exceeded these targets by 60% because of over-correction [107]. These wide MPA AUC targets and the absence of a clear association with side-effects towards the upper end of the AUC range, made it difficult to define a therapeutic window. In tacrolimus-treated patients this relationship was even more difficult to establish [108]. An MPA C_0 threshold for toxicity was determined at 3 mg/l with a high specificity (91.5%) but low sensitivity (38.7%) in tacrolimus-treated renal recipients while the corresponding AUC_{0-12} threshold was 37.6 mg h/l (sensitivity 83.3%, specificity 59.6%) [109]. We determined in a prospective study in 100 *de novo* tacrolimus-treated recipients a therapeutic window between 45 and 60 mg h/l for MPA $AUC_{0-12\ h}$, based on abbreviated AUC measurements [68]. The corresponding C_0 levels ranged from 2.5 to 4 mg/l but did not correlate completely accurate with total exposure and therefore have to be interpreted cautiously when applying to clinical practice [68]. The reason why in renal transplantation, contrary to, for example cardiac transplantation [110], a simple clear-cut relationship between MPA exposure and efficacy cannot be derived, is probably related to differences in renal function, different kinetics of MPA metabolites [29] and the fact that these relationships between exposure and clinical endpoints are time-dependent as one would expect from the natural dynamics of MPA pharmacokinetics post-renal transplantation [106].

For anaemia we could also demonstrate a relationship with MPA exposure (AUC), like others [111], as well as for leukopenia [68]. Other MMF-associated adverse events like gastrointestinal side-effects were poorly associated with MPA exposure in our study as shown by others [112].

In order to address at least some of the problems of applying abbreviated MPA AUC measurements in clinical practice, a large international multicentre study in renal transplantation has recently commenced, examining the clinical relevance of concentration-controlled MMF dose adjustments based on abbreviated (2-h) target AUC profiles versus fixed dose treatment. Whether TDM of MMF is advantageous in terms of both efficacy (prevention of acute rejection) and toxicity will be assessed in this trial.

mTOR inhibitors

It is without any doubt that TDM is necessary for clinical application of sirolimus in solid organ transplantation [113,114]. Numerous studies have demonstrated that, similar to calcineurin inhibitors, mTOR inhibitors are characterized by a narrow therapeutic window, highly variable absorption and large intra- and interindividual variability in pharmacokinetic behaviour [115,116]. In addition, the significant pharmacokinetic interaction between sirolimus and cyclosporin A [117] necessitates concentration monitoring each time dose adjustments are performed. However when sirolimus is used in a low fixed dose of 2 mg/day in combination with a full dose cyclosporin (separated by 4 h), systematic concentration monitoring can usually be omitted [115]. The latter implies a minimal obtained blood sirolimus trough concentration of 5 ng/ml (HPLC-UV) as this signifies a clinical threshold differentiating for acute rejection, at least in combination with cyclosporin [118,119]. Whether long-term association of sirolimus and

tacrolimus does not cause alterations of both drug's blood concentration, remains unclear [120,121]. Reports showing an effect of a standard dose tacrolimus on maintenance low dose sirolimus imply an interaction [121]. Interestingly, significant decreases in tacrolimus exposure were observed early after transplantation in another report using low doses of sirolimus [122].

The upper limit of sirolimus exposure, discriminating the onset of adverse events, was determined around 15 ng/ml in several studies combining the former with cyclosporin [118,119]. Especially for thrombocytopenia and hypertriglyceridaemia an exposure–response relationship could be defined. Using receiver operating characteristic curves, an inflection point for thrombocytopenia of 14 ng/ml, hypertriglyceridaemia of 11 ng/ml and hypercholesterolaemia of 13 ng/ml could be determined [119]. With everolimus, a similar exposure–response relationship could be discerned for thrombocytopenia and to a lesser extent for hypertriglyceridaemia and hypercholesterolaemia [123,124].

The above data permit us to prudently outline a therapeutic window for sirolimus between 5 and 15 g/ml in combination with cyclosporin. However, when cyclosporin is eliminated from this combination higher target levels between 12 and 20 ng/ml are advised based on the results of a large multicentre trial examining the feasibility of early calcineurin-inhibitor elimination [125]. As sirolimus seems to induce a different type of allograft nephrotoxicity, distinct from that of calcineurin inhibitors [126,127], multiple clinical studies have been performed examining the role of sirolimus in calcineurin-inhibitor free protocols [1,128,129]. The target concentration ranges for sirolimus in these trials have not yet been completely validated, especially long-term, but seem to concur with those established in the elimination trial [125]. Particularly, the combination of sirolimus with mycophenolate mofetil, corticosteroids and induction therapy with monoclonal antibodies against the IL-2 receptor has led to the preliminary definition of a clinically useful target concentration window (C_0: 10–12 ng/ml in the first 6 months post-transplantation and 5–10 ng/ml thereafter), especially with respect to avoidance of acute rejection and nephrotoxicity [1]. Combining sirolimus with tacrolimus has proven to be feasible and effective [4] but has not yet resulted in definite clinical advantages, also because recent trials have a short follow-up. A validated target therapeutic window for sirolimus in the latter combination, based on predose trough concentrations, is currently not available [4]. However, as severe acute renal failure has been reported after exposure to sirolimus and tacrolimus [130], a combined therapeutic target window for both drugs will be required.

Contrary to calcineurin inhibitors, there seems to exists a better correlation between predose trough blood sirolimus concentration and total dose-interval steady-state AUC_{0-24} [119–121]. At this point in time the clinical necessity and superiority of limited sampling concentration profiles has not been definitely established [131].

Conclusion

Therapeutic drug monitoring remains the cornerstone of today's concentration-controlled management of immunosuppressive therapy in renal transplantation. Despite the spectacular evolution in pharmacodynamic research, the progressive unravelling of the individual pharmacogenomic profile and the useful application of drug probes in order to identify drug metabolism, clinicians still have to rely on classic TDM for daily patient care. In recent years it has become clear that abbreviated AUC concentrations are stronger and more reliable predictors of concentration–exposure and exposure–response relationships for the majority of currently used immunosuppressive drugs. Comparative prospective clinical trials are necessary in order to objectively weight the advantages of abbreviated AUC monitoring against classic predose trough concentrations, in terms of cost-effectiveness, feasibility and clinical relevance with regard to long-term patient morbidity and mortality and graft survival. At the same time, it is a challenging task to further refine the therapeutic window for the individual drugs and more importantly for the increasing number of different drug combinations. In order to do so, large prospective observational pharmacokinetic studies are mandatory, carefully examining the relationship between drug exposure and clearly defined efficacy and toxicity endpoints. This relationship has to be studied in a long-term, time-dependent manner in order to recognize important changes in drug (and drug metabolite) pharmacokinetics and identify crucial time points after successful transplantation for optimal use of abbreviated AUC monitoring.

Ultimately, obtaining answers to these questions will enable clinicians to improve long-term patient and renal graft survival.

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