

The interactions of age, sex, body mass index, genetics, and steroid weight-based doses on tacrolimus dosing requirement after adult kidney transplantation

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Abstract

Purpose The aim of this study was to evaluate the effect of different clinical covariates on tacrolimus dose requirements in adult kidney transplant patients with a specific focus on drug interactions.

Patients Tacrolimus dosing requirement, normalized by drug levels and expressed as the concentration/dose (C/D) ratio as a surrogate index of tacrolimus bioavailability, was employed to identify four categories of tacrolimus dosing requirement,

namely, very high, high, small, and very-small, in very fast, fast, slow, and very slow metabolizers, respectively. Steroid weight-based doses were analyzed instead of fixed doses, and genetic analysis of cytochrome P450 (CYP) $3A5^{*1/*3}$ and multi-drug resistance 1 (*MDR1*) C3435T and C1236T polymorphisms were performed

Results Multivariate analysis on 450 adult transplant patients identified six risk factors for being slow metabolizers and therefore requiring small tacrolimus doses: male sex (OR 1.615, $p = 0.020$); age >60 years (OR2.456, $p = 0.0005$); body mass index ≥ 25 (OR1.546, $p = 0.046$), hepatitis C virus positivity (OR2.800, $p = 0.0004$); low steroid dose <0.06 mg/kg (OR3.101, $p < 0.0001$). Patients with a small tacrolimus requirement were at increased risk for multiple infections (OR 1.533, $p = 0.0008$) and higher systolic blood pressure (OR 1.385, $p = 0.022$) and showed a significant association with the *CYP3A5^{*3/*3}* genotype adjusted by *MDR1* polymorphisms C3435T and C1236T (OR8.104, $p = 0.0001$).

Conclusions Our results demonstrate the importance of the interaction among genetic and clinical factors in conditioning tacrolimus disposition, with corticosteroid weight-based dose being the only modifiable risk factor for tacrolimus requirement. As the tacrolimus dosing requirement increases with increasing tacrolimus clearance through concomitant steroid use, undesirable changes in tacrolimus levels may occur when steroid doses are tapered, predominantly in slow metabolizers. This often neglected drug interaction has to be monitored to optimize tacrolimus exposure in kidney transplant patients.

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Introduction

The aim of this study was to evaluate the effect of different clinical covariates on tacrolimus dose requirements in adult kidney transplant patients, with a specific focus on drug interaction.

Immunosuppressive therapy for the prevention of allograft rejection in kidney transplant recipients often includes tacrolimus, a lipophilic drug with high metabolic clearance which is almost completely metabolized in the liver and, to a lesser extent, in intestinal mucosa via cytochrome P450 3A (CYP3A) isoenzymes CYP3A4 and CYP3A5. Tacrolimus is also a substrate for P-glycoprotein (P-gp), a transmembrane efflux pump expressed in intestinal epithelial cells and biliary canalicular cells which affects drug absorption and excretion [1]. Eight oxidized tacrolimus metabolites have been identified: one monohydroxylated (M-IV), three mono-demethylated (M-I, M-II, M-III), and three di-demethylated (M-V, M-VI, M-VII) metabolites, in addition to a complex metabolite (31-*O*-demethyltacrolimus or M-VIII). The major metabolic pathway in human liver microsomes is based on tacrolimus transformation into M-I and subsequent production of M-VII and more polar metabolites; the first two steps are catalyzed by both CYP3A4 and CYP3A5. While M-II (31-demethyltacrolimus) is equipotent to tacrolimus in terms of immunosuppressive activity, all other metabolites have a very weak effect [2, 3]. Enzyme immunoassay (EIA) techniques employ a monoclonal antibody against tacrolimus that also cross-reacts with its eight metabolites; however, although the strength of the reactivity is similar between M-II, M-III, and M-V, the remaining metabolites show almost negligible reactivity [2, 4].

Tacrolimus has a narrow therapeutic index and large inter-individual variations in pharmacokinetics [5–7], which may partly be the consequence of metabolism by CYP3A5 and P-gp. The expression of both proteins is affected by genetic polymorphisms: for example, patients with one or two wild-type allele *CYP3A5**1 express CYP3A5, whereas homozygotes for mutant allele *CYP3A5**3 are considered to be non-expressors [8–11]. Consequently, variability in tacrolimus pharmacokinetics depends not only on interactions with concomitant drugs metabolized by CYP3A (such as ketoconazole or diltiazem) or the induction of CYP3A (such as rifampicin) [1, 3], but also on a complex genetic conditioning [8]. Polymorphism of *CYP3A5* and of the multidrug resistance 1 (*MDR1*) gene, which encodes for P-gp [9–17] are likely to play a role in this setting. Polymorphisms of *CYP3A5* would have a significant impact on tacrolimus metabolism [9, 16] while role of *MDR1* variants is more controversial [12–14].

However, until genotypic profile data become available in daily clinical practice, tools to define the drug exposure

profile other than the conventional and laborious dose-interval area under the concentration curve (AUC) would be very useful. Tacrolimus requirement may be easily normalized by drug levels and expressed as the concentration/dose (C/D) ratio, a surrogate index of tacrolimus bioavailability and pharmacokinetics that tends to change over the first months after transplantation and then stabilize after 6 months [18, 19]. This calculation evaluates metabolic efficiency and identifies different phenotypic profiles, ranging from very fast to very slow tacrolimus metabolizers. Fast and slow metabolizers treated with the same tacrolimus daily dose adjusted by body weight achieve different tacrolimus concentrations. Therefore, for any targeted trough concentration, fast and slow metabolizers need completely a different tacrolimus daily weight-adjusted dose, leading to a low and high tacrolimus C/D ratio, respectively. Fast metabolizers require high tacrolimus doses to achieve the targeted tacrolimus concentration, while low tacrolimus doses are sufficient for slow metabolizers to achieve the same concentration. Therefore, given a targeted tacrolimus concentration, the smaller the ratio, the faster the metabolic efficiency (requiring high drug doses); conversely, the higher the ratio, the slower the metabolic efficiency (requiring low drug doses).

A relationship between the C/D ratio and the *CYP3A5**1/*3 polymorphism has been shown, as homozygous *3/*3 and *1/*1 patients have been shown to have the highest and lowest C/D ratio, respectively, and the *3/*3 genotype has been determined to represent an increased risk of nephrotoxicity [20, 21].

Steroids are often concomitantly administered with tacrolimus after renal transplantation, and they share some common metabolic and transporter pathways, such as the cytochrome P450 and P-gp systems. Furthermore, corticosteroids may have an induction effect on CYP3A4 expression and reduce tacrolimus concentration by increasing tacrolimus clearance in experimental settings [22–25].

Little data are available on the clinical impact of the pharmacokinetic interaction between tacrolimus and corticosteroids following kidney transplantation [26–32]. Patients with the highest weight-adjusted steroid dose appear to require the highest tacrolimus doses to achieve the targeted trough concentrations [18]. However, while weight-adjusted steroid dose is the rule in the setting of native kidney diseases (glomerulonephritis, lupus, vasculitis), it is rarely adopted in kidney transplantation, leading to the relatively uncertain results published on this drug–drug interaction [28].

The aim of our study was to use weight-based steroid doses and the tacrolimus C/D ratio in a large group of renal transplant recipients in order to analyze factors interacting with the tacrolimus requirement and to verify if the C/D ratio is a reliable tool to improve our knowledge on

immunosuppressive drug interaction and optimize treatment with tacrolimus in this setting [33, 34].

Patients and methods

Study population

All of the patients who received a kidney transplant at our center between 4 November 1998 and 31 December 2008, were treated with tacrolimus as part of a triple immunosuppressive regimen that also included an antimetabolite (either mycophenolate mofetil or mycophenolic acid) and steroids (methylprednisolone as induction and prednisone as maintenance therapy), and who had been followed up for at least 6 months were enrolled in our study. Patients who were taking medications known to interfere with tacrolimus metabolism (such as ketoconazole, fluconazole, diltiazem, erythromycin, rifampicin) at the time of the C/D measurements were excluded. The end of the follow-up was June 2009.

Tacrolimus C/D ratio

Tacrolimus was started at doses of 0.1 mg/kg twice daily with a target trough level of 10–14 ng ml⁻¹ in the first month, subsequently tapered to 8–12 ng ml⁻¹ at the sixth month. The trough concentration of tacrolimus in whole blood was measured by microparticle enzyme immunoassay (MEIA) [35].

The tacrolimus dosing requirement, normalized by drug levels, was expressed as the C/D ratio. This surrogate index of tacrolimus bioavailability was employed to identify four categories of tacrolimus dosing requirement, namely, very high, high, small, and very small in very fast, fast, slow, and very slow metabolizers, respectively. These categories are derived from distribution statistics and the calculation of quartiles of C/D ratio. Dose-adjusted tacrolimus concentrations were calculated by dividing serum concentration at time zero (C₀) by the corresponding tacrolimus dose (mg kg⁻¹).

The ratio between drug trough serum concentration (C) and drug 24-h dose (D) normalized by the patient's weight (mg kg⁻¹ per day) was employed to study tacrolimus metabolism efficiency at 6 months post-kidney transplantation:

$$\begin{aligned} \text{C/D ratio (ng*ml}^{-1}\text{/mg*kg}^{-1}\text{)} \\ &= \frac{\text{blood Tacrolimus concentration (ng/ml)}}{\text{daily Tacrolimus dose (mg/Kg/day)}} \end{aligned}$$

Immunosuppressive regimens and concurrent medications

All patients also received either mycophenolate mofetil 2 g day⁻¹, tapered to 1 g day⁻¹ during the first post-

transplantation month, or mycophenolic acid 1,440 mg day⁻¹, tapered to 720 mg day⁻¹ over the first month. Steroid was given at a standard dosage of intravenous methylprednisolone 500 mg during surgery, 250 and 125 mg on the following days, then 20 mg of daily oral prednisone, tapered to 12.5 mg by the first month, 10 mg by the second month, 7.5 mg by the third month and 5 mg from the fourth month onward. All patients enrolled in the study received omeprazole as gastric protection and cotrimoxazole as prophylaxis for *Pneumocystis jirovecii* as well as other drugs commonly used in chronic renal failure.

Clinical data at the time of kidney transplantation and general and renal outcomes

Data on the recipients were collected at the time of kidney transplantation, including sex, age, body mass index (BMI), and hepatitis C virus (HCV) status. All complications detected before June 2009 were recorded, including malignancies, infections, and arterial hypertension. Infections only included events requiring hospitalization. Patients were stratified into two groups: those who suffered from no or only one infection and those who suffered from two or more infections ("multiple infections"). Post-transplant diabetes mellitus (PTDM) was diagnosed according to criteria defined by 2004 Consensus Guidelines on New-Onset Diabetes after Transplantation [36]. Renal outcomes included biopsy-proven acute rejection, serum creatinine (sCr) and 24-h proteinuria (Pto) at discharge and at 6 months of follow-up.

DNA genotyping

Genomic DNA was extracted from peripheral blood using the QiaAmp DNA Mini kit (Qiagen, Valencia, CA). Genotyping of the *CYP3A5*1/*3* polymorphism and polymorphisms of the *MDR1* gene (C1236T and C3435T) was performed using PCR–restriction fragment length polymorphism methods as previously described [37, 38]. Genetic analysis of the *CYP3A5*1/*3* and *MDR1* C1236T and C3435T polymorphisms was performed in patients who provided written informed consent. The protocol was reviewed and approved by an internal Review Board.

Statistical analysis

An ordinal logistic regression model (based on proportional odds) was used to analyze correlations between clinical and genetic characteristics and a slower metabolism. Each variable was assessed as a potential risk factor contributing to a trend towards a progressively slower metabolic phenotype. The odds ratio (OR) associated with each one-class shift from very fast to very slow metabolizers was

calculated. Univariate models were performed for all variables, and multivariate models were obtained that included each variable shown to be statistically significant by univariate analysis. A binary logistic regression model was adopted to estimate the probability of a single complication occurring in a metabolic phenotype, and a linear regression model was used to study the relationship between the continuous variables and metabolic phenotype. A number of continuous variables (sCr, Pto, blood pressure) were also categorized employing a threshold and analyzed with logistic regression. The survival analysis was performed using the Kaplan–Meier method, and comparisons of curves were made by log-rank test. The statistics software program used was SAS ver. 9.1 (SAS Institute, Cary, NC).

Results

General population

Of the 636 patients transplanted at our center between 4 November 1998 and 31 December 2008 450 patients (173 females, 277 males; mean age 50 ± 12 years) fulfilled the selection criteria (Table 1).

Mean follow-up time was 58 ± 28 months (minimum 6, maximum 134 months). Of the 450 patients, 13 (2.8%) died; patient survival was 100, 98, 96, and 91% at 1, 3, 5, and 10 years, respectively. Eighteen (4%) patients suffered kidney failure, with a kidney survival of 99, 98, 95, and 90% at 1, 3, 5, and 10 years, respectively.

Phenotypic profile of different tacrolimus metabolizers according to the tacrolimus C/D ratio

The tacrolimus C/D ratio was calculated at 6 months post-kidney transplantation.

An asymmetric distribution was found, with a mean value of 167 ± 108 ng ml⁻¹/mg kg⁻¹ (median 141, minimum 24.5, maximum 712 ng ml⁻¹/mg kg⁻¹) (Fig. 1).

At the same time, the trough blood concentrations of tacrolimus fell within a very narrow range (10.2 ± 2.5 ng ml⁻¹), even though the tacrolimus daily dosing requirement was very different: the weight-adjusted dose requested to achieve this targeted goal showed a large variation, ranging from 0.01 to 0.37 mg kg⁻¹ day⁻¹ (mean value 0.08 ± 0.05 mg kg⁻¹ day⁻¹) (Table 1).

Based on the clinical meaning of the C/D tacrolimus ratio, the overall population was stratified into the four quartiles of the C/D ratio values ranging from very low to very high values, and the patients belonging to these groups were defined as very fast, fast, slow, and very slow metabolizers, as they have a very high, high, small, and very small tacrolimus dosing requirement, respectively (Table 1).

Categorization of the phenotypic profile on the basis of the C/D ratio at 6 months of follow-up appeared to be reliable in predicting metabolic efficiency even over the long term, as patients categorized as very slow and slow metabolizers (considered together as one category) maintained the same profile in >85% of cases when the C/D ratio was calculated for each of 5 subsequent years: 88, 87, 86, 88, and 92% in the first, second, third, fourth, and fifth year of follow-up, respectively. Similarly, patients categorized as very fast and fast metabolizers maintained the same profile in $\geq 80\%$ of cases in each of 5 subsequent years: 85, 80, 81, 81, and 80% in the first, second, third, fourth, and fifth year of follow-up, respectively.

The patient and clinical characteristics most frequently associated with the subgroup of very slow metabolizers with a very small tacrolimus dosing requirement were male sex, HCV positivity, higher BMI, and older age (Table 1). Steroid dose normalized by body weight was significantly lower in the subgroup of patients with a very small tacrolimus dosing requirement (Table 1).

Univariate analysis with ordinal logistic regression confirmed four unmodifiable, significant risk factors for being slower tacrolimus metabolizers (Table 2): male sex, age at transplant, BMI, and HCV positivity. The only modifiable risk factor for being a slower tacrolimus metabolizer was weight-normalized steroid dose. Mean steroid dose evaluated as a continuous variable progressively decreased from very fast metabolizers to very slow ones, and the risk of being a slower metabolizer was higher for patients who received an intermediate and a low steroid dose [OR 4.716, 95% confidence interval (CI) 3.079–7.224, $p < 0.0001$] compared with reference category of patients who received a higher steroid dose.

Multivariate analysis with ordinal logistic regression (each of 5 variables adjusted for all of the others) showed that five variables significantly increased the risk of being a slower tacrolimus metabolizer with a small tacrolimus dosing requirement (Table 3): male sex, age, HCV positivity, steroid dose at 6 months of < 0.06 mg kg⁻¹ and of > 0.06 but < 0.08 mg kg⁻¹ versus > 0.08 mg kg⁻¹ (OR 1.854, 95% CI 1.171–2.935, $p = 0.0084$).

Genetic evaluation

Genetic analysis of the *CYP3A5**3/*3 and *MDR1* C1236T and C3435T polymorphisms was available for all the patients who provided written informed consent ($n = 143$). The *CYP3A5**3/*3 genotype was the most represented genotype (127/143, 88.8%), followed by the *1/3* genotype (16/143, 11%), while the *1/1* genotype was absent in our population.

The *CYP3A5**3/*3 genotype was present in 69% (23/33) of very fast tacrolimus metabolizers, 88% (30/34) of fast, 97% (36/37) of slow, and 97% (38/39) of very slow tacrolimus

Table 1 Main clinical characteristics of 450 adult kidney transplant patients at 6 months of follow-up, grouped by the values of the tacrolimus concentration/dose ratio, corresponding to different metabolic phenotypes requiring different tacrolimus doses

Clinical/ patient characteristics	General study population (n = 450 patients; 100%)	Quartiles of tacrolimus concentration/dose (C/D) ratio				p value
		Very high tacrolimus requirement (n =113)	High tacrolimus requirement (n =112)	Small tacrolimus requirement (n =113)	Very small tacrolimus requirement (n =112)	
Tacrolimus concentration (ng ml ⁻¹)						
Mean ± SD	10.2±2.5	9.2±2.3	10.4±2.2	10.7±2.5	10.5±2.8	<0.0001
Median	10	9	10.1	10.5	10.5	
Tacrolimus dose (mg kg ⁻¹)						
Mean ± SD	0.08±0.05	0.14±0.05	0.09±0.02	0.06±0.01	0.03±0.01	<0.0001
Median	0.07	0.14	0.09	0.06	0.03	
Tacrolimus concentration/dose (C/D) ratio						
Mean ± SD	167±108	65±14	114±15	172±21	315±105	<0.0001
Median	68.7	114.0	169.0	278.0		
Sex, n (%)						
Female	173 (38.4)	65 (57.5)	43 (38.4)	37 (32.7)	28 (25.0)	<0.0001
Male	277 (61.6)	48 (42.5)	69 (62.6)	76 (67.2)	84 (75.0)	
Age (years)						
Mean ± SD	50±12	46±13	48±11	50±13	54.5±11	<0.0001
Median	50	46	48	50	55.5	
BMI (kg m ⁻²)						
Mean ± SD	23.5±3.5	22.5±3.4	23±2.9	24±3.5	25±3.6	<0.0001
Median	23.5	22	23	24	25	
HCV positivity, n (%)						
Negative	402 (89.3)	106 (93.9)	104 (92.9)	98 (86.7)	94 (83.9)	
Positive	48 (10.7)	7 (6.1)	8 (7.1)	15 (13.3)	18 (16.1)	0.0065
Steroid (mg kg ⁻¹)						
Mean ± SD	0.076±0.01	0.083±0.01	0.077±0.01	0.074±0.01	0.069±0.01	<0.0001
Median	0.07	0.08	0.07	0.07	0.06	

SD, Standard deviation; BMI, body mass index; HCV, hepatitis virus C

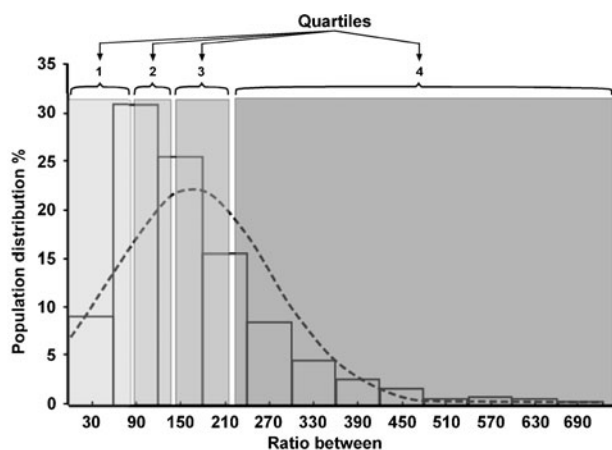


Fig. 1 Distribution of frequency of tacrolimus concentration/dose (C/D) ratio (ng ml⁻¹/mg kg⁻¹) measured at sixth month after kidney transplantation. The study population was stratified into four groups according to quartiles of the tacrolimus dose (shaded areas) and defined as very slow, slow, fast, and very fast metabolizers

metabolizers ($p < 0.001$). *MDR1* C1236T *TT, *CT and *CC genotypes were present in 32 (46/143), 44 (63/143), and 24% (34/143) of those tested, and the *MDR1* C3435T *TT, *CT, and *CC genotypes were present in 23 (33/143), 51 (73/143), and 26% (37/143) of the overall population in which genetic tests were performed. There was no different distribution found among the quartiles of different metabolizers.

Multivariate ordinal logistic regression analysis showed that the *CYP3A5**3/*3 genotype was a significant risk factor for a progressively smaller tacrolimus dosing requirement when compared with the *1/*3 genotype after adjusting for *MDR1* polymorphisms C3435T and C1236T (Table 4). On the contrary, C3435T and C1236T did not seem to be associated to a slower tacrolimus metabolism.

Multivariate linear logistic regression analysis confirmed the association between *CYP3A5**3/*3 and small tacrolimus requirement and also revealed a statistically significant, although weaker, association between the *MDR1* C3435T CC genotype and slow metabolism which had not emerged in the multivariate ordinal analysis (Table 5).

Table 2 Risk factors for requiring smaller tacrolimus doses (univariate ordinal logistic regression) among 450 adult kidney transplant patients at 6 months of follow-up

Risk factors	Quartiles of tacrolimus C/D ratio				Odds ratio	95% Confidence interval	p value
	Very high tacrolimus requirement (<i>n</i> = 113 patients; 100%)	High tacrolimus requirement (<i>n</i> = 112; 100%)	Small tacrolimus requirement (<i>n</i> = 113; 100%)	Very small tacrolimus requirement (<i>n</i> = 112; 100%)			
Sex, <i>n</i> (%)							
Female	65 (57.5)	43 (38.4)	37 (32.7)	28 (25.0)	1.00		
Male	48 (42.5)	69 (62.6)	76 (67.2)	84 (75.0)	2.464	1.738–3.493	<0.0001
Age (years)							
Mean ± SD	46±13	48±11	50±13	54,5±11			
Continuous variable					1.036	1.022–1.050	<0.0001
Categoric variable, <i>n</i> (%)							
<40	42 (37.2)	31 (27.7)	27 (23.9)	18 (16.1)	1.00		
40–60	54 (47.8)	62 (55.4)	58 (51.3)	57 (50.9)	1.721	1.152–2.570	0.008
>60	17 (15.0)	19 (16.9)	28 (24.8)	37 (33.0)	2.988	1.837–4.860	<0.0001
BMI (kg m⁻²)							
Mean ± SD	22.5±3.4	23±2.9	24±3.5	25±3.6			
Continuous variable					1.173	1.115–1.233	<0.0001
Categoric variable, <i>n</i> (%)							
<25	86 (76.1)	82 (73.9)	76 (67.3)	51 (46.4)	1.00		
≥25	27 (23.9)	29 (26.1)	37 (32.8)	59 (53.6)	2.433	1.699–3.484	<0.0001
HCV status, <i>n</i> (%)							
Negative	106 (93.9)	104 (92.9)	98 (86.7)	94 (83.9)	1.00		
Positive	7 (6.1)	8 (7.1)	15 (13.3)	18 (16.1)	2.132	1.236–3.678	0.0065
Steroid (mg kg⁻¹)							
Categoric variable, <i>n</i> (%)							
>0.08	62 (54.8)	40 (35.7)	31 (27.4)	19 (16.9)	1.00		
0.06–0.08	30 (26.6)	44 (39.3)	42 (37.2)	31 (27.7)	2.182	1.443–3.300	0.0002
<0.06	21 (18.6)	28 (25.0)	40 (35.4)	62 (55.4)	4.716	3.079–7.224	<0.0001

Patient and graft outcomes

Patient outcome Among the 13 patients (3%) who died during the follow-up period, three were very fast, one was a fast, six were slow, and three were very slow tacrolimus metabolizers. Forty-six patients (10%) developed at least one malignancy during the follow-up period. There were no significant differences between tacrolimus requirement and cumulative incidence of malignancies (OR 0.857, 95% CI 0.651–1.129, *p* = 0.273). Univariate logistic regression analysis showed that a slower tacrolimus metabolic phenotype requiring lower tacrolimus doses was associated with a significantly increased risk of developing multiple infections and systolic blood pressure >150 mmHg (Table 6).

A supplemental analysis demonstrated that very slow metabolizers had a significantly higher risk of developing multiple infections (OR 2.945, 95% CI 1.380–6.285, *p* = 0.005) compared to very fast ones. No association was found between the different phenotypic profiles of tacrolimus metabolizers and the risk of developing PTDM (OR 1.092, 95% CI 0.833–1.431, *p* = 0.5260).

Table 3 Multivariate ordinal logistic analysis of risk factors for being slower tacrolimus metabolizers having smaller tacrolimus dosing requirement

Risk factor	Odds ratio	95% confidence interval	p value
Sex			
Female	1		
Male	1.615	1.080–2.16	0.020
Age (years)			
<40	1		
40–60	1.389	0.912–2.118	0.002
>60	2.456	1.477–4.084	
HCV			
Negative	1		
Positive	2.800	1.582–4.956	0.0004
Steroid dose (mg kg⁻¹)			
>0.08	1		
0.06–0.08	1.854	1.171–2.935	0.0003
<0.06	3.101	1.787–5.379	<0.0001
BMI (kg m⁻²)			
<25	1		
≥25	1.546	1.008–2.373	0.0460

Table 4 Multivariate ordinal logistic analysis of genetic risk factors for being slower tacrolimus metabolizers (each genotype is adjusted for all the others)

Genotype	Odds ratio	95% confidence interval	<i>p</i> value
<i>CYP3A5</i> *3/*3 vs. *1/*3	8.104	2.765–23.752	0.0001
<i>MDR1</i> C3435T *CC vs. *CT+TT	1.662	0.821–3.364	0.158
<i>MDR1</i> C1236T *CC vs. *CT+TT	0.878	0.431–1.791	0.721

Multivariate logistic regression analysis adjusted for sex, age, HCV, BMI, and steroid dose confirmed that a progressively lower tacrolimus requirement significantly increased the risk of developing multiple infections (OR 1.533, 95% CI 1.201–2.008, $p = 0.0008$) and of having systolic blood pressure >150 mmHg (OR 1.385, 95% CI 1.047–1.834, $p = 0.022$),

Graft outcomes Among the 18 patients (4%) who lost their graft at a mean time of 49 ± 29 months after kidney transplant, four were very fast, six were fast, two were slow, and six were very slow metabolizers. No association was found between different phenotypic profiles of tacrolimus metabolizers and the risk of developing acute rejection (OR 0.956, 95% CI 0.675–1.354, $p = 0.798$).

Univariate linear regression analysis showed a significant association between smaller tacrolimus doses and an sCr at 6 months of follow-up ($p = 0.001$), but not with 24-h Pto at 6 months ($p = 0.678$). Univariate logistic regression analysis confirmed these results, as a progressively slower metabolic phenotype significantly increased the risk of having sCr >2 mg dl⁻¹ (Table 6), while it did not increase the risk of having Pto >1 g day⁻¹ (OR 1.297, 95% CI 0.768–2.192, $p = 0.330$).

Multivariate logistic and linear regression analysis did not confirm the associations showed by univariate analysis between slower metabolizers and sCr >2 mg dl⁻¹ (OR 1.142, 95% CI 0.644–2.027, $p = 0.649$ and 0.638, respectively).

Discussion

The results of our study demonstrate the importance of the interaction among clinical and genetic and factors in conditioning tacrolimus disposition and are in agreement with those of a previous report on pediatric kidney recipients [39]. Our data also confirm literature reports on clinical factors associated with slow tacrolimus metabolizers: male sex [40], age [41, 42], HCV positivity [43], and higher BMI [44].

Table 5 Multivariate linear regression analysis of genetic risk factors for being slower tacrolimus metabolizers (each genotype is adjusted for all the others)

Genotype	PE	Standard error	<i>t</i> value	<i>p</i> value
<i>CYP3A5</i> *3/*3 vs. *1/*3	0.606	0.145	4.18	<0.0001
<i>MDR1</i> C3435T *CC vs. *CT+TT	0.219	0.107	2.04	0.042
<i>MDR1</i> C1236T *CC vs. *CT+TT	-0.006	0.108	-0.06	0.953

However, the most significant result is that corticosteroid weight-based dose is the only modifiable risk factor for tacrolimus requirement. Several clinical studies indicate that tacrolimus dose requirement is higher when used in combination with corticosteroids [26–31]. Therefore, to avoid any undesirable alterations in tacrolimus levels, monitoring the tacrolimus level is crucial during pulse steroid therapy [26] or when tapering or withdrawing steroids [32].

Steroid interaction with tacrolimus metabolism was clearly demonstrated in our study at 6 months post-kidney transplantation, confirming previously demonstrated interactions at the first and third month post-transplant [18, 19]. The highest weight-normalized steroid doses were found in patients with the fastest tacrolimus metabolism, while the lowest steroid dose was the strongest risk factor for having a smaller tacrolimus requirement. These results confirm the theoretical premise that steroid induces tacrolimus metabolism [22–25]. This also explains why the daily tacrolimus requirement—and thus the tacrolimus C/D ratio—may change during the first months following transplantation, when steroids are rapidly tapered, but stabilize in the subsequent months [18]. It represents a key-point to be kept in mind when steroid doses have to be modified.

In slow metabolizers, characterized by a small tacrolimus dosing requirement to achieve the targeted drug concentration, tapering of the steroid will remove the component of steroid-induced tacrolimus metabolism, thus increasing the risk of toxicity due to increased drug concentrations if the concurrent tacrolimus daily dose remains unchanged. Alternatively, an increase in steroid dose will increase the risk of suboptimal drug concentration; this will occur primarily in fast tacrolimus metabolizers with a high tacrolimus requirement, who will increase their already high metabolic efficiency even more due to an enhanced steroid contribution. Therefore, this often neglected drug interaction between steroid and tacrolimus may help improve the modulation of drug exposure in kidney transplant recipients.

Another interesting aspect is that the tacrolimus C/D ratio calculated at 6 months post-kidney transplantation

Table 6 Univariate logistic regression analysis evaluating the association between different phenotypes of tacrolimus metabolizers needing different tacrolimus doses and general and renal outcome

Clinical parameter	Total (n = 450 patients)	Quartiles of tacrolimus C/D ratio				p value	Odds ratio	95% confidence interval
		Very high tacrolimus requirement (n = 113)	High tacrolimus requirement (n = 112)	Small tacrolimus requirement (n = 113)	Very small tacrolimus requirement (n = 112)			
Infections								
None or only 1	377	102 (27.0)	96 (25.5)	94 (24.9)	85 (22.5)		1	
>1	73	11 (15.1)	16 (21.9)	19 (26.0)	27 (37.0)	0.0038	1.411	(1.118–1.781)
Serum creatinine (mg dl ⁻¹) at 6th month								
≤2	346	95 (27.5)	86 (24.8)	88 (25.4)	77 (22.3)		1	
>2	96	17 (17.7)	23 (24.0)	22 (22.9)	34 (35.4)	0.010	1.311	(1.067–1.611)
Systolic blood pressure (mmHg) at 6th month								
≤50	377	101 (26.8)	96 (25.5)	94 (24.9)	86 (22.8)		1.	
>150	60	9 (15.0)	11 (18.3)	15 (25.0)	25 (41.7)	0.0018	1.505	(1.164–1.946)

identifies completely different tacrolimus dosing requirements and therefore different metabolic profiles of tacrolimus metabolizers, ranging from very fast to very slow ones. These patterns also seem to remain stable over time: in our study, >85% of our patients maintained the same pattern for up to 5 years after kidney transplantation.

The tacrolimus requirement as expressed by the tacrolimus C/D ratio is also related to genetic conditioning: homozygous *CYP3A5* *3/*3 and high tacrolimus requirement are significantly associated, as has been reported in previous studies. Our results also confirm a minor role of the *MDR1* C3435T*C variant allele and the lack of association with *MDR1* C1236T polymorphisms [12, 14, 15].

The relationship between different metabolic phenotypes and clinical outcomes is intriguing, as the slow metabolizers in our study had an increased risk of nephrotoxicity, in accordance with the results of a recent study [45, 46], and of multiple infections, hypertension, and a higher sCr. As these side effects occurred in patients who had comparable tacrolimus levels achieved by a different drug dosing requirement at 6 months post-transplantation, a possible explanation may be that patients with this very low tacrolimus requirement are at risk of achieving frequent subclinical supra-target levels which may not be detected by the clinician. This metabolic phenotype could also identify patients at risk for complications through mechanisms other than mere tacrolimus levels, in analogy with recent interpretations of calcineurin nephrotoxicity. The latter would not simply reflect systemic exposure, but most of all local, genetically conditioned drug metabolism due to gene linkage disequilibrium [45–47]. While monitoring pre-dose through concentration determinations is often insufficient to guide optimal drug therapy in clinical practice, the tacrolimus C/D ratio, expressing a normalized dosing requirement, could provide more accurate information.

There are a number of limitations to our study. First, it is a retrospective analysis of a completely Caucasian population that exhibited a high frequency of the *CYP3A5**3/*3 (127/143, 88,8%) genotype, a much lower frequency of the *1/*3 genotype (16/143, 11%), and complete absence of the *1/*1 genotype. Secondly, we analyzed genetic polymorphisms only in a sub-population of our patients (143/450) who gave their consent. Despite these limitations, our genotype prevalence frequencies are consistent with those reported in other studies on Caucasian populations, in which *CYP3A5**3 allele frequency is around 90% as compared with around 50% in African-Americans [48]. Therefore, although our results may not be applicable to different or mixed ethnical settings and are based on a relatively small subpopulation, they are in accordance with those reported in the literature and could presumably be applied to any Caucasian population. Thirdly, we did not take albumin levels and hematocrit into account, both of which can influence tacrolimus metabolism [1].

As MEIA assay was employed to measure tacrolimus levels, some degree of cross-reaction with tacrolimus metabolites M-II, M-III, and M-V can be expected [2, 3, 35]. Although differences in their pharmacokinetics may have affected overall tacrolimus concentrations as analyzed in our study, it has been shown that whole blood tacrolimus levels measured with MEIA tend to reflect those of unmodified tacrolimus in kidney transplant recipients [2].

In conclusion, the results of our study demonstrate that among the factors interacting with tacrolimus requirement, body weight-adjusted steroid doses is the only easily measurable variable that can be modified in clinical practice. The magnitude of the prednisone effect is maximum when high intravenous doses are employed in first post-transplant days, resulting in an increase in

tacrolimus clearance [26, 49]. Clinicians should be aware that the increase in tacrolimus exposure and the risk of excessively high trough levels when the steroid dose is subsequently reduced does exist for all patients, but is especially high for slow metabolizers.

We believe that, while waiting for the development of pharmacogenomic predictive approaches [50–58], pharmacokinetic profiles capable of identifying metabolic phenotypes are currently needed to optimize tacrolimus exposure. We propose that the tacrolimus dosing requirement normalized as the tacrolimus C/D ratio be tested in prospective studies as an alternative to classic methods for evaluating tacrolimus exposure [18], as already validated abbreviated AUC profiles are promising in term of “cost-effectiveness, feasibility and clinical relevance” [51]. Furthermore, we suggest that weight-normalized steroid doses be used instead of the current standardized regimen also in the setting of kidney transplant.

This integrated strategy combining the tacrolimus C/D ratio and steroid weight-based doses could prove to be a useful tool to individualize immunosuppressive treatment in kidney transplant patients [59, 60].

Conflict of interest disclosure None.

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