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

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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 were significant risk factors for reaching the composite end point of failure [P-values for

Variability in tacrolimus clearance as a risk factor for graft loss


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

doi: 10.1093/ndt/gfq096
Advance Access publication 26 February 2010
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
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.

\[
\frac{[\text{mean} - X_1] + (\text{mean} - X_2) + \ldots + (\text{mean} - X_n)}{n} \times 100 = \text{within-patient variability (%)}
\]

Formula 1: Formula used for calculating the intrapatient variability. \(X_{\text{mean}}\) is the mean Tac concentration of all available samples, \(X_1\) is the first available Tac concentration measurement, \(X_2\) is the second, etc., etc.

The patients were divided into low and high intrapatient 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

![Graph showing variability in tacrolimus clearance](image-url)
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 $1849 \pm 585$ 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 MPA 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 1.** Baseline characteristics of kidney transplant patients reaching the composite end point of failure and controls

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

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

<table>
<thead>
<tr>
<th>Intraindividual variability</th>
<th>Tac</th>
<th>MMF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (263)</td>
<td>Failure (34)</td>
</tr>
<tr>
<td>Low</td>
<td>138/263</td>
<td>10/34</td>
</tr>
<tr>
<td></td>
<td>52.5%</td>
<td>29.4%</td>
</tr>
<tr>
<td>High</td>
<td>125/263</td>
<td>24/34</td>
</tr>
<tr>
<td></td>
<td>47.5%</td>
<td>70.6%</td>
</tr>
</tbody>
</table>
patients 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.

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.
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 univariate 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, MFD 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, splanchic 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 medication. 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 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.