Tacrolimus overexposure in kidney transplant recipients during the first post-operative week: caution is required in older patients

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Tacrolimus overexposure in kidney transplant recipients during the first post-operative week: caution is required in older patients

Running title: Tacrolimus overexposure in kidney transplantation during the first week

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ABSTRACT

In liver transplantation, tacrolimus trough concentrations (Cmin) above 20 ng/mL during the first days led to worse outcome at 1 year but data in the kidney transplant (KT) era are scarce.

The aim of this study was to evaluate the impact of tacrolimus overexposure during the first week post-transplantation on the kidney function (KF) of KT recipients.

In this retrospective study, 105 KT recipients were attributed to overexposure group (OG) or normal group according to their Cmin during the first week of treatment. KF was evaluated by comparing the rate of Delayed Graft Function (DGF) and by collecting plasma creatinine from day-1, 2, 3, 4, 5, 6, 7, 14, 21, 28 and at 1 year. Risk factors for developing DGF were also investigated using a multivariate model.

DGF was more frequent in OG (43% of patients) (p=0.027) which has higher plasma creatinine on day-7, -14 and 21. OG patients were older with more extended criteria donor's grafts. In the multivariate analysis, only cold ischemia time (CIT) remained associated with DGF (OR = 1.003), while TAC overexposure did not reach significance (p = 0.06; OR = 3.9).

In this study, we confirmed the predominant role of CIT as a risk factor for the onset of DGF in kidney transplantation. 43% of KT recipients were overexposed with more DGF, especially older patients.

Keywords: Kidney, Transplantation, Tacrolimus, Pharmacokinetics, Early Allograft Dysfunction

INTRODUCTION

Graft rejection prevention in renal transplant recipients is ensured by an association of immunosuppressive (IS) drugs administered to patients. Among these drugs, tacrolimus (TAC), a calcineurin inhibitor inhibiting NFAT nuclear translocation in the T-lymphocyte and subsequently inhibiting interleukin-2 synthesis, appears as the main drug of IS therapy [1]. Despite its potent effect, TAC is also associated with adverse events which are frequently presented as concentration-dependent [2]. Thus, TAC overexposure is associated with nephrotoxicity, neurotoxicity, diabetes and onset of malignancies or infections [2]. It has been shown, in liver transplant recipients that whole-blood trough concentrations above 20 ng/mL during the first two weeks post-transplantation led to worse outcome at 1 year [3]. Higher blood concentrations of TAC have been reported to reduce renal afferent arterioles blood flow due to vasoconstriction and to conduct to acute tubulopathy due to the vacuolization of the tubular cytoplasm leading to decrease glomerular filtration rate [4]. Therefore, TAC overexposure has to be avoided and therapeutic drug monitoring of TAC blood level is mandatory in all organ transplant patients. In kidney transplant recipients, nephrotoxicity is particularly threatening as it exerts a toxic pressure on the newly transplanted organ. During the first post-transplantation days, the kidney function slowly improves. However, this function recovery may be inadequate leading to graft dysfunction. Indeed, Delayed Graft Function (DGF) is defined as the need for dialysis in kidney transplant recipients during the first week post-transplantation [5]. This state should be avoided as it is associated with worse outcome including graft loss and death [6]. Thus, while other factors can also be responsible for DGF, exerting a nephrotoxic pressure during the first post-transplantation days by exposing patients to high level of TAC appears theoretically inappropriate.

However, while nephrotoxicity can be a cause of concern in kidney transplant recipients, to date, the relationship between early overexposure and renal dysfunction has not been fully elucidated.

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The aim of this study was to evaluate the impact of tacrolimus overexposure during the first week post-transplantation on the kidney function of 105 transplant recipients.

METHOD

Patients and data

All patients receiving a first kidney transplant with an organ harvested from a brain-dead patient between January 2013 and December 2015 and treated with TAC between day-2 and day-28 where retrospectively included in the study. Patients receiving a living-donor organ, combined organ transplantation, not treated with TAC, switched to another IS regimen during the first month, with insufficient measurement of TAC blood levels, receiving chronic medication by TAC before transplantation and treated with an inhibitor or an inducer of TAC metabolism were excluded from the study. IS treatment consisted initially of TAC 0.2 mg/kg/day administered as two doses separated by 12 hours initiated a few hours before the transplantation, mycophenolate mofetil 1000mg twice a day initiated just before the transplantation. Patients received an induction treatment with an IL-2 receptor inhibitor or a lymphocyte-depletant agent. Patients also received an IV bolus of methylprednisolone as an induction and then 125mg on day-1. From day-2 oral prednisolone 20mg was administered to patients on the morning tapered to 15mg from day-15 to day-30. Donor positive/recipient negative patients for cytomegalovirus also received a prophylactic treatment consisting of valganciclovir 450 mg/day and for *Pneumocystis jiroveci* consisting of sulfamethoxazole 800 mg and trimethoprime 160 mg administered once a day. Therapeutic drug monitoring of TAC blood concentration was conducted every day during the first two weeks and twice a week until the end of the first month. TAC dosage was adapted to reach the target trough concentration of 10-15 ng/mL during the first 6 weeks of treatment.

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The following data were gathered from patient files, biological database or CRISTAL national database that is managed by the French government-funded "Agence de la BioMedicine" (ABM): TAC blood levels on day-2, 3, 4, 5, 6, 7, 14, 21, 28, serum creatinine collected on day-1, 7, 14, 21, 28 and at 1 year, donor age, cold ischemia time, Extended Criteria Donor (ECD), recipient age and sex, bilirubin, ASpartate aminoTransferase (AST), ALanine aminoTransferase (ALT), Gamma GlutamylTransferase (GGT), ALkaline Phosphatase (ALP), albumin, protein, hematocrit. All the biological measures (apart from TAC concentrations and serum creatinine) were retrieved on day 1. TAC concentrations measured after drug intake or more than 2 hours before drug intake were excluded from the analysis.

Overexposure was defined as at least one measure of TAC trough concentration above 20 ng/mL between day-2 and day-7. Patients were then attributed to Overexposure Group (OG) or Normal Group (NG).

The primary endpoint of the study was the rate of DGF defined as the need for dialysis during the first week post-transplantation, between the groups. DGF was collected with CRISTAL national database that is managed by the French government-funded ABM and that prospectively collects the demographic, clinical and biological data of all organ transplant candidates and donors in France.

Secondary endpoints were: the relationship between DGF and: Tacrolimus overexposure, Creatinine, Donor Age, Cold Ischemia Time, Extended Criteria Donor, Recipient Age, Bilirubin, AST, ALT, GGT, ALP, Albumin, Protein, Hematocrit.

Statistical analysis

Continuous variables were expressed as median and interquartile range, and categorical variables were reported as counts and percentages. The characteristics of the patients of both groups were compared, using the Wilcoxon-Mann-Whitney test (after verification of non-normal distribution

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using the Shapiro-Wilk normality test) for continuous variables and the Chi-squared test (or Fisher's exact test when appropriate) for categorical variables and proportions. A multivariate logistic regression analysis was performed to identify independent predictors of DGF. The variables showing a p-value of <0.1 at the univariate step were included in the multivariate model. Then, a stepwise selection of the variables based on the reduction of the Akaike's Information Criterion (AIC) was performed. The statistical analysis was performed using R 3.0.3.

The impact of overexposure on kidney function was evaluated by comparing the rate of DGF in both groups and by comparing plasma creatinine on day-7, 14, 21, 28 and at 1 year.

RESULTS

One hundred and forty patients were assessed for eligibility and 105 patients were finally included in this study. A workflow displaying the reasons for patients to be excluded from the study is presented in figure 1. Baseline demographic and biological parameters of the patients are presented in table 1. Forty five patients (43%) have been overexposed during the first week and formed the OG (figure 2). Recipients ($p < 0.001$) and donors ($p < 0.001$) age as well as percentage of donors displaying ECD ($p = 0.001$) differed between OG and NG patients with patients in the OG group being older, with older donors and more ECD. Overall, 12 patients (11.4%) experienced DFG. The univariate analysis revealed that the cold ischemia time was longer in the DGF group (1126 min *versus* 881 min, $p<0.001$) and the frequency of overexposure to TAC was higher in the DGF group (75.0% *versus* 38.7%, $p=0.037$). The patients with or without DGF were comparable regarding the other characteristics. In the multivariate logistic regression analysis, cold ischemia time remained significantly linked to the occurrence of DGF ($p=0.0066$, OR=1.003), whereas overdose was close to significant ($p=0.062$, OR=3.9) (table 2).

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DGF was more frequent in OG (20.0%) compared to NG (5.0%) (p=0.027). OG patients presented a higher plasma creatinine on day-7 (273 [IQR 121-440] vs 176 [IQR 105-173] µM; p=0.003), day-14 (197 [IQR 124-223] vs 162 [IQR 112-169] µM; p=0.04) and 21 (177 [IQR 137-184] vs 143 [IQR 117-164] µM; p=0.01) but not on day-28 (171 [IQR 129-174] vs 144 [IQR 116-155] µM; p=0.18) and at 1 year (135 [IQR 106-165] vs 132 [IQR 110-146] µM; p=0.93) while creatinine on day-1 was not different between the two groups (585 [IQR 440-734] vs 520 [IQR 372-639] µM; p = 0.12) (figure 3). Creatinine was roughly the same in the two groups at one year (135 [IQR 106-165] vs 132 [IQR 110-146] µM; p = 0.93). Hematocrit was slightly higher in the OG (35.3 [IQR 30.9-39.5] vs 33.4 [IQR 30.3-35.4] %; p=0.06). Cold ischemia time was also slightly higher in the OG although not reaching statistical significance (1067 [IQR 840-1310] vs 970 [IQR 777-1145]; p = 0.09).

DISCUSSION

In the present study, TAC overexposure, defined by the occurrence of at least one measure of whole-blood trough concentration of TAC above 20 ng/mL within the first week after renal transplantation, was frequent with more than 40% of the patients experiencing it within the early period post transplantation. Overexposed patients were older and received graft from older donors. As older grafts are often allocated to older patients, this finding is not so surprising. The donors in the OG belonged more frequently to ECD group particularly because of their age. There was also a trend to a longer cold ischemia time in the OG. Older renal transplant recipients have already been shown to exhibit higher TAC concentrations than younger recipients while being exposed to the same dosage. In the studies of Jacobson *et al.* and David-Neto *et al.*, the authors reported higher TAC-normalized concentrations for patients of more than 65 years compared to young and middle age patients [7,8]. Indeed, TAC is highly metabolized and then predominantly eliminated through biliary excretion. We could then speculate that older patients might have reduced TAC metabolism and elimination. Hepatic dysfunction has already been reported to be associated with TAC

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pharmacokinetics [2]. This should at least partly explain the accumulation of TAC in the OG. However, none of the biological marker of hepatic dysfunction was different between the two groups. Neither AST and ALT nor bilirubin appeared to be higher in the OG. Nevertheless, TAC should be initiated cautiously in older patients and these patients should receive lower dosage than younger patient.

In addition, while non-statistically significant, there was a trend to higher hematocrit in the OG. Hematocrit has already been shown to explain part of the variability of TAC pharmacokinetics which is consistent with the high level of binding of TAC to red blood cells [7].

The main finding of that study is that patients overexposed to TAC during the first week of treatment displayed more DGF (20% versus 5%, p = 0.027). Moreover, while being comparable on day-1, plasma creatinine was higher in OG during the first month post-transplantation meaning that glomerular filtration might be negatively impacted by overexposure periods during the first week of treatment. Assuming the fact that patients in OG were older, this raises the hypothesis of a faster accumulation leading to a concentration-dependent toxicity ending up in increase toxicity in a sub-population of patients with an increase renal frailty. Thus, exerting a toxic pressure on renal function during the first days after kidney transplantation has probably a deleterious effect on the graft function recovery. A similar conclusion has been raised by Barraclough *et al.* in a study whose aim was to correlate TAC but also mycophenolic acid and prednisolone exposure with DGF and new onset diabetes after transplantation [9]. In this study, 120 kidney transplant recipients benefited of an estimation of area under the curve (AUC_{0-12}) of IS drugs concentrations on day-4 and at month 1 of their treatment. The authors explored the relationship between TAC AUC_{0-12} and treatment outcomes. Interestingly, DGF was observed in 20% of their patients and AUC_{0-12} and trough concentrations of TAC were higher in patients developing DGF. A receiver operational characteristic curve showed that a trough concentration of 9.9 ng/mL was a cut-off allowing prediction of DGF with a sensitivity of 81% but a weaker specificity of 57%. Kuypers *et al.* highlighted that, in kidney

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transplant recipients treated with TAC 0.2 mg/kg/day, mean TAC trough concentrations were higher during the first 4 days in patients experiencing DGF [10]. Interestingly, in this prospective, open-label, observational clinical cohort study conducted in 304 kidney transplant recipients, most of the findings evidenced by the author appears in agreement with our study. Indeed, while the rate of DGF was lower than in the present work (9.9% versus 20.0%), transplant recipients developing DGF in the study of Kuypers *et al.* had a mean TAC trough concentrations above 20 ng/mL on day-2 of their treatment. Moreover, in the univariate analyses, these patients were also older with an older donor, a longer cold ischemia time and a higher serum creatinine (on day-30, 60 and 90).

A nephrotoxic association between higher concentrations of TAC and renal function has also been reported by Ekberg and colleagues one year after kidney transplantation [11]. In a retrospective study pooling data obtained from three clinical trials conducted in *de novo* kidney transplant recipients (Symphony, FDCC and Opticept), the authors showed a significant inverse relationship between TAC concentrations measured between 6 and 12 months and the creatinine clearance at 1 year. It should be noted that patients in the Symphony and Opticept studies were treated on a minimization protocol for TAC with an objective of trough concentrations of 3-7 ng/mL and 8-12 ng/mL respectively during the first month.

We defined overexposure using a threshold of trough concentration of 20 ng/mL as it is usually considered as the toxic threshold when undertaken therapeutic drug monitoring of TAC in solid organ recipients. A threshold of 15 ng/mL has also been proposed in a retrospective study conducted by Rehman *et al.* aiming at evaluating different levels of TAC on early outcomes after kidney transplantation [4]. The incidence of DGF in this study appeared also higher in patients displaying a trough concentration above 15 ng/mL, but also in patients with trough concentration between 12 and 15 ng/mL and below 10 ng/mL, during the first two weeks of treatment when compared with patients with a maximum trough concentration between 10 and 12 ng/mL. This should mean that nephrotoxic effect could appear at concentrations as low as 12 ng/mL and might

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also be related to low immunosuppressive drug exposure. Of note, the patients in the highest TAC exposure arm (i.e above 15 ng/mL) had a mean maximum TAC trough concentration above 20 ng/mL (20.2 +/- 5.5 ng/mL) which renders these patients very similar to our OG patients.

All these elements might legitimate the use of minimization protocols in immunosuppressive treatment in kidney transplantation including during the very first post-operative days particularly in older patients. As these patients exhibit higher TAC exposure, they would benefit from a lower initial TAC dose in order to avoid reaching toxic threshold.

We then try to highlight the risk factors for developing DGF in our population study by using univariate and multivariate analysis. We identify cold ischemia time ($p < 0.001$, OR = 1.003) and TAC overexposure during the first week of treatment ($p = 0.04$, OR = 3.9) in the univariate analysis. Cold ischemia time was the only factor remaining associated with DGF in the multivariate analysis ($p = 0.007$). Ischemic period is known to exacerbate microvascular vasoconstriction, thrombosis and inflammation leading to kidney tubule damages and increasing the time to cell proliferation leading to the recovery of the tubules in denuded areas of the baseline membrane [6]. DGF rate can be as high as 25.5% in deceased-donor recipients while only 4% of living-donor recipients usually develop DGF [12,13]. Numerous risk factors have been associated with DGF such as male gender, black race, body mass index, previous transplantation, diabetes for recipient; female gender, age, body mass index, deceased donor, donation after cardiac arrest, increased serum creatinine for donors; as well as cold and warm ischemia time, sensitization and HLA mismatches [6]. In our study, cold ischemia time was the major determinant of DGF. While it did not reach significance in the multivariate analysis, TAC overexposure during the first week might also be negatively related to DGF and should certainly be avoided. Thus, delaying TAC treatment onset and minimizing TAC exposure could be valuable strategies to decrease the risk of DGF. Disappointingly, delaying calcineurin inhibitor exposure with an interleukin-2 receptor inhibitor as an induction treatment does not seem to reduce the risk of DGF [14]. On the opposite, reduction of calcineurin inhibitor exposure (considering

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altogether minimization, delay and avoidance strategies) has been shown to be associated with a reduced rate of DGF in a meta-analysis when pooling data from 45 studies (n = 9456 kidney transplant recipients) [15]. However, most of these studies have been conducted with cyclosporine.

Our study has several limitations. The main one is that this is a retrospective, monocentric study with a limited number of patients included. We also arbitrary decide to define TAC overexposure by displaying a trough concentration above 20 ng/mL between day-2 and day-7 while a threshold of 15 ng/mL has been proposed in other studies. As the recommended range of TAC trough concentration in our center is 10-15 ng/mL during the first week, and because many of our patients exhibited TAC trough concentration above 15 ng/mL during this period, we choose to define overexposure by having a clear overshot concentration. Interestingly, all patients developing DGF in our study had at least one measured TAC concentration above 15 ng/mL. The most questionable point of the study is maybe the differences between OG and NG groups. Thus, OG patients were older, received older graft and more frequently from ECD. These render the two populations not entirely comparable. Causality of worst recovery in the renal function in OG is then hard to claim as it could also be related to patient's age or donor's age. TAC overexposure also failed being recognized as an independent risk factor in the multivariate analysis which decreases the impact of this finding. The retrospective design of the study and the relatively small number of patients, are certainly responsible for such a difference in groups but, despite this limitation, the fact that our conclusions seem to be supported by pre-cited works conducted on a similar subject is reassuring. Another limitation of our work is that some recognized factors related to DGF had not been included in the analysis. Thus, diabetes for recipients, increased serum creatinine for donors as well as sensitization and HLA mismatches had not been gathered. However, some of these covariates such as donation after cardiac arrest and increased serum creatinine in donors are included in the definition of extended criteria donors which is collected for the study and then indirectly taken into account in the analysis. Nevertheless, one cannot exclude a potential bias in the interpretation of the results due to the lack of these data. After all, it would have been relevant to collect patient's genotype

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particularly for cytochrome P450 3A5 (*CYP3A5*) which has been recognized to be a major determinant of the pharmacokinetics of TAC in solid organ transplant patients [2,16]. Indeed, *CYP3A5* non-expressers are likely to reach supratherapeutic concentrations because of a decrease in TAC clearance. On a basis of dose-weight dosage, these patients might be more likely overexposed when compared with *CYP3A5* expressers. Not to mention that older patients may display reduced cytochrome P450 metabolism resulting in decreased TAC clearance. Unfortunately, *CYP3A5* genotype determination is not currently performed in our center while it could help preventing TAC overexposure particularly in the early post-operative period.

CONCLUSION

In this study, we confirmed the predominant role of cold ischemia time as a risk factor for the onset of delayed graft function in kidney transplantation. Moreover, we found that 43% of kidney transplant recipients appeared to be overexposed during the first week of treatment with TAC. While there must be confounding factors, this might have an impact on kidney function as more patients displayed delayed graft function in the overexposed group of patients. Overexposed patients were older so that caution is required in defining drug dosage in these old patients. Plasma creatinine also appeared to be more elevated during the first 3 weeks post-operative period in overexposed patients. These results might be an appraisal for an a priori individualization of TAC dosage particularly in older transplant recipients, such as using a 0.15 mg/kg dosage in *CYP3A5* non-expressers, to avoid overexposure during the first week of treatment.

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TABLES

Table 1: Patients characteristics in overexposed group and normal group

	Overexposed Group	Normal Group	p value
Patients (n(%))	45 (42.9)	60 (57.1)	
Delayed Graft Function (n,(%))	9 (20.0%)	3 (5.0%)	**0.027**
Baseline Characteristics			
Recipients			
Creatinine (µM)	585 ± 226	520 ± 204	0.12
Age (y)	59.2 ± 12.7	48.5 ± 14.8	**<0.001**
Bilirubin (µM)	5.2 ± 2.3	5.3 ± 3.9	0.36
AST (IU/mL)	25.9 ± 17.0	23.0 ± 11,8	0.39
ALT (IU/mL	22.6 ± 14.1	21.8 ± 12,4	0.84
GGT (IU/mL)	41.7 ± 44.1	41.6 ± 64.1	0.08
ALP (IU/mL)	62.2 ± 22.6	82.9 ± 56.3	0.06
Albumin (g/L)	36.2 ± 6.2	37.7 ± 7.6	0.37
Protein (g/L)	62.8 ± 7.7	64.7 ± 8.9	0.22
Hematocrit (%)	35.3 ± 5.4	33.4 ± 4.3	0.06
Donor			
Age (y)	60.1 ± 15.7	50.4 ± 14.8	**<0.001**
Cold ischemia (min)	1067 ± 364	970 ± 296	0.09
Extended Criteria (%)	67	35	**0.001**

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyltransferase; ALP: Alkaline phosphatase

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Table 2: Univariate and multivariate analysis for Delayed Graft Function risk factors

No DGF	DGF	p (univariate)	p (multivariate)	
57 (39 - 64)	62 (48 - 66,5)	0,25		
21 (15 - 45)	22 (19,5 - 26,5)	0,88		
64 (50 - 82)	54,5 (46,5 - 59,75)	0,11		
21 (16 - 26)	19,5 (13,75 - 42,5)	0,81		
19 (14 - 28)	20,5 (13,25 - 21,5)	0,68		
4 (4 - 6)	5 (4 - 7,25)	0,40		
34,1 (30,9 - 38,3)	34,45 (30,5 - 37,4)	0,90		
63 (58 - 69)	61 (57,75 - 63)	0,35		
35 (31,5 - 42,5)	36,9 (30,2 - 42,4)	0,73		
56 (45 - 67)	60,5 (54,5 - 66,25)	0,32		
45 (48,4%)	6 (50,0%)	1,00		
881 (777 - 1159)	1226 (1088 - 1565)	0,00072	**0,0066**	

GGT: Gamma glutamyltransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ECD: Extended criteria donor; TAC: Tacrolimus

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FIGURES

Figure 1:

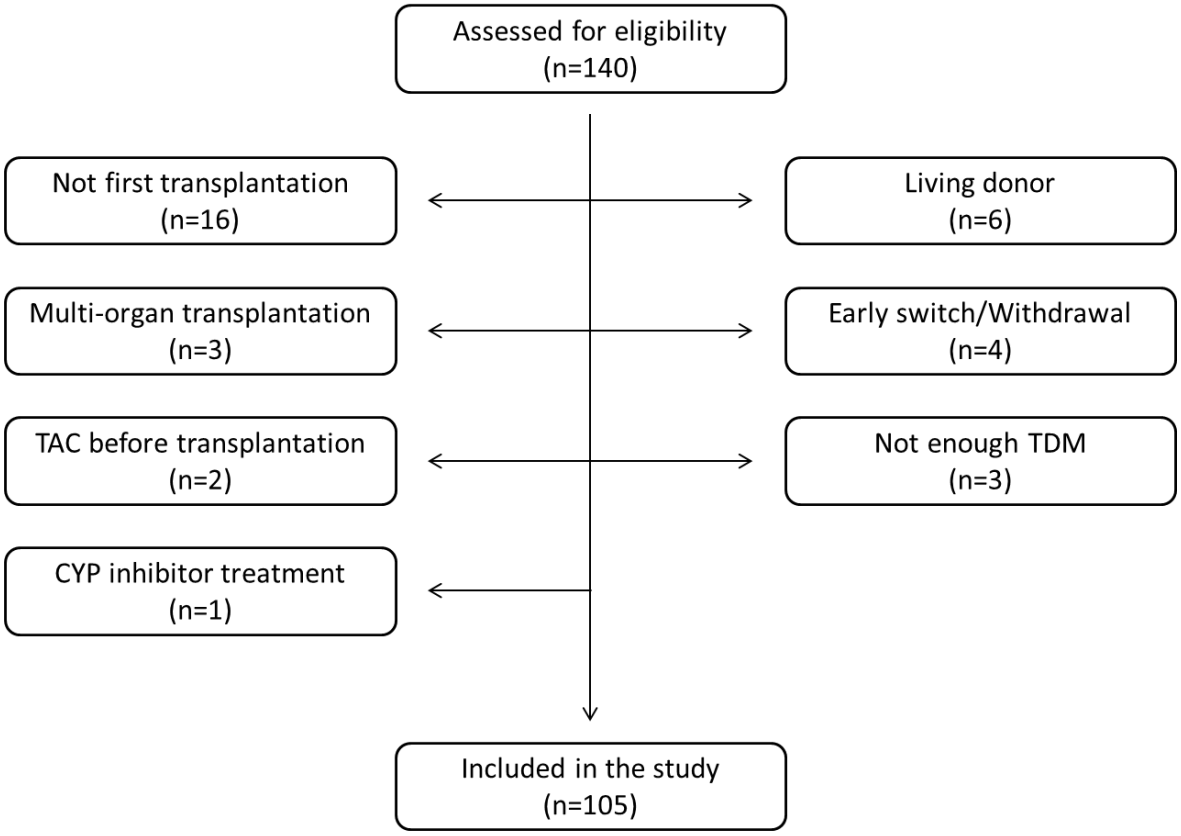

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Figure 2:

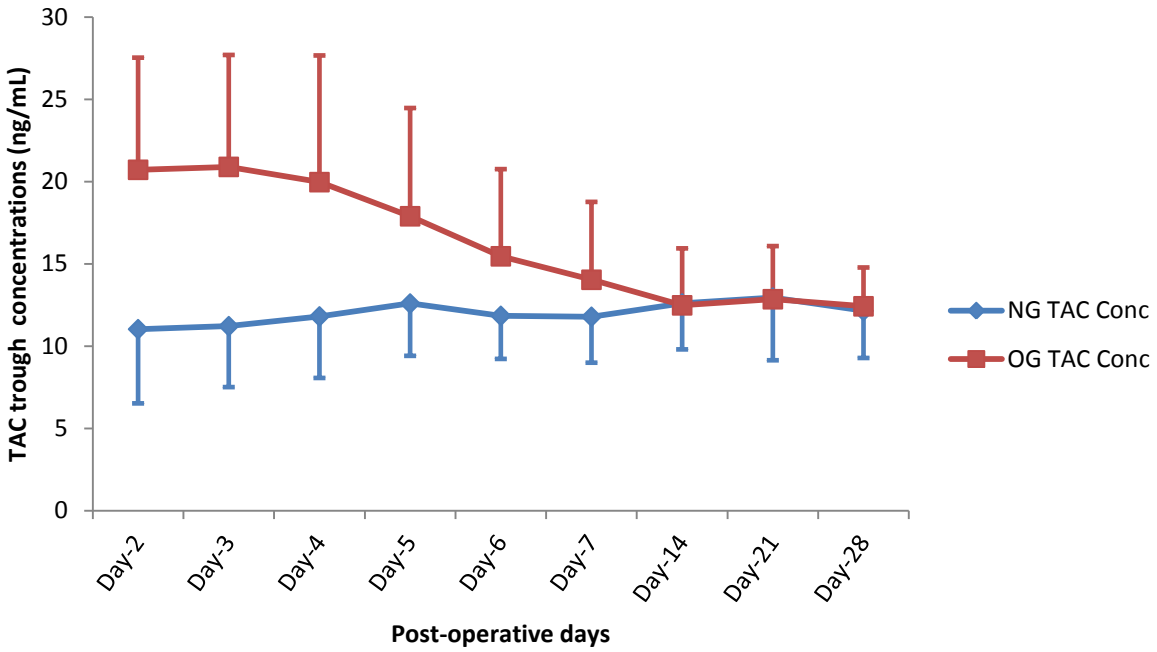

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Figure 3:

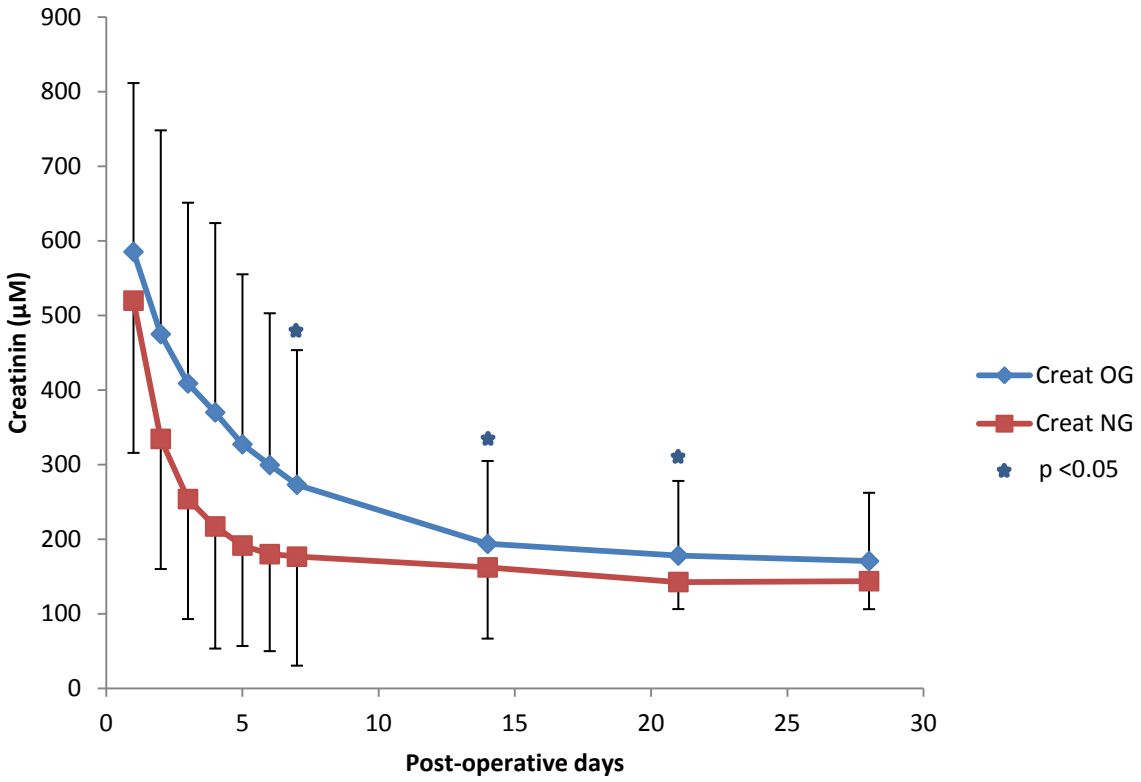

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FIGURE LEGENDS

Figure 1: Study workflow

TAC: Tacrolimus; TDM: Therapeutic drug monitoring; CYP: Cytochrome P450

Figure 2: Tacrolimus trough concentrations according to post-operative days in overexposed group patients and patients with normal tacrolimus exposure

TAC: Tacrolimus; NG: Normal group; OG: Overexposed group

Figure 3: Plasma creatinine according to post-operative days in overexposed group patients and patients with normal tacrolimus exposure (Stars point statistically significant difference between the groups ($p<0.05$))

NG: Normal group; OG: Overexposed group

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