ORIGINAL ARTICLE

A high intrapatient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation

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SUMMARY

Tacrolimus is a critical dose drug with a considerable intrapatient variability (IPV) in its pharmacokinetics. We investigated whether a high IPV in tacrolimus exposure is associated with adverse long-term renal transplantation outcomes. Tacrolimus IPV was calculated from predose concentrations measured between 6 and 12 months post-transplantation of 808 renal transplant recipients (RTRs) transplanted between 2000 and 2010. One hundred and eighty-eight (23.3%) patients reached the composite end point consisting of graft loss, late biopsy-proven rejection, transplant glomerulopathy, or doubling of serum creatinine concentration between month 12 and the last follow-up. The cumulative incidence of the composite end point was significantly higher in patients with high IPV than in patients with low IPV (hazard ratio: 1.41, 95% CI: 1.06–1.89; $P = 0.019$). After the adjustment for several factors, the higher incidence of the composite end point for RTRs with a high IPV remained statistically significant (hazard ratio: 1.42, 95% CI: 1.06–1.90; $P = 0.019$). Younger recipient age at transplantation, previous transplantation, worse graft function (at month 6 post-transplantation), and low mean tacrolimus concentration at 1 year post-transplantation were additional predictors for worse long-term transplant outcome. A high tacrolimus IPV is an independent risk factor for adverse kidney transplant outcomes that can be used as an easy monitoring tool to help identify high-risk RTRs.

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Key words
intrapatient variability, kidney transplantation, tacrolimus, therapeutic drug monitoring, transplant survival

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Introduction

Tacrolimus (Tac) is widely used as part of the immunosuppressive regimen for kidney transplantation. It is a critical dose drug with a considerable intrapatient variability (IPV) in its pharmacokinetics, which is defined as the fluctuation in Tac concentrations within an individual patient over a certain period of time during which the Tac dose is unchanged [1,2]. A high IPV in Tac exposure may be caused by behavioral factors, interacting co-medication, food [1–5], and to a lesser extent, genetic factors [6–8]. Whatever the cause, a high Tac IPV may result in a Tac exposure, which is outside the therapeutic window. These patients may be at risk of underexposure and rejection, or Tac toxicity in the case of overexposure.

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Late allograft rejection and graft loss remain important problems in the field of solid organ transplantation. The first evidence for the clinical importance of Tac IPV was obtained by Borra *et al.* [9]. In this study, it was demonstrated that a high Tac IPV was associated with the reduced kidney transplant survival. In a Korean study, it was shown that renal transplant recipients (RTRs) with a high Tac IPV had a significantly higher risk to develop a biopsy-proven acute rejection (BPAR) than patients with a low Tac IPV (hazard ratio: 2.66; 95% CI: 1.39–5.06; $P = 0.003$) [10]. Recently, Sapir-Pichhadze *et al.* [11], in a study that included 356 adult RTRs, observed that a higher Tac IPV was associated with more late allograft rejection, transplant glomerulopathy, graft loss, and death with a functioning transplant. In pediatric kidney transplantation, a high Tac IPV has also been associated with increased late rejection and graft loss [12–14].

A limitation of the above-mentioned studies was their limited sample size and the relatively short follow-up period. The small number of events may have hampered the multivariate analyses of the obtained data. This prompted us to substantially enlarge our original study population [9] and extend the duration of clinical follow-up to evaluate in this extended population whether a high Tac IPV is associated with a composite end point consisting of late acute rejection, transplant glomerulopathy, graft loss, or doubling of serum creatinine.

Subjects and methods

Patients and setting

This was a retrospective cohort study. The study cohort included RTRs who were transplanted and followed at the Erasmus MC, University Medical Centre Rotterdam, the Netherlands, between January 2000 and December 2010. Adult (age >18 years) RTRs were included if they were treated with Tac and mycophenolate mofetil (MMF) in the period between 6 and 12 months after the kidney transplantation, survived the first post-transplant year, and had an estimated glomerular filtration rate (eGFR) of ≥25 ml/min at month 12 after the transplantation. Patients who were treated between months 6 and 12 with an immunosuppressive regimen that did not consist of Tac plus MMF or who received a multi-organ transplant were not included. Usage of low-dose prednisolone, which is given in our center in the first three postoperative months as a component of the routine immunosuppressive regimen, was not an exclusion condition.

Tac concentrations were determined in the whole blood by several kinds of immunoassays. Details on the sensitivity and reproducibility of the EMIT assay in our laboratory have been published previously [15]. Proficiency samples were obtained from the United Kingdom Quality Assessment Scheme (Dr. Holt, St George's Hospital Medical School, London, UK). The laboratory successfully participates in international proficiency testing schemes. The target Tac C_0 between 6 and 12 months post-transplantation was between 4–10 ng/ml.

End points

Because we hypothesized that a high IPV in Tac exposure could result in frequent under-immunosuppression, the outcome of interest was a composite end point named "event," which consisted of graft failure [defined as re-transplantation, (re)start of dialysis, or an eGFR ≤15 ml/min], late BPAR (i.e., occurring after month 12), histologically confirmed transplant glomerulopathy, or doubling of serum creatinine concentration in the period between month 12 after the transplantation and the last follow-up, taken the serum creatinine concentration at month 12 as a reference. Biopsies were performed for cause only. Patients who died with a functioning graft and who did not have signs of transplant glomerulopathy or acute rejection were considered not to have reached the end point and were censored.

Intrapatient variability and outcome variables

The variable of interest was the IPV of Tac. For its calculation, at least 3 predose Tac concentrations (C_0) for an individual patient had to be available. A median of 5 (range: 3–11) Tac C_0 measurements were used to calculate Tac IPV. Because RTRs are not on a stable Tac dose in the first phase after transplantation and because they often use interacting drugs [such as antibiotics and (pulse) glucocorticoids] in this period, only data on Tac exposure measured at outpatient clinic visits in the period of 6–12 months post-transplantation were collected. Tac concentration measurements obtained during hospitalization were not considered. As not all patients received a constant drug dose between months 6–12, the obtained C_0 were corrected for the corresponding daily Tac dose (C_0/D). The IPV in Tac exposure (from now on referred to as "Tac IPV") between months 6 and 12 post-transplantation was calculated as:

$$\text{IPV\%} = \frac{1}{T}\sum_{t=1}^{T}\frac{\text{abs}(X_t - \bar{X})}{\bar{X}} \times 100,$$

where $\bar{X}$ is the mean C_0/D of all available samples in the period of months 6–12 after the transplantation; X_t is an individual value of C_0/D measured in the period mentioned; and T is the number of all available values for an individual patient.

Statistical analysis

The distribution of baseline characteristics is reported using summary statistics and frequency tables for continuous and categorical variables, respectively. The sample was divided into groups by a dichotomized version of Tac IPV, using the median as threshold. The probability to have reached the composite end point as a function of the time since year one after the transplantation was calculated using the Kaplan–Meier method and compared between the groups using the log-rank test.

Univariable and multivariable Cox regression analyses were performed to study the association between Tac IPV, other clinical variables, and the composite end point. The time origin for the survival analysis was 1 year post-transplantation. Besides Tac IPV (coded as a dichotomous variable), the Cox regression analyses included the following covariates: recipient age at transplantation, recipient gender, recipient ethnicity, primary kidney disease, panel-reactive antibody level, donor type (living or deceased donor), transplant number (1 vs. >1), the number of HLA mismatches, transplant year, delayed graft function, eGFR at 6 months post-transplant, acute rejection in the first year, and the mean of the average Tac concentrations measured for an individual patient in the period between 6 and 12 months after the transplantation. The covariates in the multivariable Cox regression model were selected from these variables using a backward elimination method with a threshold for the removal of $P = 0.20$. We assessed the proportional hazard assumption by testing for an interaction between time and covariates in a multivariable Cox regression with time-dependent covariates.

To test our hypothesis that high Tac IPV could put the patients who are usually exposed to low Tac concentrations at higher risk to lose their graft than patients who are usually at optimal Tac exposure, effect modification was tested by including the interaction term of IPV and Tac concentration as a covariate in the multivariable Cox regression model. This interaction term was tested in a model that included the main effects of IPV and Tac concentration as covariates (irrespective of the associated P-values), as well as covariates that were selected using backward elimination.

Finally, we considered the possibility of differential effects of Tac IPV in the first 2 years of follow-up (i.e., between 12 and 36 months after the transplantation) versus the remaining follow-up period, by adding a time-dependent covariate to the Cox regression and testing its significance. This covariate was defined as the Tac IPV (which was measured between 6 and 12 months after the transplantation) between 12 and 36 months after the transplantation, and as 0 after 36 months.

Patients with missing data for one or more covariates were dropped from the multivariable Cox regression. Statistical analyses were performed using IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA). All tests were two-sided, and a P-value <0.05 was considered statistically significant.

Results

Between January 2000 and December 2010, a total of 1232 adult patients were transplanted and started on Tac/MMF-based immunosuppression. A total of 424 patients were excluded from the present analysis, leaving a final study cohort of 808 patients. The characteristics of these patients are presented in Table 1. The reasons for not including the 424 patients were the following: death within the first year after the transplantation ($n = 31$); graft failure within first year after the transplantation ($n = 70$); GFR below 25 ml/min at month 12 after the transplantation ($n = 50$); multiorgan transplant ($n = 4$); no treatment with tacrolimus and MMF ($n = 179$); less than 3 Tac C_0 measurements available ($n = 31$); and insufficient data available ($n = 59$).

The median follow-up was 1993 days (5.5 years) with a range of 23–5130 days (0.06–14.1 years) beyond the first year after the transplantation. A total of 188 events (23.3%) were documented during 4823 person-years at risk: 68 cases of graft loss, 69 cases of late BPAR, 39 cases of transplant glomerulopathy, and 12 cases of doubled serum creatinine.

At 12 months after the transplantation, the median Tac dose was 4.2 mg/day (0.10–28.0 mg/day). Among patients who did not reach the composite end point, the median Tac dose was 4.2 mg/day (1.0–28.0), whereas this was 4.4 mg/day (0.10–22.7) among patients

Table 1. Characteristics of renal transplant recipients in the group patients without and with events.

	Number of patients (n = 808)	Summary measure
Gender recipient		
Male/Female	521/287	64.5%/35.5%
Age of recipient (years)	808	51 (18–77)
Ethnicity		
Caucasian	618	76.5%
Asian	84	10.4%
Black	61	7.5%
Other	45	5.6%
Primary kidney disease		
Diabetic nephropathy	98	12.1%
Polycystic kidney disease	105	13.0%
Glomerulonephritis	202	25.0%
Hypertensive nephropathy	175	21.7%
Reflux disease/chronic pyelonephritis pylonephritis	68	8.4%
Other	91	11.3%
Unknown	69	8.5%
Number of kidney transplantation		
1st	662	81.9%
2nd	117	14.5%
≥3rd	29	3.6%
Donor type		
Living/deceased	519/289	64.2%/35.8%
Delayed graft function		
Yes/no	148/658	18.3%/81.4%
Acute rejection in the first post-transplant year		
Yes/no	165/643	20.4%/79.6%
PRA%	803	0.0 (0.0–96.0)
Peak PRA%	804	4.0 (0.0–100.0)
HLA mismatches	807	3 (0–6)
Transplant year		
2000–2005	328	40.6%
2006–2010	480	59.4%
Serum creatinine (μmol/l) at 6 months	808	125 (43–273)
eGFR (ml/min/1.73 m^2) at 6 months	808	50 (21–90)
Tac C_0* (ng/ml)	808	7.2 (1.8–16.5)

PRA, panel-reactive antibodies; eGFR, estimated glomerular filtration rate.

*Mean of the average Tac concentrations measured in the period between 6 and 12 months after the transplantation. The summary measure for non-normally distributed variables is the median (range). For binary or categorical variables, the summer measure is the proportion.

who reached the composite end point. The corresponding median Tac C_0 was 7.2 ng/ml (1.8–16.5). The median Tac C_0 was 7.4 ng/ml (1.8–16.5) and 6.9 ng/ml (2.3–15.5) in patients who didn't reach and patients who reached the composite end point, respectively.

The median Tac IPV was 16.2% (range: 1.1%–76.0%; Fig. 1). Dividing patients into two groups based on their variability, using the median as cutoff, resulted in 404 patients in the low-variability group, with a mean variability of 11.0% (median = 11.6%, range: 1.1%–16.1%), and 404 patients with high variability, with a mean IPV of 25.1% (median = 22.6%, range: 16.2%–76.0%).

To visualize the association between Tac IPV and the composite end point, a Kaplan–Meier curve was constructed for patients with low and high Tac IPV (Fig. 2). Kaplan–Meier analysis demonstrated a cumulative incidence of the composite end point of 41.8% by 14 years post-transplant for the composite end point in patients with low Tac IPV compared with 49.5% in patients with high Tac IPV. As shown in Fig. 2, long-term transplant outcomes were significantly worse in patients with high Tac IPV (P = 0.018).

Survival analysis

To determine whether the Tac IPV is a predictor for poor transplant outcome, univariable and multivariable Cox regression analyses were performed. In the univariable analyses, significance was found for six covariates, including Tac IPV (Table 2). Univariable analyses showed a 41.3% (hazard ratio: 1.413, 95% CI: 1.059–1.886; P = 0.019) increase in the risk for the composite end point for patients with high Tac IPV compared with those with low Tac IPV.

Only four patients (0.5%) were dropped from the multivariable Cox regression because of missing covariate data. The multivariable Cox regression analysis confirmed that high Tac IPV was associated with poor kidney transplant outcome (hazard ratio: 1.42, 95% CI: 1.059–1.903; P = 0.019, Table 3). Also using Tac IPV as a continuous variable, the multivariable Cox regression analysis demonstrated a 1.4% increase in the hazard for composite end points for every one-unit (1%) increase in Tac IPV (hazard ratio: 1.014, 95% CI: 1.000–1.028; P = 0.043). Allowing for the differential effects of Tac IPV during the first 2 years of follow-up yielded an estimated hazard ratio of 2.03 during the first 2 years of follow-up and 1.20 during the remaining follow-up period. However, the difference between these two hazard ratios was not statistically significant (P = 0.10).

Recipient age at transplantation, eGFR at 6 months post-transplantation, transplant number, and the average Tac C_0 measured in the period between 6–12 months post-transplantation were also found to be

independent predictors for transplant outcome (Table 3). The proportional hazards assumption was not violated, suggesting that the hazard ratios were constant with time.

Based on the mean Tac C_0 at 12 months after the transplantation (baseline), the patients were divided into four groups using the quartiles of mean Tac C_0 as cutoff values. The interaction term of Tac C_0 subgroup and Tac IPV was added to the multivariable Cox proportional hazards model to determine the statistical significance of the resulting interaction term. Dividing patients into groups using the quartiles of the mean Tac C_0 at 12 months post-transplantation as cutoffs resulted in four groups with Tac C_0 as follows: group 1 with Tac $C_0 \leq 6.2$ ng/ml; group 2: 6.2 ng/ml < Tac $C_0 \leq 7.2$ ng/ml; group 3: 7.2 ng/ml < Tac $C_0 \leq 8.2$ ng/ml; and group 4 with Tac $C_0 > 8.2$ ng/ml. There was no significant ($P = 0.59$) modification of the association between Tac IPV and the primary composite end point by patients in the four Tac C_0 groups. This was also the case when effect modification was tested by including the interaction term of IPV and Tac concentration (coded as a continuous variable) as a covariate in the multivariable Cox regression model ($P = 0.35$).

The estimated hazard ratios as a function of Tac IPV and mean Tac concentrations are shown in Figs 3a and b. This figure shows (based on the results of the multivariable model) the influence of Tac IPV and Tac C_0, respectively, as continuous variables on the risk of developing the composite end point. It is clear that the risk of reaching the composite end point (graft failure, late BPAR, transplant glomerulopathy, or doubling of serum creatinine concentration) censored for death increases with increasing Tac IPV and decreasing Tac concentrations.

Discussion

This study demonstrates that a high Tac IPV is associated with inferior long-term outcomes after the kidney transplantation. Patients with a high Tac IPV had a 1.4 times higher risk of reaching the composite end point

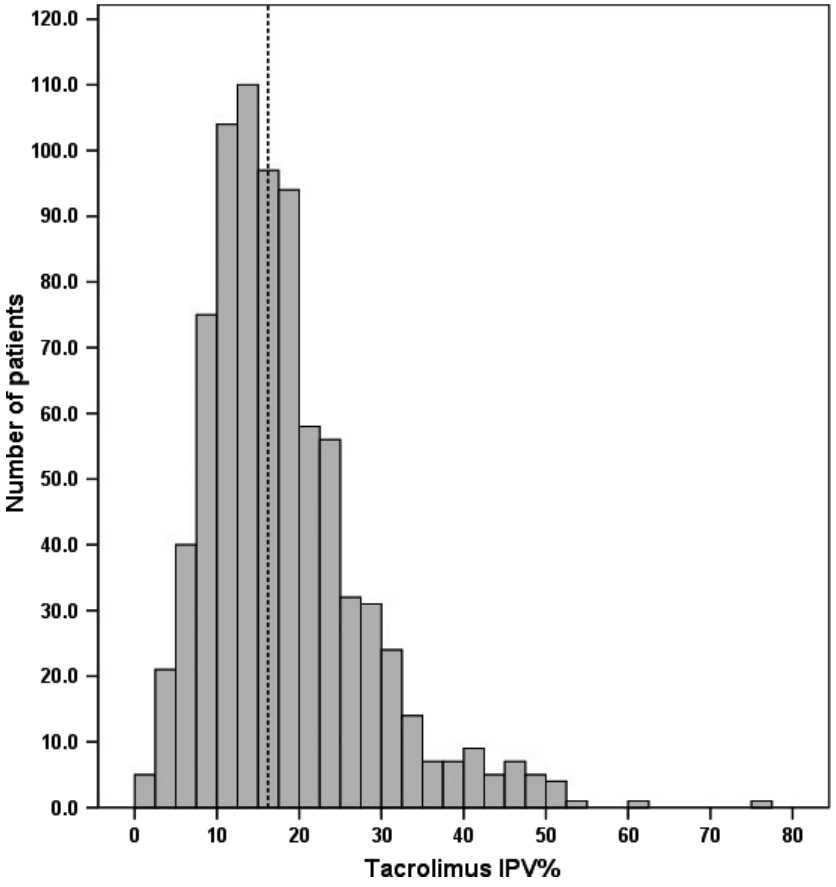

Figure 1 Distribution of Tac IPV in the studied cohort ($n = 808$). The mean Tac IPV was 18.1% (±9.7); the median (shown by dotted line) Tac IPV was 16.2% (1.1–76.0%).

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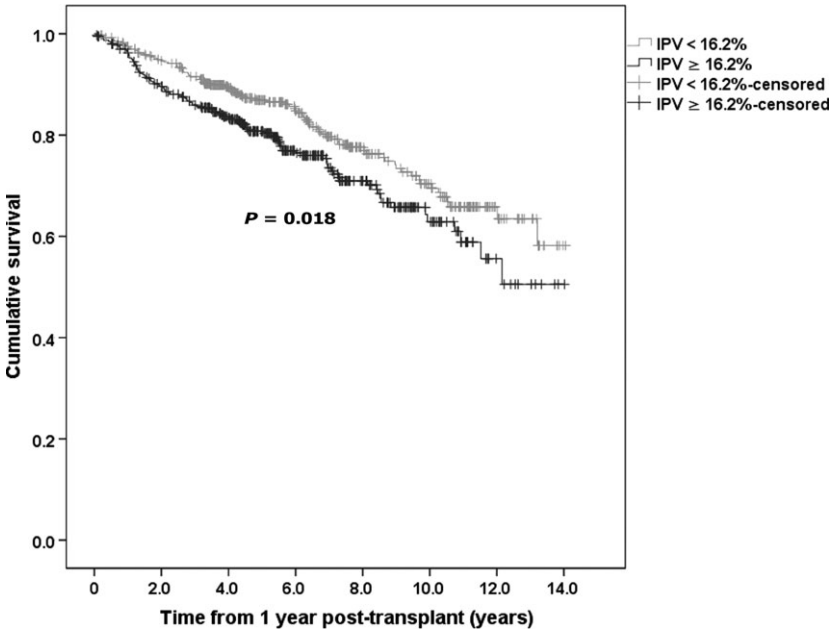

Figure 2 Kaplan–Meier survival curves for patients with low (<16.2%) and high (≥16.2%) Tac IPV. These groups were compared using the log-rank test.

of graft failure, late BPAR, transplant glomerulopathy, or doubling of serum creatinine concentration. The multivariate analysis showed that the effect of Tac IPV was independent of other known risk factors for poor outcome, such as lower recipient age [16], the number of transplantations, and an impaired renal allograft function [17].

This study was an extension of the previously published study of Borra *et al.* [9] and has an almost threefold larger study population and a twofold longer follow-up period. The present findings are in line with our previous findings, although the association between Tac IPV and long-term graft failure as reported previously was stronger than the association observed here. In the study of Borra *et al.*, patients with a high Tac IPV had a threefold higher risk of developing the composite end point, whereas it was 1.4-fold higher in the present study.

The smaller effect size observed here can be explained by the fact that in the present study, the composite end point was modified and differed from that used by Borra *et al.* In the latter study, "biopsy-proven chronic allograft nephropathy (CAN)" was included in the composite end point in addition to the graft loss and doubling of serum creatinine concentration. CAN may be caused by several clinical entities including, among others, calcineurin inhibitor nephrotoxicity, (antibody-mediated) rejection, and chronic pyelonephritis [18]. Because the definition of the histopathology of CAN has changed through the years and the histopathologic picture of CAN is not specific, the item "biopsy-proven CAN" was changed into the more specific diagnoses of late BPAR and transplant glomerulopathy in the present study. Moreover, a longer follow-up in the present study could be another reason for the smaller effect size we found. This study provides some indication that the effect of Tac IPV on the risk of developing the composite end point decreases with time. As has been mentioned previously, patients with high Tac IPV had a twofold higher risk than patients with low Tac IPV to develop an event in the first 2 years of follow-up, whereas this risk was only 1.20-fold higher during the remaining follow-up period. This finding suggests that the longer follow-up period in the present study may partially explain the smaller effect size we found.

Apart from Tac IPV, three other factors proved to be related to long-term kidney transplant failure in multivariate analysis: the recipient's age at the transplantation, graft function at 6 months after the transplantation, the transplant number, and the mean of the average Tac concentrations measured between 6 and 12 months after the transplantation. An advanced age of the recipient at the time of transplantation was found to be a protective factor. This may be explained by the lower immunological activity of elderly patients [16–19]. It is also not surprising that graft function (eGFR) at baseline predicts the survival time of the graft [20].

Table 2. Univariable Cox proportional hazards analyses for the influence of clinical variables on the outcome of graft failure censored for death.

	Hazard ratio (95% CI)	*P*-value
eGFR at 6 months (ml/min)	0.988 (0.979–0.998)	0.016
Recipient age at transplantation (year)	0.982 (0.972–0.992)	<0.001
Mean Tac concentration (ng/ml)	0.890 (0.819–0.967)	0.006
Transplant number (1st)	1.296 (1.073–1.565)	0.007
Tac IPV% (low versus high)	1.413 (1.059–1.886)	0.019
Tac IPV% (continuous variable)	1.015 (1.001–1.028)	0.030
Acute rejection in the first year	1.425 (1.021–1.989)	0.037
Peak PRA (%)	1.005 (1.000–1.010)	0.052
PRA (%)	1.005 (0.997–1.013)	0.196
Ethnicity		0.452
Caucasian	Reference	
Asian	1.285 (0.826–1.999)	0.266
Black	1.327 (0.791–2.228)	0.284
Other	0.831 (0.424–1.631)	0.591
Primary kidney disease		0.138
Diabetic nephropathy	Reference	
Polycystic kidney disease	0.710 (0.381–1.323)	0.281
Glomerulonephritis	0.923 (0.550–1.550)	0.762
Hypertensive nephropathy	0.892 (0.519–1.535)	0.681
chronic pyelonephritis	1.544 (0.861–2.767)	0.145
Other	0.799 (0.432–1.478)	0.475
Unknown	0.692 (0.331–1.445)	0.327
HLA mismatch (none)	1.058 (0.967–1.157)	0.217
Transplant year (per year)	1.018 (0.965–1.074)	0.518
Recipient gender (male)	0.927 (0.686–1.252)	0.620
Delayed graft function (no)	0.923 (0.631–1.350)	0.679
Donor type (living)	1.045 (0.778–1.404)	0.770

Table 3. Results of the multivariable Cox regression analysis. Impact of Tac intrapatient variability on the composite end point (graft failure, late biopsy-proven acute rejection, transplant glomerulopathy, or doubling of serum creatinine concentration) censored for death.

	Hazard ratio (95% CI)	*P*-value
Recipient age at transplantation (year)	0.980 (0.970–0.991)	<0.001
eGFR at 6 months (ml/min)	0.985 (0.976–0.995)	0.002
Tac IPV% (high)	1.420 (1.059–1.903)	0.019
Transplant number (>1)	1.505 (1.066–2.125)	0.020
Mean Tac concentration (ng/ml)	0.913 (0.839–0.994)	0.036
HLA mismatch (none)	1.087 (0.989–1.194)	0.084
DGF	0.736 (0.473–1.146)	0.175
Donor type (deceased)	0.791 (0.555–1.127)	0.194

Salvadori *et al.* [17] demonstrated in a multivariate analysis that the effects of several highly relevant parameters from univariable analysis (such as acute rejection and delayed graft function) on 5-year GFR were fully explained by their influence on 1-year GFR. They showed that 1-year GFR was the most relevant predictor for 5-year allograft function. They also demonstrated that immunological risk factor like previous transplantation has an ongoing effect on graft survival beyond year 1 [17]. In our study, a low mean of the average Tac C_0 measured in the period between 6 and 12 months after the transplantation was found to be another significant predictor for inferior long-term kidney transplantation outcomes. This finding is in line with the results presented by Naesens *et al.* [21]. They demonstrated in a multivariate analysis that low mean Tac exposure was independently associated with higher increase in biopsy-proven chronicity scores [calculated as the sum of the four basic "chronic" Banff qualifiers (chronic glomerular damage, interstitial fibrosis, tubular atrophy, and vascular intimal thickening)] between 3 and 12 months after the transplantation. Recently, in the DeKAF study, a lower Tac exposure after month 3 was also associated with an increased risk of acute rejection [22]. The association between the Tac IPV and poor kidney transplantation outcome was not significantly modified within four patients subgroups based on their mean Tac C_0. Contrary to previous reports [9,23] that suggested that an episode of acute rejection is one of the major factors for inferior graft outcome, our multivariate analysis did not confirm that. The reason probably is that the population we studied is a selection that survived at least 1 year after the transplantation with the acceptable renal function. Recently, a multivariate analysis performed in 739 living donor recipients found steroid-resistant acute rejection, but not any acute rejection episode, to be significantly associated with death-censored graft loss [24]. Unfortunately, in this retrospective analysis, we were unable to distinguish between several types of acute rejection. The major reason for this is that this was a retrospective study and that in the period between year 2000 and year 2010 the Banff classification for kidney transplant rejection was frequently changed.

This multivariable analysis in a large patient population with long follow-up underlines the importance of IPV as a predictor of long-term outcome after the kidney transplantation. In our analysis, the median IPV value was used as a cutoff value. It remains unclear whether there is a critical threshold for IPV above which the risk of graft loss increases. The cutoff values

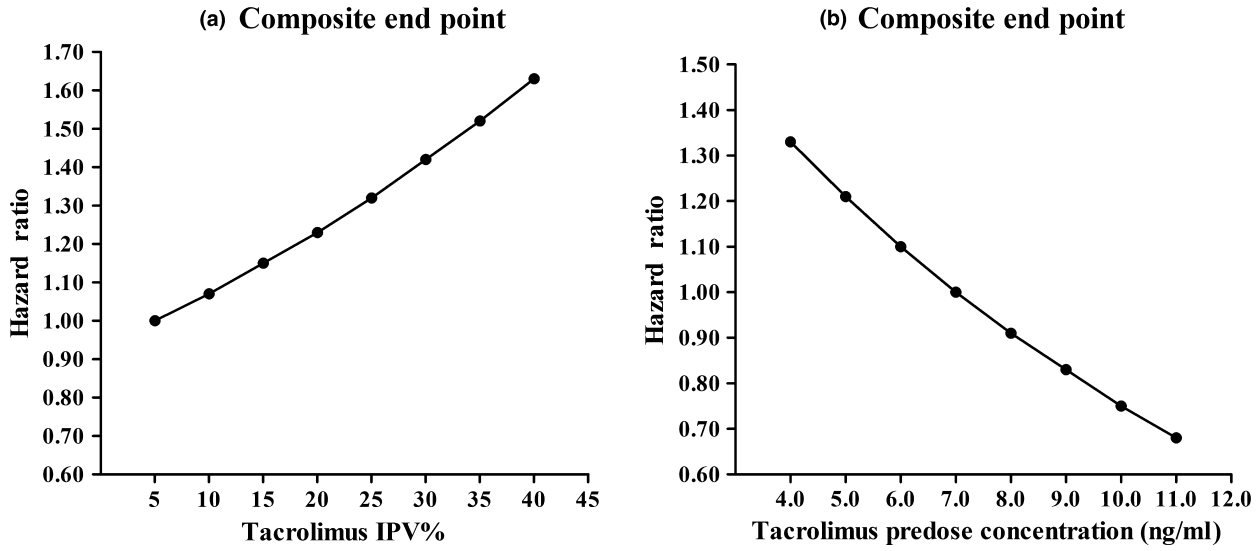

Figure 3 Calculated hazard ratios of the composite end point with increasing Tac IPV (a) and decreasing Tac predose concentrations (b). Example: a patient with a high Tac IPV (25%) and low Tac predose concentration (4.0 ng/ml) has a higher risk (1.32 × 1.33 = 1.76) to reach the composite end point than a patient with the same Tac IPV, but a higher Tac predose concentration (7.0 ng/ml; 1.32 × 1.00 = 1.32).

in the studies by Borra *et al.* [9] and Ro *et al.* [10] (14.9% and 18.0%, respectively) were close to the Tac IPV cutoff value of this study, namely 16.2%.

This study provides good evidence that high Tac IPV increases the risk of poor kidney transplantation outcome. Also, the mean Tac concentration at month 12 after the transplantation was a significant predictor of long-term outcome after the kidney transplantation. From Figs 3a and b, it can be suggested that in patients with a high Tac IPV (>16.2%) it is judicious to strive for a Tac C_0 of ≥7.0 ng/ml, to reduce the risk of poor kidney transplantation outcomes.

Calculation of Tac IPV is an easy and cheap monitoring tool that may help to identify high-risk patients during the routine follow-up visits to the outpatient clinic. Incorporating algorithms that calculate IPV into electronic patient files may assist physicians to recognize these patients. Once a patient is recognized as having a high IPV, physicians need to find out what is the underlying cause, and try to resolve the problem. It is interesting to speculate on the potential causes of Tac IPV [5]. Nonadherence to the therapy is considered an important cause of high variability [25] and has been repeatedly associated with poor transplant outcome [26]. Concomitant diet, over-the-counter medications, and a repetitive substitution of different (generic) Tac formulations may also contribute to Tac IPV. To avoid a high IPV in Tac exposure, patients should be instructed to take their Tac in a consistent manner, with respect to the meal content and timing of ingestion relative to the consumption of meals. Moreover, the use of interacting substances should be addressed and substitution of the innovator drug for generic Tac or one generic formulation for another has to be avoided. Some investigators have reported an improved adherence after switching from the twice-daily to the once-daily, modified-release Tac formulation [27]. Others also showed that Tac IPV decreased following a switch to a once-daily formulation [28,29]. This has, however, not been a universal finding, and at present, it is unknown whether switching to a once-daily Tac formulation will improve the long-term kidney transplantation outcome [30].

In conclusion, in the largest sample size studied so far, a high Tac IPV was found to be associated with adverse long-term renal transplant outcome. In patients with fluctuating tacrolimus concentrations despite a stable dose, physicians should discuss drug adherence with the patient. To quantify the variability, the IPV can be calculated but most likely there is not a critical threshold above which clinical outcome is impaired. In order to collect more evidence, a prospective evaluation of the use of IPV monitoring to see whether it can indeed improve outcomes is needed.

Authorship

NS: contributed to the research design, participated in performing the research, analyzing data and writing of the article. LS: participated in performing the research and writing of the article. JvR: participated in analyzing data and revision of the article. JIR: contributed to the research design, participated in performing the research

and revision of the article. LCPB: participated in performing the research and revision of the article. WW: contributed to the research design and participated in revision of the article. DAH: contributed to the research design and participated in the revision of the article. TvG: contributed to the research design and participated in performing the research and writing of the article.

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Conflicts of interest

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. An example from clinical practice to illustrate intrapatient variability in whole blood Tac C_0.

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