sensus Conference: American College of Medical Genetics. *Am J Med Genet* 1997; 72: 468–477
3. EBPG EGoRT. European Best Practice Guidelines for Renal Transplantation. Section IV: long-term management of the transplant recipient. IV.11 Paediatrics (specific problems). *Nephrol Dial Transplant* 2002; 17: 55–58
4. Wolfe AR, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY *et al*. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999; 341: 1725–1730
5. Levenson JL, Olbrisch ME. Psychosocial evaluation of organ transplant candidates. A comparative survey of process, criteria, and outcomes in heart, liver, and kidney transplantation. *Psychosomatics* 1993; 34: 314–323
6. Ohta T, Motoyama O, Takahashi K, Hattori M, Shishido S, Wada N *et al*. Kidney transplantation in pediatric recipients with mental retardation: clinical results of a multicenter experience in Japan. *Am J Kidney Dis* 2006; 47: 518–527
7. Martens MA, Jones L, Reiss S. Organ transplantation, organ donation and mental retardation. *Pediatr Transplant* 2006; 10: 658–664
8. Baqi N, Tejani A, Sullivan EK. Renal transplantation in Down syndrome: a report of the North American Pediatric Renal Transplant Cooperative Study. *Pediatr Transplant* 1998; 2: 211–215
9. Luckasson R, Reeve A. Naming, defining, and classifying in mental retardation. *Ment Retard* 2001; 39: 47–52
10. Medina-Pestana J. Organization of a high-volume kidney transplant program—the "assembly line" approach. *Transplantation* 2006; 81: 1510–1520
11. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41
12. Benedetti E, Asolati M, Dunn T, Walczak DA, Papp P, Bartholomew AM *et al*. Kidney transplantation in recipients with mental retardation: clinical results in a single-center experience. *Am J Kidney Dis* 1998; 31: 509–512
13. 2007 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients. Transplant Data 1997–2006. *Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation*. Rockville, MD, USA, 2007
14. Hitzler JK. Acute megakaryoblastic leukemia in Down syndrome. *Pediatr Blood Cancer* 2007; 49: 1066–1069
15. Andrés A. Cancer incidence after immunosuppressive treatment following kidney transplantation. *Crit Rev Oncol Hematol* 2005; 56: 71–85
16. Ugazio AG, Maccario R, Notarangelo LD, Burgio GR. Immunology of Down syndrome: a review. *Am J Med Genet* 1990; 7: 204–212
17. de Hingh YC, van der Vossen PW, Gemen EF, Mulder AB, Hop WC, Brus F *et al*. Intrinsic abnormalities of lymphocyte counts in children with Down syndrome. *J Pediatr* 2005; 147: 744–747
18. Wu O, Levy AR, Briggs A, Lewis G, Jardine A. Acute rejection and chronic nephropathy: a systematic review of the literature. *Transplantation* 2009; 87: 1330–1339

Received for publication: 27.7.09; Accepted in revised form: 9.2.10

Nephrol Dial Transplant (2010) 25: 2757–2763
doi: 10.1093/ndt/gfq096
Advance Access publication 26 February 2010

High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation

Lennaert C.P. Borra[1], Joke I. Roodnat[2], Judith A. Kal[2], Ron A.A. Mathot[1], Willem Weimar[2] and Teun van Gelder[1,2]

[1]Department of Hospital Pharmacy, Molewaterplein 40, 3015GD Rotterdam, The Netherlands and [2]Department of Internal Medicine, Erasmus Medical Center, P.O. Box 2040, 3000 CA, Rotterdam, The Netherlands

Correspondence and offprint requests to: Teun van Gelder; E-mail: t.vangelder@erasmusmc.nl

Abstract

Background. We hypothesized that a high within-patient variability in clearance of tacrolimus and mycophenolate mofetil (MMF) would put patients at risk for periods of over- or underimmunosuppression and would thus lead to long-term chronic allograft nephropathy and graft loss after transplantation.

Methods. From 297 patients transplanted between 1 January 2000 and 31 December 2004, the within-patient variability in clearance was calculated from tacrolimus whole-blood concentrations and mycophenolic acid (MPA) plasma concentrations drawn between 6 and 12 months post-transplantation. As a primary outcome, a composite end point consisting of graft loss, biopsy-proven chronic allograft nephropathy and 'doubling in plasma creatinine concentration in the period between t = 12 months post-transplantation and last follow-up' was used.

Results. In the study population of 297 patients, 34 patients reached the primary end point of graft failure. The within-patient variability in the clearance of tacrolimus and three other covariates are significant risk factors for reaching the composite end point of failure [P-values for

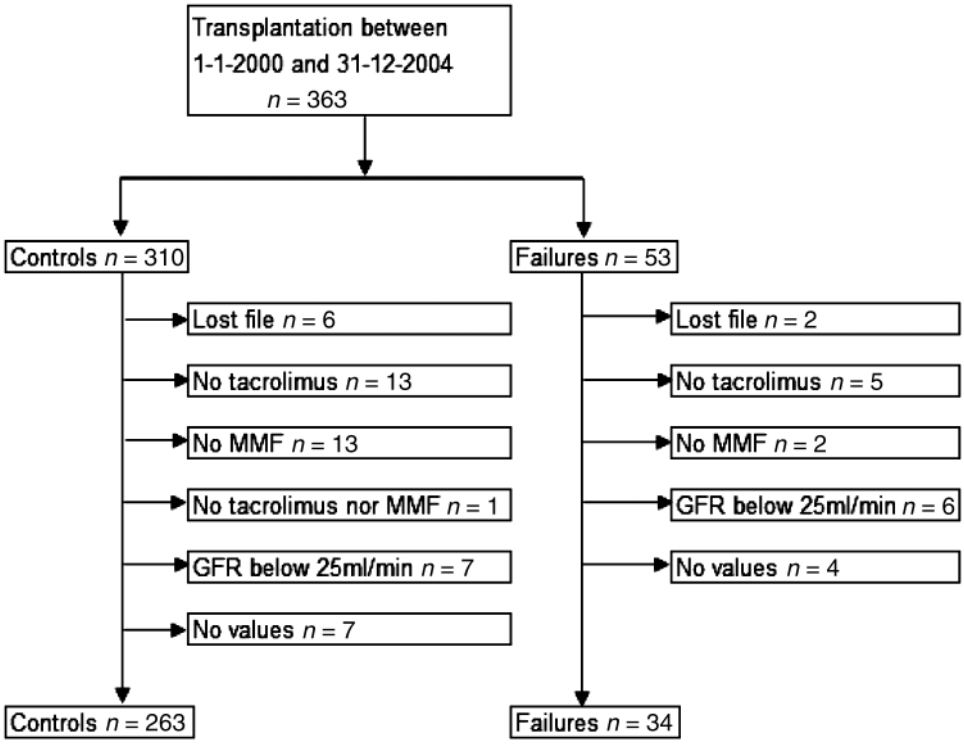

Fig. 1. Patients recruited for the study and overview of the reasons for exclusion censored for patient death.

intraindividual tacrolimus variability = 0.003, biopsy-proven acute rejection (BPAR) = 0.003, recipient age at transplantation = 0.005]. The mean tacrolimus concentration for controls [7.4 (± 2.9) ng/mL] and for failures [6.9 (± 2.5) ng/mL] was similar. Within-patient variability in the clearance of MPA was not related to reaching the composite end point of failure.

Conclusions. This study shows a significant relationship between the high within-patient variability in the clearance of tacrolimus, but not for MPA, and long-term graft failure.

Keywords: tacrolimus; variability; mycophenolate mofetil; pharmacokinetics; clearance

Introduction

The use of the currently available immunosuppressive drugs for solid organ transplantation is complicated by a considerable variability in the pharmacokinetics between individual patients [1]. As these drugs are considered to have a narrow therapeutic window, a routine therapeutic drug monitoring is performed for the calcineurin inhibitors cyclosporine (CsA) and tacrolimus (Tac), for the mammalian target of rapamycin (mTOR) inhibitors sirolimus (SRL) and everolimus (ERL), and increasingly also for mycophenolate mofetil (MMF) [2]. The determinants for this variability between patients have been extensively studied and include genetic and non-genetic factors [3–9].

In contrast to the large number of studies investigating between-patient variability in the pharmacokinetics of these critical dose drugs, there are very few studies that focused on within-patient variability (also known as 'inter-occasion variability'). Drugs with a high within-patient day-to-day variability may be less suited for therapeutic drug monitoring (TDM) compared to drugs with a high between-patient but low within-patient variability [10]. The intrapatient variability is visualized by fluctuating concentrations of the immunosuppressive drug within a certain period of time during which drug dosage was unchanged. A high within-patient variability complicates TDM as the drug concentrations will frequently be above or below the therapeutic window, putting the patient at risk for toxicity in the case of overexposure or for acute rejection in the case of drug concentrations below the lower threshold of the therapeutic window. It is therefore logical to expect that long-term outcome is impaired in patients with higher within-patient variability.

In the literature, however, there is little support for this assumption. One of the few studies that investigated the clinical importance of within-patient variability of cyclosporine pharmacokinetics is the study by Kahan *et al.* [11]. In this study, the role of within-patient variability of CsA exposure in predisposing renal transplant recipients to the occurrence of chronic rejection was investigated. The incidence of chronic rejection over a period of 5 years was 24% among the less variable *versus* 40% among the variable cohort. The degree of variability displayed by any individual could only be predicted by serial measurements of CsA concentrations and not by demographic features, laboratory determinations, clinical characteristics, individual or mean values of any observed CsA concentration, or other pharmacokinetic parameters calculated following a

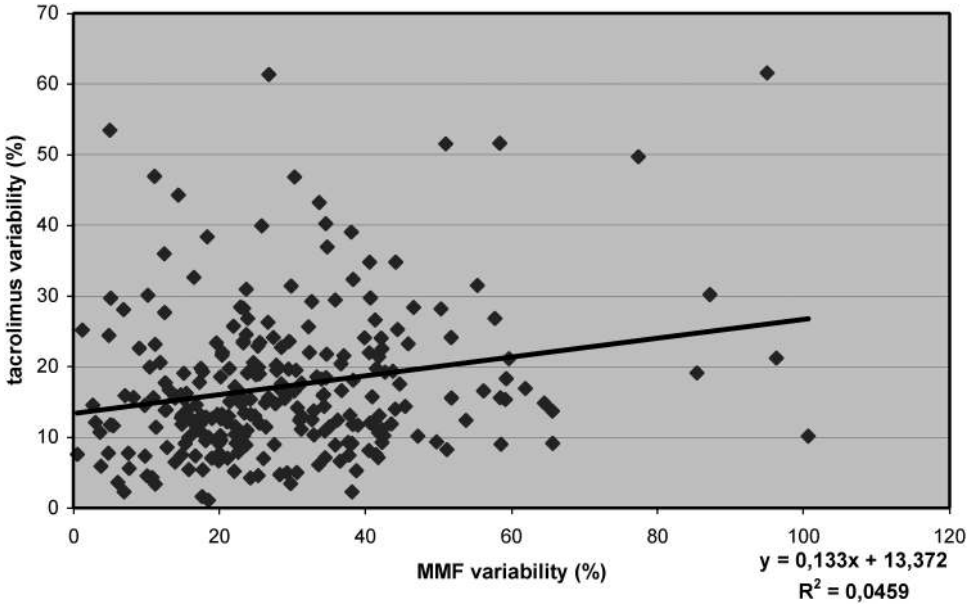

Fig. 2. MPA within-patient variability in clearance *versus* Tac within-patient variability in clearance.

single drug exposure. The authors concluded that strategies that reduce within-patient variability of CsA exposure over time may lead to reductions in chronic allograft loss.

For Tac and MMF, there are no data that indicate that within-patient variability of the pharmacokinetics is a predictor of poor outcome. Given the increasing use of Tac as the calcineurin inhibitor of first choice, in combination with MMF, we decided to investigate the clinical relevance of a high within-patient variability for these drugs. We hypothesized that a high within-patient variability for Tac and/or MMF would put patients at risk for periods of over- or underimmunosuppression and would thus lead to long-term chronic allograft nephropathy and graft loss after transplantation.

Materials and methods

For this study, all 297 patients transplanted in our centre between 1 January 2000 and 31 December 2004 were included in the study. The main inclusion criteria were as follows: (i) treatment with the immunosuppressive drugs Tac and MMF in the period between 6 and 12 months postrenal transplantation; (ii) presence of a functioning graft at 12 months, with an estimated glomerular filtration rate (GFR) [abbreviated Modification of Diet in Renal Disease (aMDRD) formula] of 25 mL/min or higher. Figure 1 shows the total number of patients who received a kidney transplant between 1 January 2000 and 31 December 2004 and the reasons for excluding some of these patients from the analysis. Patients were followed up until reaching the end points graft loss, death or until the last follow-up. All data were collected retrospectively. The routine immunosuppressive regimen in our centre is the combination of Tac and MMF, with prednisone treatment given for the first three post-operative months only.

Calculation of variability

Predose Tac whole-blood concentrations and predose mycophenolic acid (MPA) plasma concentrations were measured using an enzyme-multiplied immunoassay (EMIT, Dade-Behring, Germany). The EMIT method has a lower limit of quantification of 0.5 mg/L and an intratest variability of 6.5%. Our laboratory participates in the international proficiency testing scheme of the Analytical Unit of the St George's Hospital in London. All patients were on oral and twice daily administration of Tac and MMF therapy. For 297 Tac- and for 262 MMF-treated patients, we collected all the results of drug concentrations measurements for all outpatient visits within the period of 6–12 months post-transplantation. For all the patients, there were at least three samples available for calculation of the within-patient variability. The mean number of samples per patient was 4.6 ± 1.8 for Tac and 4.1 ± 1.7 for MMF. As not all patients were treated with a stable dose during the period between 6 and 12 months post-transplantation, the reached whole-blood Tac and plasma MPA concentrations were corrected for the drug dosage. The resulting quotient for the concentration divided by dose is a measure of apparent oral clearance. The within-patient variability in Tac and MPA apparent oral clearance (in the rest of this manuscript referred to as clearance) between 6 and 12 months post-transplantation (Tx) was calculated using the formula shown below.

$$\{[(X_{\text{mean}} - X_1) + (X_{\text{mean}} - X_2) \ldots\ldots + (X_{\text{mean}} - X_n)]$$
$$/n\}/\ X_{\text{mean}} * 100 = \text{intra individual variability (\%)}$$

Formula 1: Formula used for calculating the intraindividual variability. X_{mean} is the mean Tac concentration of all available samples, X_1 is the first available Tac concentration measurement, X_2 is the second…, etc.

The patients were divided into low and high intraindividual variability groups using the median variability for each of the two drugs as the cutoff value. As a primary outcome, a composite end point consisting of graft loss, biopsy-proven chronic allograft nephropathy and doubling of plasma creatinine concentration in the period between 12 months post-Tx and last follow-up was used. In this paper, we have chosen to use 'graft failure' to refer to this composite primary end point. A total of 16 patients in the study died after 12 months post-Tx, with a functioning graft and without signs of chronic allograft nephropathy. These patients were considered to have not reached the primary end point and were censored. In Figure 1, the patient numbers shown are censored for death. In order to examine the acceptability of this procedure, all analyses have been repeated for the patient population without censoring for death. The number of patients reaching the primary end point for those analyses was (34 + 16 =) 50.

Regression analysis

Subsequently, a Cox regression analysis was performed using graft failure as time indicator. The included covariates besides within-patient variability of Tac and MMF were sex, age, episode of biopsy-proven acute rejection in the first year post-Tx, the most recent percentage of panel-reactive antibodies (%PRA) pretransplantation, serum creatinine at 12 months, highest % PRA, number of previous transplantations, living donor, pre-emptive

Table 1. Baseline characteristics of kidney transplant patients reaching the composite end point of failure and controls

Baseline characterisrics	Controls (n = 263)	Failures (n = 34)	P-value
Sex	62% male	71% male	0.349
Mean age recipient at Tx	48 years	41 years	0.122
Mean Tx number	1.21	1.38	0.015
Pre-emptive transplantation	10%	9%	0.793
Living donor	54%	47%	0.471
Mean number of HLA mismatches	2.64	2.62	0.894
Delayed graft function	22%	24%	0.846
Acute rejection in first year	9%	24%	0.011
Mean recent %PRA	5%	13%	0.001
Mean historic highest %PRA	14%	26%	0.022
Mean creatinine (μmol/L) at 12 months	121	137	0.138
Mean Tac concentration	7.4	6.9	0.07
Mean MPA concentration	2.37	2.27	0.602

transplantation, human leukocyte antigen (HLA) mismatch and delayed graft function post-Tx.

Results

The mean follow-up of the 297 patients in this study was 1849 ± 585 days (range 1096–2811 days). Of the 34 patients who reached the composite primary end point, 29 patients suffered from graft loss, 3 patients had biopsy-proven chronic allograft nephropathy (not yet leading to graft loss) and 2 patients had a doubled creatinine (the latter patients with a doubled creatinine had creatinine values of 481 and 1016 μmol/L, respectively, and were clearly well on their way to graft loss). The three patients with biopsy-proven chronic allograft nephropathy (not yet leading to graft loss) all suffered from a clinically evident renal function loss, with the new appearance of proteinuria and creatinine values of 214 (2 years after Tx), 162 (3 years after Tx) and 308 (3 years after Tx). At the last follow-up, the mean creatinine value in controls was 133 μmol/L.

The within-patient variability in Tac clearance was lower compared to the within-patient variability in MPA clearance. For both Tac and MPA, there was a wide range of values, with some individuals having a within-patient variability <5%, while others had a variability of >50%. The mean within-patient variability for Tac was 17.0% (median 14.9%), and for MPA 28.8% (median 25.5%). For both Tac and MPA, the patients were distributed into two groups based upon their variability using the median as a cutoff. For Tac, this resulted in 148 patients in the low-variability group, with a mean variability of 9.6%, and in 149 patients with high variability, with a mean variability of 24.2%. For MPA, the mean variability in the low-variability group (n = 131) was 16.5%, and for the group with high variability (n = 131) 41.1%. There was no difference in the incidence of acute rejection in the first post-Tx year between patients with a low *versus* a high within-patient variability: for Tac 12% *versus* 9% and for MMF 11% *versus* 11%, respectively. The patients with high variability had a higher mean number of Tac dose changes in the 6-month observation period compared to patients with low variability (0.75 ± 0.89 *versus* 0.47 ± 0.60 dose changes; P = 0.002). Numerically, patients reaching the primary end point had more dose changes compared to controls, but this difference did not reach statistical significance (0.79 ± 0.91 *versus* 0.59 ± 0.74 dose changes; P = 0.193).

To check whether the same patients who showed a high within-patient variability in Tac clearance also had a high variability in MMF clearance, a plot was made with MPA variability on the *x*-axis *versus* Tac variability on the *y*-axis. The results are shown in Figure 2. Clearly, there is only a poor correlation between MPA and Tac variability. Apparently, patients with a high within-patient variability for Tac do not necessarily also have a high within-patient variability for MPA clearance. Baseline characteristics (the list of items shown in Table 1) did not show differences between patients with a low *versus* a high variability for Tac nor for MPA (data not shown).

For Tac, in the group of patients reaching the primary end point (failures), there were significantly more patients with a high within-patient variability (24/34 = 70.6%) compared to low within-patient variability (10/34 = 29.4%) (P = 0.011) (Table 2). In contrast to the failures, in the controls (patients not reaching the primary end point), there was no difference in the proportions of pa-

Table 2. Distribution of controls and failures in the low and high intraindividual variability group for Tac and MMF, respectively

	Tac		MMF	
Intraindividual variability	Control (263)	Failure (34)	Control (230)	Failure (32)
Low	138/263	10/34	116/230	15/32
	52.5%	29.4%	50.4%	46.9%
High	125/263	24/34	114/230	17/32
	47.5%	70.6%	49.6%	53.1%

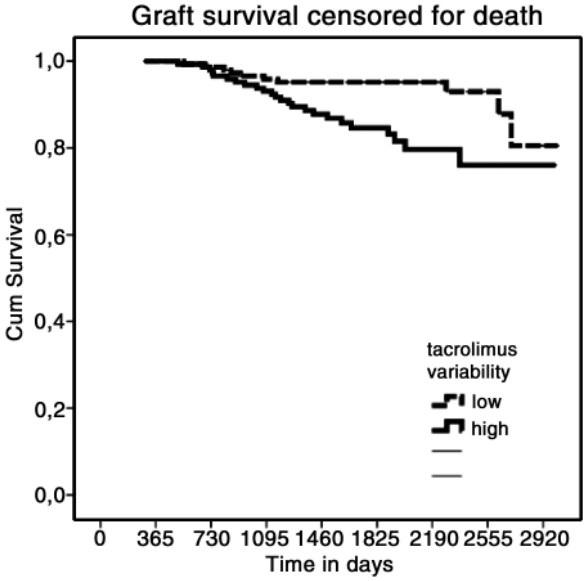

Fig. 3. Graft survival for patients with a low and a high within-patient variability for Tac clearance (P = 0.003).

tients with high *versus* low variability (47.5% *versus* 52.5%). The mean Tac concentration for patients in the controls was 7.4 (± 2.9) ng/mL, and for the failures 6.9 (± 2.5) ng/mL (P = 0.07). For MMF, we did not find an over-representation of patients with a high within-patient variability among the patients reaching the primary end point. In the 32 patients reaching the primary end point, 52.1% had a high and 46.9% a low variability for MPA clearance (P = 0.78).

In order to visualize the clinical importance of the observed effect of within-patient variability of Tac clearance on graft failure, a Kaplan–Meier curve was drawn for patients with a low and a high variability for Tac clearance (Figure 3). The clinical impact of differences in within-patient variability of Tac clearance on graft failure (P = 0.003) is comparable with the well-known impact of experiencing an acute rejection episode within the first year post-Tx (P = 0.003), a finding that we confirm in our data set, as shown in Figure 4. However, sensitivity and specificity, with a cutoff of 14.9%, were 70.6% and 52.9%, respectively. The receiver-operating characteristic (ROC) area is 0.59 [95% confidence interval (CI) 0.50–0.69], which means that the discriminative value is limited.

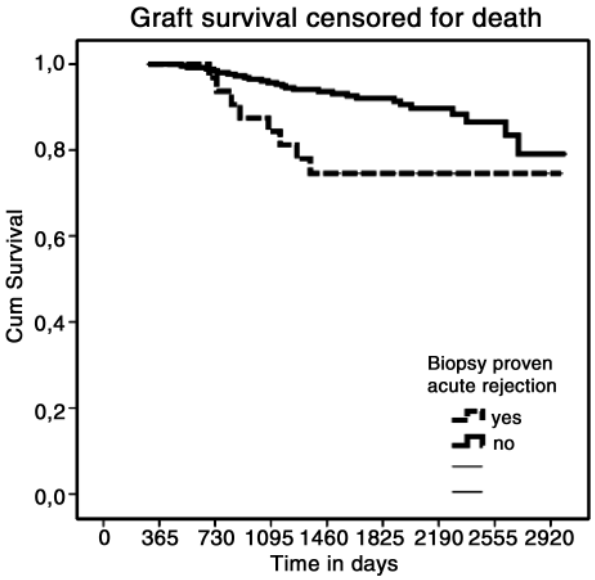

Fig. 4. Graft survival for patients with *versus* without an acute rejection episode within the first post-Tx year (P = 0.003).

Table 3. Univariate Cox proportional hazards analysis on the influence of covariates related to the outcome of graft failure censored for death

	P-value	exponent B
Tac variability	0.001	4.237
BPAR in first year	0.006	3.567
Recipient age at Tx	0.018	1.030
Creatinine at t = 12 months	0.118	1.008
Sex	0.144	2.046
%PRA, most recent	0.326	1.011
%PRA, maximum	0.378	1.008
Living donor	0.472	1.408
HLA mismatch	0.579	1.175
Tx number	0.629	1.183
Pre-emptive transplantation	0.743	1.248
Delayed graft function	0.756	1.182
MMF variability	0.809	1.100

BPAR, biopsy-proven acute rejection.

Not unexpectedly, the patients who reached the primary end point (failures) differed from controls in a number of covariates known to be associated with poor outcome. In Table 1, these covariates and the differences between the failures and controls are shown. All of these parameters are weighted in the final analysis to correct for confounding. A chi-square test was performed to calculate the significance of the difference in covariates between the control and failure groups.

To determine whether the within-patient variability of Tac clearance is a predictor of poor outcome, a univariate and multivariate Cox regression analyses were performed. In the univariate analysis, the significance was found for three covariates, including the within-patient variability of Tac clearance, as listed in Table 3. In the multivariate Cox regression analysis, we show that the covariate within-patient variability of Tac clearance is indeed associated with the occurrence of graft failure. This influence proved to be independent of the influence of any of the other variables with a significant influence on graft failure. When the data were uncensored for patient death, still a significant relation was found for the within-patient variability of Tac clearance (data not shown).

Table 4. Results of the multivariate Cox regression analysis: graft failure censored for death

	P-value	exponent B
Tac variability	0.003	3.125
BPAR in first year	0.003	3.390
Recipient age at Tx	0.005	1.031

BPAR, biopsy-proven acute rejection.

Discussion

In this study, a significant correlation is found between the within-patient variability of Tac clearance and reaching the primary composite end point of graft failure. Although between-patient variability has been studied extensively, there are hardly any studies in which the importance of within-patient variability of immunosuppressive drugs for clinical outcome has been investigated. To the best of our knowledge, this is the first study to demonstrate that high within-patient variability for Tac clearance leads to a reduced graft survival.

Patients with high variability in clearance have more fluctuation in the measured Tac concentrations. As a result, the blood concentrations will more often be outside the therapeutic window causing either toxicity (in the case of overexposure) or an episode of acute rejection (in the case of underexposure). This may explain the poor outcome associated with high within-patient variability.

In this study, we only analysed the variability in Tac clearance between 6 and 12 months post-Tx and correlated this variability to the composite end point of graft failure in the following 12 months. We decided not to take all Tac concentrations available from all outpatient visits, as impairment of renal function during follow-up after the first year may have impacted on Tac dosing. We also decided to include only the data obtained during outpatient visits, as we expected that the Tac pharmacokinetics of patients admitted to the hospital might have been influenced by interventions such as antibiotics or pulse corticosteroid therapy. Within the first post-Tx year, there was no difference in the incidence of acute rejection between patients with a low *versus* a high within-patient variability.

One of the well-known risk factors for chronic allograft nephropathy is an episode of acute rejection [12]. In this study, we also find that acute rejection is a significant risk factor for treatment failure. We show that the influence of a high within-patient variability in Tac clearance on graft survival is similar to that of a history of biopsy-proven acute rejection in the first year post-Tx. This underlines the importance of variability for a poor long-term outcome. Sensitized patients were disproportionately over-represented in the failures (Table 2), but the uni- and multivariate analyses did not find that historic or recent %PRA was associated with graft failure (Tables 3 and 4).

Apart from the within-patient variability of Tac and an episode of acute rejection, another factor that proved to be significantly related to the composite end point of renal graft failure was the age of the recipient at transplantation. Our data on Tac within-patient variability are in line with the published results of CsA within-patient variability. Kahan *et al.* also showed a significantly increased risk of chronic rejection in patients with higher variability. An economic analysis was also part of that study, and cost was higher in patients with higher variability. We did not assess the cost in our study, but evidently, more graft loss will undoubtedly lead to disproportionately higher cost. A weakness of our study is that, within the group of patients reaching the primary end point of graft failure, we cannot distinguish between the immunologically caused loss of function and the drug-induced nephrotoxicity. Due to the retrospective nature of the analysis, we cannot provide data such as donor-specific antibodies (DSA) to distinguish between an immunological or non-immunological pathogenesis.

In the present study, the variability for MPA was almost double the variability for Tac. This is not unexpected, as the pharmacokinetic profile of MPA is complex. MPA, as opposed to most other agents utilized in transplantation, undergoes glucuronidation as the first step in its metabolism [13]. In addition, it undergoes extensive enterohepatic recirculation, and its clearance is highly dependent on protein binding. Moreover, due to the changes in the clearance, MPA exposure will rise in the first year after transplantation, adding to the overall within-patient variability. In this study, we could not find differences in baseline characteristics between patients with a low *versus* a high variability for Tac or MPA. Future prospective studies should focus on analysing the determinants of intraindividual variability for these drugs, including registration of diet and measures of adherence to medication.

The significant difference in the number of failures between the low and high within-patient variability groups as found in this study for Tac was not found for MMF. This difference between Tac and MMF was unexpected. In the past few years, a number of studies examining the effects of MMF have been carried out. Those studies showed a significant effect of MMF on both short- and long-term outcomes. Apart from the impact of MMF on acute rejection and graft survival, the exposure to the active metabolite, MPA has also been shown to be related to the risk of acute rejection. For MPA exposure, certain target concentrations have been defined. One would expect that patients with high variability have MPA exposures outside the target range more often than patients with a low variability. Therefore, a high within-patient variability in MPA clearance could potentially lead to more graft failures in the long term. The results of this study do not show a significant correlation between within-patient variability of MPA clearance and graft failure. Possibly, this is explained by a wider therapeutic window for MMF compared to Tac. Due to the wider therapeutic window of MMF, even patients with a high within-patient variability would not pay a penalty for the fluctuation of their MPA levels. Another possibility is that, in our study, MMF dose was rather high, so that even patients with episodes of lower exposure to MPA are not below the lower limit of the therapeutic window. MMF is not a nephrotoxic drug, and thus, exposure to levels above the therapeutic window would not compromise renal function. The mean MPA predose concentration in this patient cohort was rather high: at 6 months, the mean MPA concentration was 2.46 (± 2.0)mg/L, and at 12 months, the mean MPA concentration was 2.18 (± 1.7)mg/L. This supports the explanation that the relatively wide margin of MPA exposure above the lower threshold of the presumed therapeutic window for MPA prevents against any detrimental effects of high variability.

The most relevant result of this study is the significant correlation between the within-patient variability in Tac clearance and graft survival. Because of this correlation, it is interesting to speculate on the potential causes for this variability. The first thing that comes to mind is patient compliance, which has been previously suggested to play

a critical role in the outcome of transplantation. In this study, however, patient non-compliance may not be the main underlying cause of within-patient variability. If patient compliance were indeed the main underlying cause for a high within-patient variability, one would expect a better correlation between the within-patient variability for Tac and MMF. As shown in Figure 3, a high within-patient variability for Tac does not necessarily mean an equally high variability for MMF. It is unlikely that a patient is correctly taking one of his drugs while being non-compliant for the other drug. Future studies should investigate the influence of non-compliance on within-patient variability.

According to some investigators, concomitant diet and over-the-counter medication were found to be more likely factors to contribute to a high within-patient variability [14]. The mechanism that is thought to be responsible for this is the interaction between immunosuppressive drugs and intestinal physicochemical factors such as pH, polarity, particle size, splanchnic blood flow, bile flow and bowel content. This hypothesis is supported by studies showing a lower variability for a microemulsion of CsA in comparison to the regular formulation [15]. Nevertheless, it is important to emphasize that it is necessary to make patients aware of the importance to take their medication at fixed times every day and that not doing so can result in a higher risk of graft loss.

If intestinal physicochemical interactions with the immunosuppressive drugs are indeed the main factors causing a high within-patient variability, it is worthwhile to consider better monitoring of the patient's diet and comedication. When a patient shows an unexplained high within-patient variability in Tac clearance, switching Tac to another immunosuppressive drug may be considered, in order to prevent a dismal outcome.

Finally, the coefficient of variation for the assay used to measure Tac concentrations, the EMIT method, is ~5% for Tac concentrations between 5 and 10 ng/mL [16]. This variation will have contributed equally to the overall variability found in both the low- and the high-variability patients and did not influence the results of this study.

In conclusion, this study shows a significant relationship between high within-patient variability in clearance of Tac and reaching the composite end point of graft failure. We acknowledge that the data presented should be regarded as an alert to indicate the clinical relevance of intrapatient variability. The exact cutoff value for variability that may lead to detrimental outcome is to be established in prospectively designed studies. The results of this study did not show any relation between within-patient variability of MPA clearance and long-term graft failure.

Conflict of interest statement. None declared.

References

1. Srinivas TR, Meier-Kriesche HU, Kaplan B. Pharmacokinetic principles of immunosuppressive drugs. *Am J Transplant* 2005; 5: 207–217
2. Kahan BD, Keown P, Levy GA, Johnston A. Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. *Clin Ther* 2002; 24: 330–350
3. Saint-Marcoux F, Marquet P, Jacqz-Aigrain E, Bernard N, Thirty P, Le Meur Y. Patient characteristics influencing ciclosporin pharmacokinetics and accurate Bayesian estimation of ciclosporin exposure in heart, lung and kidney transplant patients. *Clin Pharmacokinet* 2006; 45: 905–922
4. Van Hest RM, van Gelder T, Vulto AG, Mathot RA. Population pharmacokinetics of mycophenolic acid in renal transplant recipients. *Clin Pharmacokinet* 2005; 44: 1083–1096
5. Hesselink DA, van Gelder T, van Schaik RH, Balk AH, van der Heiden IP, van Dam T. Population pharmacokinetics of cyclosporine in kidney and heart transplant recipients and the influence of ethnicity and genetic polymorphisms in the MDR-1, CYP3A4, and CYP3A5 genes. *Clin Pharmacol Ther* 2004; 76: 545–556
6. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients. *Clin Pharmacokinet* 2007; 46: 13–58
7. Dansirikul C, Morris RG, Tett SE, Duffull SB. A Bayesian approach for population pharmacokinetic modelling of sirolimus. *Br J Clin Pharmacol* 2006; 62: 420–434
8. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet* 2004; 43: 623–653
9. Djebli N, Rousseau A, Hoizey G, Rerolle JP, Toupance O, Le Meur Y. Sirolimus population pharmacokinetic/pharmacogenetic analysis and bayesian modelling in kidney transplant recipients. *Clin Pharmacokinet* 2006; 45: 1135–1148
10. Budde K, Glander P. Pharmacokinetic principles of immunosuppressive drugs. *Ann Transplant* 2008; 13: 5–10
11. Kahan BD, Welsh M, Urbauer DL *et al*. Low intraindividual variability of cyclosporin A exposure reduces chronic rejection incidence and health care costs. *J Am Soc Nephrol* 2000; 11: 1122–1131
12. Joosten SA, van Kooten C, Sijpkens YW, de Fijter JW, Pail LC. The pathobiology of chronic allograft nephropathy: immune-mediated damage and accelerated aging. *Kidney Int* 2004; 65: 1556–1559
13. West-Thielke P, Kaplan B. Therapeutic monitoring of mycophenolic acid: is there clinical utility? *Am J Transplant* 2007; 7: 2441–2442
14. Kahan B. High variability of drug exposure: a biopharmaceutic risk factor for chronic rejection. *Transpl Proc* 1998; 30: 1639–1641
15. Kahan BD, Dunn J, Fitts C, Van Buren D, Wombolt D, Pollak R. Reduced inter- and intrasubject variability in cyclosporine pharmacokinetics in renal transplant recipients treated with a microemulsion formulation in conjunction with fasting, low-fat meals, or high-fat meals. *Transplantation* 1995; 59: 505–511
16. Hesse CJ, Baan CC, Balk AH, Metselaar HJ, Weimar W, van Gelder T. Evaluation of the new EMIT enzyme immunoassay for the determination of whole-blood tacrolimus concentrations in kidney, heart, and liver transplant recipients. *Transplant Proc* 2002; 34: 2988–2990

Received for publication: 28.7.09; Accepted in revised form: 8.2.10