The effects of the CYP3A5*3 variant on tacrolimus pharmacokinetics and outcomes in Tunisian kidney transplant recipients

Les effets du variant CYP3A5*3 sur la pharmacocinétique du tacrolimus et le pronostic de transplantés rénaux tunisiens

Rim Charfi[1], Mohamed Mongi Bacha[2], Myriam Ben Fadhla[3], Khouloud Ferchichi[1], Hanene El Jebari[1], Emna Gaies[1], Anis Klouz[1], Ezzeddine Abderrahim[2], Fathi Ben Hamida[2], Taieb Ben Abdallah[2], Sameh Trabelsi[1], Yosr Gorgi[3], Imen Sfar[3]

1. University of Tunis El Manar, Faculty of de Medicine of Tunis. National Centre Chalbi Belkahia of Pharmacovigilance, Department of clinical pharmacology, Research Laboratory of Clinical and Experimental Pharmacology (LR16SP02), Tunis, Tunisia
2. University of Tunis El Manar, Faculty of de Medicine of Tunis. Charles Nicolle hospital -Department of nephrology and internal medicine, Research Laboratory of Renal Pathology (LR00SP01), Tunis, Tunisie
3. University of Tunis El Manar, Faculty of de Medicine of Tunis. Charles Nicolle hospital -Department of immunology, Research Laboratory of Immunology of Renal Transplantation and Immunopathology (LR03SP01), Tunis, Tunisia

Abstract

Introduction: Tacrolimus, exhibits interindividual pharmacokinetic variability and a narrow therapeutic index. The influence of the CYP3A5 6986A>G single nucleotide polymorphism (SNP) on this variability remains a topic of debate.
Aim: To assess the impact of the aforementioned SNP on tacrolimus area under curve (AUC0-12h), adverse drug reactions (ADRs), and kidney graft outcomes.
Methods: Blood samples were collected from Tunisian kidney transplants over a five-year period during either the early (<3 months) or late (>3 months) post-transplant phases. Through blood concentration (C0) and AUC0-12h of tacrolimus were measured. Patients were prospectively followed to assess graft outcomes. Polymerase chain reaction of restriction fragment length polymorphism was used for CYP3A5 6986A>G genotyping.
Results: Fifty Tunisian kidney recipients receiving tacrolimus were enrolled in the study. Acute and chronic graft rejections were observed in eight and three patients, respectively. Twenty-one patients (42%) reported ADRs. C0 and AUC0-12h, showed a significant difference between CYP3A5*1 carriers (mean C0=4 ng.mL-1 and AUC0-12h=94.37 ng.h.mL-1) and CYP3A5*3/3 or poor metabolizers carriers (mean C0=7.45 ng.mL-1; AUC0-12h=151.27 ng.h.mL-1) (p=0.0001; p=0.003, respectively). Supratherapeutic tacrolimus levels were significantly more common in poor metabolizers (p=0.046; Odds-ratio =1.3; confidence interval 95% [1.12-1.66]). The impact of SNP was significant on C0, AUC0-12h, C0/Dose and AUC0-12h/Dose, only in the late phase (p=0.01, 0.002, 0.012, 0.003 respectively).
Conclusion: CYP3A5*3 variant was significantly associated with tacrolimus pharmacokinetics but had no impact on graft outcomes.

Key words: Adverse drug reaction, kidney grafting, pharmacokinetics, rejection, SNPS, tacrolimus

Résumé

Introduction: Le tacrolimus présente une variabilité pharmacocinétique interindividuelle et un index thérapeutique étroit. L'influence du single nucleotide polymorphism (SNP) CYP3A5 6986A>G dans cette variabilité est controversé.
Objectif: Etudier l'effet du SNP suscité sur l'aire sous la courbe du tacrolimus (ASC0-12h), les effets indésirables (EI) et la survie du greffon.
Méthodes: Des prélèvements sanguins étaient effectués chez des transplantés rénaux tunisiens pendant cinq ans, soit précocement ou tardivement après la transplantation. La concentration sanguine résiduelle (C0) et l'ASC0-12h de tacrolimus étaient mesurées. Un suivi prospectif pour établir la survie des greffons et un génotypage classique étaient effectués.
Résultats: Cinquante transplantés rénaux tunisiens recevant du tacrolimus étaient inclus dans l'étude. Un rejet aigu était observé chez huit patients et un dysfonctionnement chronique du greffon chez trois patients. Vingt et un patients (42%) présentaient des EI. Il y avait une différence significative de la C0 et l'ASC0-12h entre les porteurs du CYP3A5*1 (C0 moyenne=4 ng.mL-1; ASC0-12h=94,37 ng.h.mL-1) et les métaboliseurs lents ou porteurs du CYP3A5*3/3 (C0 moyenne=7,45 ng.mL-1 ; ASC0-12h=151,27 ng.h.mL-1) (p=0,0001;p=0,003, respectivement). Les C0 suprathérapeutiques étaient significativement plus fréquentes chez les métaboliseurs lents (CYP3A5*3/*3) (p=0,046; rapport des cote =1,3; intervalle de confiance 95% [1,12-1,66]). L'impact de ce SNP était significatif sur la C0, l'ASC0-12h, la C0/Dose et l'ASC0-12h/Dose, uniquement dans la phase tardive (p=0,01, 0,002, 0,012, et 0,003).
Conclusions: Le variant CYP3A5*3 était significativement associé à la pharmacocinétique du tacrolimus mais n'avait aucun impact sur la survie du greffon.

Mots clés: Effet indésirable, greffe rénale, pharmacocinétique, rejet, SNPS, tacrolimus

Correspondance
Rim Charfi
National Centre Chalbi Belkahia of Pharmacovigilance, Department of clinical pharmacology.
Email: rim.charfi@fmt.utm.tn

LA TUNISIE MEDICALE-2023; Vol 101 (10): 738-744

INTRODUCTION

Kidney transplantation is the best alternative among renal replacement therapies for end-stage renal failure due to its association with a better life quality and survival rate of kidney transplant recipients (KTRs) [1]. However, the main challenge is to prevent acute rejection in post-transplantation by establishing effective immunosuppression therapy with less induced adverse drug reactions (ADRs). Calcineurin inhibitors, namely cyclosporine and tacrolimus remain the best option as they represent, the cornerstone of immunosuppressant therapy in kidney transplantation [2,3]. Nevertheless, these drugs are characterized by a narrow therapeutic index and large inter-individual and intra-individual variability (5-93%) [4], justifying their mandatory therapeutic drug monitoring [3].
Factors such as age, race, weight, time since transplant, kidney and liver functions, drug interactions, and genetic factors are known to influence both cyclosporine and tacrolimus pharmacokinetics and pharmacodynamics [3,5]. The cytochromes P450 with both CYP3A isozymes (CYP3A4 and CYP3A5) contribute to both calcineurin inhibitors' metabolism. Previous studies have shown that CYP3A5 is the predominant enzyme for tacrolimus metabolism [4,5]. Several single nucleotide polymorphisms (SNP) of the CYP3A5 gene were identified, including the CYP3A5 6986A>G (rs776746). This variant is believed to be associated with high inter-individual variability of tacrolimus bioavailability [6]. Homozygotes CYP3A5*3/*3 are considered poor metabolizers while CYP3A5*1/*1 and *1/*3 define rapid metabolizers [7]. Rapid metabolizers are exposed to graft rejection risk because of decreased tacrolimus bioavailability under usual doses administration [7]. Thus, we highlight the relevance of CYP3A5 genetic profile-based dose adjustment of tacrolimus in the management of KTRs [7]. The topic has as much interest as there are currently no alternative immunosuppressants to calcineurin inhibitors [8]. To the best of the authors' knowledge, only one study has already studied the influence of this SNP on tacrolimus pharmacokinetics variation in Tunisian KTRs [9]. However, the mentioned study did not assess ADRs or the impact of this SNP on graft survival.
Thus, the aim of this study was to assess prospectively the impact of CYP3A5*3 variant on tacrolimus pharmacokinetic parameters, ADRs and on the graft survival.

METHODS

Study design

This was an analytical, longitudinal study including KTRs, followed in the transplant unit of the nephrology internal medicine Department. Patients were recruited over a five-year period (from April 2010 to May 2015) for the pharmacogenetic study. Included patients were prospectively followed until October, 2018. This study was carried out within the framework of personalized medicine [10] between three hospital-university departments.

Study population

We included unrelated Tunisian patients, aged from 18 to 70 years, who were candidates for their first kidney transplantation from living or cadaveric ABO-compatible donors and receiving a tacrolimus treatment. All patients must have a renal function (assessed by creatinine clearance calculation) within the normal range for at least three months and must have reached the steady state before every tacrolimus blood measurement which is defined as a minimum of three days under the same tacrolimus daily dose.
Non-inclusion criteria were: liver dysfunction (assessed by aminotransferase serum levels upper the lab recommended range), experiencing diarrhea or vomiting immediately after drug taking, poor compliance and polypharmacy causing potential clinically significant drug interactions with calcineurin inhibitors (macrolides, imidazole antifungals, antidepressants and antiretroviral) [11].
Exclusion criteria were switching to another immunosuppressant drug during the study and lost follow-up patients (Figure 1).

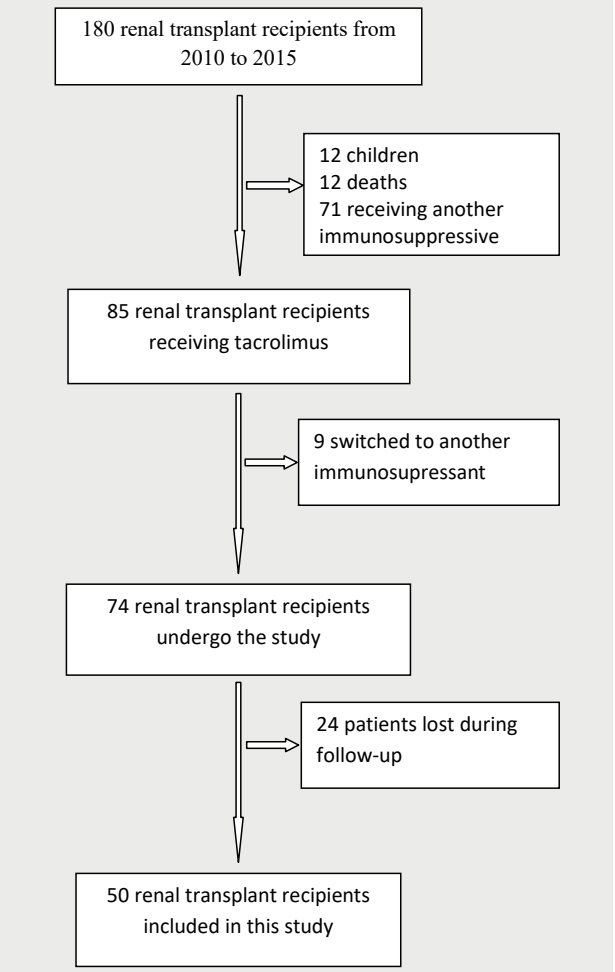

Figure 1. Flow chart of the study population

Data collection

For all patients, we collected medical history, demographic, clinical and biological data, pre-transplant assessment, the kidney transplantation date, received immunosuppressants, induced ADRs, associated medication and graft outcome. The graft outcome was assessed histologically based on the Banff criteria about graft acute rejection or by a return to hemodialysis due to chronic graft dysfunction [12]. We distinguished two group of patients according to age of the graft: the early phase corresponding to the first three months post kidney transplantation and the late phase > 3 months post kidney transplantation [13,14].

Pharmacokinetic study

Blood samples were collected in heparin tubes and addressed to the clinical pharmacology department for tacrolimus blood concentration measurement. The tacrolimus bioavailability was evaluated using the following

pharmacokinetic parameters correlated with calcineurin inhibitors systemic exposure [11,15]:

- **C_0**: Trough blood concentration, before the first daily intake.
- **AUC_{0-12h}**: Tacrolimus area under the curve using the limited sampling strategy (C_0, C_{1h}, C_{3h}) [16].
- **D**: Normalized dose (mg/kg/day)
- **C_0/D**, **AUC_{0-12h}/D**: Dose normalized C_0 and AUC_{0-12h} to eliminate the dose-effect.

We used the following tacrolimus therapeutic ranges:

- For early phase treatment: C_0= 5 to 10 ng.mL-1, AUC_{0-12h} 180 to 270 h.µg.L-1 [17].
- For late-phase treatment: C_0 10 to 15 ng.mL-1, AUC_{0-12h} 100 to 190 h.µg.L-1 [17].

Tacrolimus concentrations were measured using a fluorescence polarization immunoassay method (limit of quantification: 2 ng.mL-1).

Genetic study

This part of the study was carried out in the immunology laboratory. The substitution of adenosine with guanine at position 6986 of the intron 3 of the CYP3A5*3 variant was investigated by polymerase chain reaction-restriction fragment length polymorphism using SspI restriction enzyme [18].

Statistical analysis

Parametric and non-parametric tests were used according to variable distribution. Pearson coefficient was used to establish correlations. Odds ratios (OR) and confidence interval (CI) 95% were calculated to stratify genetic results according to the therapeutic range. The p significance threshold was fixed to 0.05. The determination coefficient (R2) was assessed for certain variables. An R2 value between 0 and 0.3 was typically considered to represent a low strength of relationship between the independent and dependent variables. An R2 value between 0.3 and 0.7 was considered to indicate a moderate strength of relationship. An R2 value between 0.7 and 1 was considered to represent a strong strength of relationship. For graft survival curves, the comparison of one, two, five and 10-year graft survival rates was determined by the Kaplan Meier method. SPSS version 22.0 software was used.

Ethical considerations

All patients have given their informed consent for participation before they have been included in the study. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the local Ethics Committee.

RESULTS

Descriptive study

This study included a total of 50 patients from the initial sample of 180 patients (Figure 1), with patient characteristics summarized in table 1.

Tacrolimus treatment was initiated on the day of kidney transplantation. Of the patients, 24% received induction therapy with tacrolimus alone, while 11% received interleukin-2 receptor antagonist therapy (20 mg on days 0, and 4 post-transplant) and 65% received anti-lymphocyte immunoglobulin therapy.

ADRs were observed in 42% of patients. The most reported ADRs were tremors (30%), high blood pressure (22%) and gastrointestinal troubles (22%).

Acute graft rejection was observed in eight patients. Three of these patients had a tacrolimus C0 in the therapeutic range.

Table 1. Patients' characteristics.

Characteristics		Patients (n=50)
Sex-ratio (male/female)		3.54
Age (years)		40±10[a]
Weight (kg)		67 ± 13[a]
Post-transplant delay (months)		10 [3-41][b]
Early phase (n)		19 (38%)[c]
Late phase (n)		31 (62%)[c]
Age of transplantation at the end of the study (months)		4-21
End-stage renal failure etiology (n)		
	Glomerular nephropathy	18 (36%)[c]
	Vascular nephropathy	5 (10%)[c]
	Tubulo-interstitial nephropathy	11 (22%)[c]
	Uropathies	3 (6%)[c]
	Polycystic kidney disease	1 (2%)[c]
	Undetermined causes	12 (24%)[c]
Kidney transplantation from a living donor		43 (84.6%)[c]
Serum creatinine (µmol.L^{-1})		125 ± 63[a]
Hematocrit level (%)		37.8 ± 7.9[a]
HLA A, B, DR mismatches <3		86%
Negativity of DSA* before transplantation		100%
Mean normalized dose of tacrolimus (mg/kg/day):		
	Initial dose	0.1
	Maintenance dose of tacrolimus	
	In the early phase	0.10 ± 0.03[a]
	In the late phase	0.069 ± 0.03[a]
Mycophenolate mofetil (g/day):		
	Induction dose	2
	Maintenance dose	1.5
Corticosteroids (mg/kg/day):		
	Induction dose	1
	Maintenance dose, for life	
	In the early phase (mg/day)	13.5 ± 6.5[a]
	In the late phase (mg/day)	10 ± 3[a]
Patients under calcium channel blockers (n, percentage)		9 (18%)[c]
	Acute rejection	6 (12%)[c]
	Chronic graft dysfunction	2 (4%)[c]

DSA: Donor specific antibodies, HLA: human leukocyte antigens. Data were [a] Means ± standard deviation ; [b] Median [interquartile range], and [c] Number (%).

Chronic graft dysfunction was observed in three patients. One of these patients had a tacrolimus C0 in the therapeutic range.

No significant association was found between AUC0-12h or C0 and acute graft rejection or chronic graft dysfunction with p=0.4, 0.3 and 0.78, respectively.

Pharmacokinetic study

Results identified significantly higher AUC0-12h/D in the late-phase transplant than in the early phase (Table 2). Regardless of the post-transplant phase, there was a correlation between C0 and AUC0-12h of tacrolimus, R2=0.81 (p=0.01). Among patients, 47% had sub-therapeutic C0, 65% in the early phase and 25% in the late one. Patients had C0 of tacrolimus in the therapeutic range, in 35% of the early phase and in 45% of the late one. There was no significant association between C0, AUC0-12h and the occurrence of ADRs (p=0.98 and p=0.75 respectively). In addition, no association between supra-therapeutic C0 and the occurrence of ADRs was observed (p=0.4). No significant correlation between the variation in C0, AUC0-12h and the occurrence of acute graft rejection episodes was oted with p=0.4 and 0.3, respectively. Furthermore, the occurrence of chronic graft dysfunction was not associated with variations in tacrolimus pharmacokinetic parameters.

Table 2. Tacrolimus pharmacokinetic parameters according to post-transplant phases (n=50).

	Patients	Early phase	Late phase	p
C_0 (ng.mL^{-1})	6 (5-10)	6.9 (5.5-9.8)	6.5 (4.5-10)	0.7
AUC_{0-12h} (h.µg.L^{-1})	137 (104-178)	136 (115-194)	146 (95-177)	0.8
C_0/D	93 (56-178)	78 (54-98)	117 (55-207)	0.08
AUC_{0-12h}/D	1723(1256-2685)	1507 (1269-1954)	2198(2189-3986)	**0.018**

AUC_{0-12h}: Area under the curve of tacrolimus, C_0: Blood trough concentration of tacrolimus, D: daily weight dose of tacrolimus (mg/kg/day). Data were median (interquartile range).

Genetic study

Regardless of the post-transplant phase and knowing that the mean dose of tacrolimus administered to rapid and poor metabolizers was comparable, the analysis of the pharmacokinetic parameters according to the different genotypes, revealed that C0, AUC0-12h, C0/D and AUC0-12h/D were significantly lower in rapid metabolizers (Table 3).

Table 3. Pharmacokinetic parameters according to CYP3A5*3 variants (n=50).

	CYP3A5 *1/*1 and *1/*3	CYP3A5 *3/*3	p
Global results			
Genotypic frequencies	13 (26%) [c]	37 (74%) [c]	
D (mg/kg/day)	0.078 ± 0.029 [a]	0.08 ± 0.03 [a]	0.8
C_0* (ng.mL^{-1})	4 (3-6) [b]	7.5 (5.6-10.4) [b]	**0.000**
AUC_{0-12h} ** (h.µg.L^{-1})	94 (57-148) [b]	151 (122-193) [b]	**0.003**
C_0/D	66 (40-90) [b]	98 (67-197) [b]	**0.013**
AUC_{0-12h} /D	1136 (960-1689) [b]	2007 (1418-3558) [b]	**0.021**
Results according post-transplant phases			
Early phase			
D (mg/kg/day)	0.090 ± 0.029 [a]	0.10 ± 0.03 [a]	0.3
C_0 (ng.mL^{-1})	4.5 ± 3.8 [a]	7.2 ± 2.7 [a]	0.12
AUC_{0-12h}(h.µg.L^{-1})	112.5 ± 75.4 [a]	144.4 ± 57.5 [a]	0.3
C_0/D	57.7 ± 50.9 [a]	72.0 ± 45.8 [a]	0.4
AUC_{0-12h}/D	1354 ± 983 [a]	1581 ± 923 [a]	0.6
Late phase			
D (mg/kg/day)	0.075 ± 0.030 [a]	0.070 ± 0.050 [a]	0.2
C_0 (ng.mL^{-1})	4.40 ± 1.97 [a]	8.46 ± 4.14 [a]	**0.01**
AUC_{0-12h} (h.µg.L^{-1})	95 ± 42 [a]	165 ± 82 [a]	**0.002**

AUC_{0-12h}: Area under the curve of tacrolimus, **C_0**: Blood trough concentration of tacrolimus, **D**: Daily normalized dose of tacrolimus (mg/kg/day). Data were [a] Means ± standard deviation; [b] Median [interquartile range], and [c] Number (percentage)

Furthermore, stratification of genetic results according to the therapeutic range showed that in poor metabolizers, compared to rapid ones, the prevalence of sub-therapeutic C0 (41 vs 67%) was significantly lower (p= 0.037, OR=0.22 CI95% [0.05-0.96]), while that of supratherapeutic C0 (23 vs. 0%) was significantly higher (p=0.046, OR=1.3 CI95% [1.12-1.66]). R2 between CYP3A5*3 variant and C0 variance was low at 0.25 and R2 between CYP3A5*3 variant and the AUC0-12h variance was low at 0.21. During the early phase, no statistically significant difference in the distribution of the studied pharmacokinetic parameters between poor and rapid metabolizers was observed (Table 3). In contrast, during the late phase, the pharmacokinetic parameters were significantly higher in poor metabolizers for similar D of tacrolimus (Table 3). However, a statistically significant association between tacrolimus C0, hematocrit levels (p=0.01) and corticosteroid doses (p=0.029), was found during the early phase. All patients with nephrotoxicity were poor metabolizers (n=5). Whereas, we did not find a significant association between this SNP and the onset of ADRs (p=0.35), in particular, nephrotoxicity (p=0.5). There was no impact of this SNP on acute rejection episodes (p=0.6), chronic graft dysfunction (p=0.59) and graft survival beyond 10 years (p=0.46) (Figure 2).

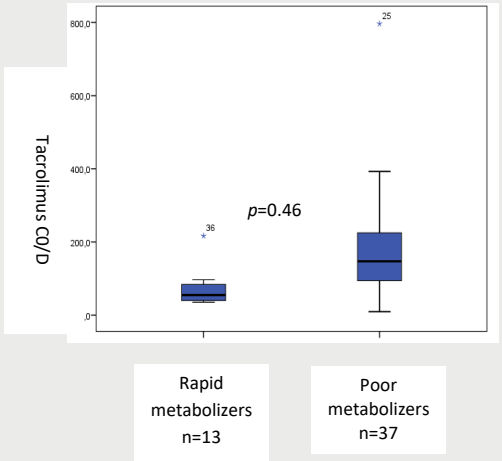

Figure 2. Graft survival according to CYP3A5*3 SNP's variant
C_0: Blood trough concentration of tacrolimus (ng.mL-1), **D**: Normalized dose (mg/kg/day)

DISCUSSION

Fifty Tunisian KTRs receiving tacrolimus were enrolled in the study. Acute graft rejections were observed in eight patients and chronic graft dysfunction in three patients. Twenty-one patients (42%) reported ADRs. C0 and AUC0-12h, showed a significant difference between CYP3A5*1 carriers (mean C0=4 ng.mL-1 and AUC0-12h=94.37 ng.h.mL-1) and CYP3A5*3/3 or poor metabolizers carriers (mean C0=7.45 ng.mL-1; AUC0-12h=151.27 ng.h.mL-1) (p=0.0001; p=0.003). Supratherapeutic tacrolimus levels were significantly more common in poor metabolizers (p=0.046; OR=1.3 CI95% [1.12-1.66]). The impact of this SNP was significant on C0, AUC0-12h, C0/D and AUC0-12h/D, only in the late phase (p=0.01, 0.002, 0.012, 0.003 respectively).

Therapeutic drug monitoring of calcineurin inhibitors, including tacrolimus, is essential to minimize ADRs and optimize the immunosuppressive effect in KTRs [11,15,16]. In our study, we found a strong correlation between the tacrolimus C0 and AUC0-12h. This result is consistent with some studies in the literature, but there is heterogeneity in the optimal pharmacokinetic parameter for monitoring tacrolimus therapy [11,15]. The 2017 guidelines of the clinical practice guidelines committee recommended C0 monitoring of tacrolimus (grade 2C) [14]. However, the therapeutic range of tacrolimus varies among studies and depends on several factors, including the type of induction therapy (eg; anti-thymocyte globulin or interleukin-2 receptor antagonist), immunological risks, and the occurrence of resistant acute or chronic rejection [11,13-15]. In our study, we observed a relatively high prevalence of subtherapeutic concentrations of tacrolimus, particularly in the early phase after transplantation. However, this prevalence decreased during the late phase, when the risk of acute rejection is lower, and dose adjustment is easier. These findings underscore the importance of regular monitoring of tacrolimus concentrations, especially in the early phase after transplantation, to optimize immunosuppression and prevent ADRs.

In 2015, a Tunisian study reported subtherapeutic C0 of tacrolimus in 47.3% of patients during the early phase post-kidney transplantation and 22.6% during the late phase [9]. Additionally, a significant increase in the area under the AUC0-12h/D ratio was observed in the late phase compared to the early phase (p=0.018), likely due to a decrease in tacrolimus clearance over time [2]. Interestingly, despite low tacrolimus blood concentrations, this study did not report an increase in rejection episodes or ADRs compared to other studies [11,19]. The correlation between tacrolimus pharmacokinetic parameters and clinical

outcomes remains controversial [11,15,19]. Some studies suggest that nephrotoxicity, arterial hypertension, and neurotoxicity may be more closely associated with pharmacokinetic parameters than diabetes mellitus or gastrointestinal complications [20]. Other studies have found no correlation between tacrolimus trough levels and renal impairment, suggesting that other factors such as initial kidney disease and ischemia-reperfusion injury may play a role [21].

In our cohort, we found that the frequency of poor metabolizers (CYP3A5 *3/*3) was significantly higher than that of rapid metabolizers (CYP3A5 *1/*1), consistent with the results reported by Aouam et al. [9] and with frequencies ranging from 81 to 96% in the European population [22]. Our analysis showed that the CYP3A5*3 variant was significantly associated with variability in tacrolimus pharmacokinetic parameters, in line with the majority of published studies. For example, a meta-analysis by Terrazino et al. [23] found that rapid metabolizers had lower tacrolimus trough levels to dose ratio, and this effect remained stable over time (two years) regardless of ethnicity. Another meta-analysis published in 2016 reported that 31 out of 37 studies found a significant association between C0/D and the CYP3A5*3 variant at one, three-, and 12-months post-transplant [24]. In our cohort, the CYP3A5*3 variant accounted for 25% of the variance in tacrolimus trough concentrations and 21% of the variance in AUC0-12h. In the literature, this SNP has been reported to explain between 25% [25] and 30% [26] of the variability in tacrolimus pharmacokinetic parameters, although some studies have reported lower values ranging from 3 to 6% [27]. These discrepancies may be due to differences in the characteristics of the studied populations and ethnic variability. Several studies have also shown that rapid metabolizers require an initial tacrolimus dose that is twice as high as that of other patients and take longer to reach therapeutic calcineurin inhibitor concentrations [28,29]. A randomized controlled trial including 280 patients [ie; group 1 received an initial tacrolimus dose of 0.1 mg/kg/day and group 2 received a dose adjusted according to CYP3A5 genotype (rapid metabolizers received 0.15 mg/kg/day and poor metabolizers received 0.075 mg/kg/day)] found that patients in group 2 had a significantly higher prevalence of therapeutic tacrolimus concentrations, reached these concentrations more quickly, and required fewer dose adjustments.

In our cohort, we investigated the impact of the CYP3A5*3 variant on tacrolimus pharmacokinetics according to the post-transplant phase. During the early phase post-transplant, we did not observe a significant association between the CYP3A5*3 variant and tacrolimus concentrations, contrasting with most studies' data [30,31]. This discrepancy may be due to the low number of rapid metabolizers sampled during the early phase in our cohort and the influence of other factors on tacrolimus bioavailability that may have masked the effect of the CYP3A5*3 variant during this period. In contrast, during the late phase post-transplant, we observed a significant association between the CYP3A5*3 variant and tacrolimus pharmacokinetics. During this phase, the gradual increase in tacrolimus clearance may expose poor metabolizers to the risk of drug toxicity, necessitating appropriate dose reduction [32]. Results from other studies during the early phase post-transplant are more variable. Some studies, including meta-analyses, have reported a significant association between the CYP3A5*3 variant and tacrolimus pharmacokinetic parameters [23,24,32,33], while others have not [9]. Additionally, some studies have suggested that the effect of the CYP3A5*3 SNP on tacrolimus pharmacokinetics may increase gradually during the first few months post-transplant [31], although this has not been consistently observed during the late phase.

While the impact of the CYP3A5*3 single nucleotide polymorphism on tacrolimus bioavailability has been well documented, it only partially explains the observed variability. In this context, our study found a significant association between low hematocrit and high corticosteroid doses with tacrolimus bioavailability during the early phase post-transplant [34], supporting the hypothesis that other variables may mask the effect of the CYP3A5*3 variant during this phase. Several studies have attempted to develop equations incorporating various variables, including CYP3A5 genotype, to predict the required tacrolimus dose [35], but the included variables and results have been heterogeneous. In our cohort, we did not observe an association between the CYP3A5*3 variant and the occurrence of ADRs, particularly renal ADRs, consistent with most published studies [23,33,36,37]. However, some studies have reported an association between the CYP3A5*3 variant and long-term nephrotoxicity [24,32]. The lack of association in our study may be due to a low incidence of nephrotoxicity and a potential lack of correlation between blood and intra-renal tacrolimus concentrations [38].

In our study, although we found a statistically significant correlation between the CYP3A5*3 variant and tacrolimus pharmacokinetics, we did not observe an impact on graft outcomes. This may be due to the low prevalence of histologically diagnosed transplant rejections and a limited sample size. Our results are consistent with several other studies [33,39,40]. For example, a meta-analysis by Terrazzino et al. [23] did not find an impact of the CYP3A5*3 variant on allograft rejection. However, some studies have reported an association between the CYP3A5*3 variant and earlier onset of rejection in rapid metabolizers [41]. Another study found no difference in graft survival according to the CYP3A5*3 variant over five years of follow-up [42], while a few studies have reported an association between this SNP and rejection [28]. A meta-analysis by Tang et al. [33] reported that the risk of rejection was significantly higher in rapid metabolizers only in KTRs at one-month post-transplant. Another meta-analysis by Rojas et al. [27] identified no association between the CYP3A5*3 variant and biopsy-proven rejection but did find an association with clinically diagnosed rejection. In 2015, the clinical pharmacogenetics implementation consortium [43] did not recommend routine administration of tacrolimus based on the CYP3A5*3 variant due to uncertainty regarding its contribution to improving outcomes in KTRs, particularly in reducing rejection and calcineurin inhibitor-induced nephrotoxicity.

To the best of the authors' knowledge, this study is the first one analyzing the impact of this SNP on ADRs, on graft survival and on the risk of rejection in addition to tacrolimus pharmacokinetics variation in KTRs treated by tacrolimus.

It is important to note that our study has some limitations that should be considered when interpreting the results. These limitations include the single-center design, the small number of KTRs included, and the cross-sectional nature of the pharmacokinetic analysis. Additionally, the prospective follow-up of KTRs was limited to monitoring for chronic graft dysfunction or a return to hemodialysis, which defined graft survival [9].

CONCLUSION

Pre-transplant genotyping for the CYP3A5 6986A>G variant may assist clinicians in tailoring tacrolimus doses to individual patients' genetic profiles, potentially facilitating the rapid achievement of therapeutic drug concentrations. This approach could save time and healthcare resources

by enabling more effective dosing of calcineurin inhibitors. However, given the absence of a clear prognostic benefit observed in our cohort, the routine use of CYP3A5*3 genotyping in pre-transplant clinical practice cannot be unequivocally recommended.

REFERENCES

1. Gomes RM, Guerra Junior AA, Lemos LL, Costa Jde O, Almeida AM, Alvares J, et al. Ten-year kidney transplant survival of cyclosporine or tacrolimus treated patients in Brazil. Expert Rev Clin Pharmacol 2016;9(7):991-9.
2. Shrestha BM. two decades of tacrolimus in renal transplant: basic science and clinical evidences. Exp Clin Transplant 2017;15(1):1-9.
3. 3. Barbarino JM, Staatz CE, Venkataramanan R, Klein TE, Altman RB. Cyclosporine and tacrolimus pathways. Pharmacogenet Genomics 2013;23(10):563-85.
4. Antignac M, Barrou B, Farinotti R, Lechat P, Urien S. Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. Br J Clin Pharmacol 2007;64(6):750-7.
5. Roy JN, Lajoie J, Zijenah LS, Barama A, Poirier C, Ward BJ, et al. Cyp3A5 genetic polymorphisms in different ethnic populations. Drug Metab Dispos 2005;33(7):884-7.
6. Crettol S, Venetz JP, Fontana M, Aubert JD, Pascual M, Eap CB. CYP3A7, CYP3A5, CYP3A4, and ABCB1 genetic polymorphisms, cyclosporine concentration, and dose requirement in transplant recipients. Ther Drug Monit 2008;30(6):689-99.
7. Meng XG, Guo CX, Feng GQ, Zhao YC, Zhou BT, Han JL, et al. Association of CYP3A polymorphisms with the pharmacokinetics of cyclosporine A in early post-renal transplant recipients in China. Acta Pharmacol Sin 2012;33(12):1563-70.
8. 8. Cheng Y, Li H, Meng Y, Liu H, Yang L, Xu T, et al. Effect of CYP3A5 polymorphism on the pharmacokinetics of Tacrolimus and acute rejection in renal transplant recipients: experience at a single centre. Int J Clin Pract2015;183:16-22.
9. Aouam K, Kolsi A, Kerkeni E, Ben Fredj N, Chaabane A, Monastiri K, et al. Influence of combined CYP3A4 and CYP3A5 single nucleotide polymorphisms on tacrolimus exposure in kidney transplant recipients: a study according to the post-transplant phase. Pharmacogenomics 2015;16(18):2045-54.
10. Charfi R, Mzoughi K, Boughalleb M, et al. Response to clopidogrel and of the cytochrome CYP2C19 gene polymorphism. Tunis Med. 2018;96(3):209–18
11. Wallemacq P, Armstrong VW, Brunet M, Haufroid V, Holt DW, Johnston A, et al. Opportunities to optimize Tacrolimus therapy in solid organ transplantation: report of the european consensus conference. Ther Drug Monit 2009;31(2):139-52.
12. Haas M, Sis B, Racusen LC, Solez K, Glotz D et al. Banff 2013 meeting report: inclusion of c4dnegative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014;14:272-83.
13. Ekberg H, Bernasconi C, Silva H, Vitko S, Hugo C, Demirbas A, et al. Calcineurin inhibitor minimization in the symphony study: observational results 3 years after transplantation. Am J Transplant 2009;9(8):1876-85.
14. Baker RJ, Mark PB, Patel RK, Stevens KK, Palmer N. Renal association clinical practice guideline in post-operative care in the kidney transplant recipient. BMC Nephrol 2017;18(1):174-5.
15. Schiff J, Cole E, Cantarovich M. Therapeutic monitoring of calcineurin inhibitors for the nephrologist. Clin J Am Soc Nephrol.2007;2(2):374-84.
16. Barraclough KA, Isbel NM, Kirkpatrick CM, Lee KJ, Taylor PJ, Johnson DW, et al. Evaluation of limited sampling methods for estimation of tacrolimus exposure in adult kidney transplant recipients. Br J Clin Pharmacol2011;71(2):207-23.
17. Marquet P. Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments. Therapeutic drug monitoring for dose drug adjustement Paris: Elsevier; 2004.
18. Tsuchiya N, Satoh S, Tada H, Li Z, Ohiyama C, Sato K, et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetic of tacrolimus in renal transplant recipients. Transplant 2004;78(8):1182-7.
19. Mangray M, Vella JP. Hypertension after kidney transplant. Am J Kidney Dis 2011;57(2):331-41.
20. Ekberg H, Bernasconi C, Noldeke J, Yussim A, Mjornstedt L, Erken U, et al. Cyclosporine, tacrolimus and sirolimus retain their distinct toxicity profiles despite low doses in the symphony study. Nephrol Dial Transplant 2010;25(6):2004-10.
21. Krejci K, Tichy T, Bachleda P, Zadrazil J. Calcineurin inhibitor induced renal allograft nephrotoxicity. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2010;154(4):297-306.
22. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 2001;27(4):383-91.
23. Terrazzino S, Quaglia M, Stratta P, Canonico PL, Genazzani AA. The effect of CYP3A5 6986A>G and ABCB1 3435C>T on tacrolimus dose-adjusted trough levels and acute rejection rates in renal transplant patients: a systematic review and meta-analysis. Pharmacogenet Genomics 2012;22(8):642-5.
24. Rojas L, Neumann I, Herrero MJ, Boso V, Reig J, Poveda JL, et al. Effect of CYP3A5*3 on kidney transplant recipients treated with tacrolimus: a systematic review and meta-analysis of observational studies. Pharmacogenomics J 2015;15(1):38-48.
25. Lunde I, Bremer S, Midtvedt K, Mohebi B, Dahl M, Bergan S, et al. The influence of CYP3A, PPARA, and POR genetic variants on the pharmacokinetics of tacrolimus and cyclosporine in renal transplant recipients. Eur J Clin Pharmacol 2014;70(6):685-93.
26. Jonge H, Loor H, Verbeke K, Vanrenterghem Y, Kuypers DR. In vivo CYP3A4 activity, CYP3A5 genotype, and hematocrit predict tacrolimus dose requirements and clearance in renal transplant patients. Clin Pharmacol Ther 2012;92(3):366-75.
27. Jacobson PA, Oetting WS, Brearley AM, Leduc R, Guan W, Schladt D, et al. Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. Transplant 2011;91(3):300-8.
28. Chen L, Prasad GVR. CYP3A5 polymorphisms in renal transplant recipients: influence on Tacrolimus treatment. Pharmgenomics Pers Med 2018;11:23-33.
29. Dai Y, Hebert MF, Isoherranen N, Davis CL, Marsh C, Shen DD, et al. Effect of CYP3A5 polymorphism on tacrolimus metabolic clearance in vitro. Drug Metab Dispos. 2006;34(5):836-47.
30. Li JL, Liu S, Fu Q, Zhang Y, Wang XD, Liu XM, et al. Interactive effects of CYP3A4, CYP3A5, MDR1 AND NR1I2 polymorphisms on tracrolimus trough concentrations in early postrenal transplant recipients. Pharmacogenomics 2015;16(12):1355-65.
31. Niioka T, Kagaya H, Saito M, Inoue T, Numakura K, Habuchi T, et al. Capability of utilizing CYP3A5 polymorphisms to predict therapeutic dosage of tacrolimus at early stage post-renal transplantation. Int J Mol Sci 2015;16(1):1840-54.
32. Kuypers DR, Jonge H, Naesens M, Lerut E, Verbeke K, Vanrenterghem Y. CYP3A5 and CYP3A4 but not MDR1 single-nucleotide polymorphisms determine long-term tacrolimus disposition and drug-related nephrotoxicity in renal recipients. Clin Pharmacol Ther 2007;82(6):711-25.
33. 33. Tang HL, Xie HG, Yao Y, Hu YF. Lower tacrolimus daily dose requirements and acute rejection rates in the CYP3A5 nonexpressers than expressers. Pharmacogenet Genomics 2011;21(11):713-20.
34. Jain AB, Venkataramanan R, Cadoff E, Fung JJ, Todo S, Krajack A, et al. Effect of hepatic dysfunction and tube clamping on FK506 pharmacokinetics and trough concentrations. Transplant Proc 1990;22(1):57-9.
35. Zuo XC, Ng CM, Barrett JS, Luo AJ, Zhang BK, Deng CH, et al. Effects of CYP3A4 and CYP3A5 polymorphisms on tacrolimus pharmacokinetics in chinese adult renal transplant recipients: a population pharmacokinetic analysis. Pharmacogenet Genomics 2013;23(5):251-61.
36. Glowacki F, Lionet A, Buob D, Labalette M, Allorge D, Provot F, et al. CYP3A5 and ABCB1 polymorphisms in donor and recipient: impact on tacrolimus dose requirements and clinical outcome after renal transplantation. Nephrol Dial Transplant. 2011;26(9):3046-50.
37. Gervasini G, Garcia M, Macias RM, Cubero JJ, Caravaca F, Benitez J. Impact of genetic polymorphisms on Tacrolimus pharmacokinetics and the clinical outcome of renal transplantation. Transpl Int. 2012;25(4):471-80.
38. Gelder T, Hesselink DA. Dosing tacrolimus based on CYP3A5 genotype: will it improve clinical outcome? Clin Pharmacol Ther 2010;87(6):640-1.
39. Flahault A, Anglicheau D, Loriot MA, Thervet E, Pallet N. Clinical impact of the CYP3A5 6986A>G allelic variant on kidney transplantation outcomes. Pharmacogenomics. 2017;18(2):165-73.
40. Qiu XY, Jiao Z, Zhang M, Zhong LJ, Liang HQ, Ma CL, et al. Association of MDR1, CYP3A4*18B, and CYP3A5*3 polymorphisms with cyclosporine pharmacokinetics in chinese renal transplant recipients. Eur J Clin Pharmacol. 2008;64(11):1069-84.
41. Macphee IA, Fredericks S, Tai T, Syrris P, Carter ND, Johnston A, et al. The influence of pharmacogenetics on the time to achieve target tacrolimus concentrations after kidney transplantation. Am J Transplant 2004;4(6):914-9.

42. Satoh S, Saito M, Inoue T, Kagaya H, Miura M, Inoue K, et al. CYP3A5*1 allele associated with tacrolimus trough concentrations but not subclinical acute rejection or chronic allograft nephropathy in japanese renal transplant recipients. Eur J Clin Pharmacol 2009;65(5):473-81.

43. Birdwell KA, Decker B, Barbarino JM, Peterson JF, Stein CM, Sadee W, et al. Clinical pharmacogenetics implementation consortium guidelines for cyp3A5 genotype and tacrolimus dosing. Clin Pharmacol Ther. 2015;98(1):19-24.