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Research Article

Hemoglobin and Cholesterol Affect Apparent Tacrolimus Clearance in Pediatric Transplant Recipients – a Retrospective Cohort Study

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Abstract

Introduction: Tacrolimus has a narrow therapeutic index with substantial inter- and intra-patient variability. Factors beyond genetic and developmental factors are poorly understood. Recent adult studies suggest that hemoglobin affects the apparent clearance (CL/F), whereas this and other potential factors in children are understudied.

Methods: After ethics approval, we performed a single center retrospective cohort study of pediatric renal transplant recipients, who were followed between January 1st, 2004, and June 30th, 2018. Patients without tacrolimus therapy or concomitant sirolimus were excluded. The aim was to show the impact of hemoglobin, albumin, cholesterol and HDL on the apparent tacrolimus clearance (CL/F = Dose/AUC). Data were collected from electronic health record. We used 12-point pharmacokinetic (PK) profiles. Results: Thirty-three patients were included. Median age at transplantation was 10 years, 52% were female, the median tacrolimus area under the curve (AUC) was 133 ng*h/mL. CL/F mainly correlated with hemoglobin (n=1,257, r=-0.3767, p<0.0001), HDL-cholesterol (n=236, r=-0.3973, p<0.0001) and total cholesterol (n=373, r=-0.1821, p=0.0004).

Conclusion: The present study suggests a moderate impact of the biochemical factors studied in the tacrolimus CL/F. Lower hemoglobin seems to increase it, while higher cholesterol decreases it. Physicians should be aware of this association during the TDM follow up.

Key words: kidney transplantation; pediatric; tacrolimus; apparent total clearance; high-density lipoprotein cholesterol; hemoglobin and immunosuppression

Introduction

There is growing evidence that under-immunosuppression after renal transplantation; both early and late, may contribute to graft loss. Tacrolimus is a critical dose drug with a narrow therapeutic index for which therapeutic drug monitoring (TDM) is routinely performed. Usually, we aim for a lower tacrolimus concentration as time after transplant increases. Previous studies have shown that there is a

significant correlation between the levels and the risk of acute graft rejection [1].

There are multiple factors that affect the pharmacokinetics of tacrolimus. Limited studies in adults suggest that lower hemoglobin increases tacrolimus clearance [2]. The rigor of addressing chronic kidney disease (CKD) related changes such as renal anemia is poorer after transplantation when compared to the management of CKD in native kidneys [3]. The impact of hemoglobin and other biochemical parameters regarding apparent clearance of tacrolimus has been understudied in children. Our study aimed at identifying the possible confounders and their impact on the apparent tacrolimus clearance (CL/F). It was hypothesized that, similar to adults, hemoglobin and albumin would have an effect on tacrolimus clearance. We also hypothesized that similar to adults, total cholesterol and HDL cholesterol would inversely correlate with tacrolimus clearance [4].

Materials and Methods

The Western University Research Ethics Board (HSREB FileNumber 105148) approved the study and it was conducted in accordance with the declaration of Helsinki.[5-8] we retrospectively analyzed all existing data on 33 pediatric renal transplant recipients who were followed between January 1st, 2004, and June 30th, 2018. This study cohort included all pediatric patients who had undergone renal transplantation <18 years of age, including re-transplants (n=2), had a functioning graft, received tacrolimus and mycophenolate mofetil (MMF) for immunosuppression, and were followed at the Children's Hospital at the London Health Sciences Centre, a tertiary care center with a catchment area of 629,000 children across southwestern and northern Ontario. Patients without tacrolimus therapy or concomitant sirolimus or cyclosporine therapy were excluded. Also, patients with less than 3 months of follow-up after transplantation were excluded. Patients were identified using a divisional database.

Clinical information

Gender, underlying diagnosis leading to end-stage renal failure, age at transplant, follow-up time after transplantation, daily concomitant immunosuppressive and other medication, and anthropometric data (height, weight) were obtained from the patient's paper and electronic charts. Along with all available tacrolimus concentrations, we used our electronic health record to obtain the following laboratory parameters: cystatin C, creatinine, albumin, hemoglobin, total cholesterol, and high-density lipoprotein (HDL) cholesterol and triglyceride levels. None of the patients had elevated transaminases or elevated bilirubin. Estimated glomerular filtration rate (eGFR) was calculated using the Filler formula [9].

Calculation of the apparent clearance (CL/F) based on estimated Area under the time-concentration curve (AUC)

We had 122 complete 12-point tacrolimus PK profiles of pediatric renal transplant patients which had been described in a previous paper for the calculation of the estimated AUC.[10] We used linear regression to calculate a regression line based on the trough level (see results). The estimated AUC was expressed as

$$Y=11.8118 * C_0+40.56$$

Where C_0 is the pre-dose trough level.

CL/F was calculated using the dose for a dosing interval divided by the AUC using the formula:

$$CL/F = Dose/AUC$$

For the CL/F per body weight, we divided CL/F by the body weight. CL/F/kg was calculated dividing CL/F by the body weight of the patient.

Tacrolimus concentration measurements and adjustments

All patients were treated with tacrolimus with concomitant MMF. Tacrolimus was started at 0.15 mg/kg/dose and modified according to the patient's trough concentrations. We did not adjust for genotype expresser status [11]. Target tacrolimus concentrations were 10-20 ng/mL in the first month post-transplantation, followed by a gradual decrease of the trough concentration to 5-10 ng/mL at one year and 4-6 ng/mL thereafter. Data were only included until the 18th birthday.

Concomitant MMF was started at a dose of 1200 mg/m^2 body surface area in 2 divided doses [12]. A higher starting dose was occasionally chosen for small children.

Tacrolimus concentrations were measured with the HPLC/MS/MS. The lower limit of quantification for the tacrolimus concentrations was 2 ng/mL. The total imprecision for all tests was less than 5%.

Statistical analysis

Data were tested for normality using the D'Agostini Pearson Omnibus test. Descriptive data are represented as mean ± one standard deviation (for normal distribution) or as median and 25th and 75th percentile (for non-normal distribution). AUC was measured from a 12-point PK profile using the trapezoid rule [10]. As most parameters were not normally distributed, we used Spearman rank correlation tests to determine which parameters correlated with the apparent clearance (CL/F) in a univariate approach. No adjustments were made for missing values. After the univariate approach, we used mixed effect multivariate regression analysis because of the interdependencies of several factors, for instance, lower albumin yields higher total cholesterol or lower eGFR yields lower hemoglobin, albumin and higher triglycerides [13, 14]. CL/F was used as dependent variable while all significant factors from the univariate analysis were used as independent variables. In the final model, we excluded HDL cholesterol because of the small number of measurements. Constant term was not suppressed, and a correlation matrix was reported. We also compared our CL/F results with the existing literature, using the one sample t-test. All analyses were performed using Excel (Office for Mac 2011, version 14.3.8), GraphPad Prism for Mac OS X version 5 (GraphPad Software, San Diego, CA, USA) and STATA version 11.2 for Mac (StataCorp, College Station, TX, USA). A p-value <0.05 was considered statistically significant.

Results

Patient population for the estimated tacrolimus clearance study

Out of the 42 pediatric renal transplant recipients followed during the study period, 33 qualified for the study (Figure 1). The demographic characteristics of the entire cohort are described in table 1. Seventeen of these 33 were female. The median age at transplant was 10.5 years, with a median follow-up of 9.2 years (25th percentile 3.7, 75th percentile 10.0 years). Each patient had a median of 82 tacrolimus trough concentrations (range 10-264). The underlying diagnoses were: renal dysplasia=12, obstructive uropathy or reflux nephropathy=9, focal and segmental glomerulosclerosis=2, autosomal recessive polycystic kidney disease=3, glomerulonephritis= 2, spina bifida=2, cystinosis=1, bilateral Wilms Tumor=1, bilateral renal vein thrombosis=1. Demographic data are summarized in table 1.

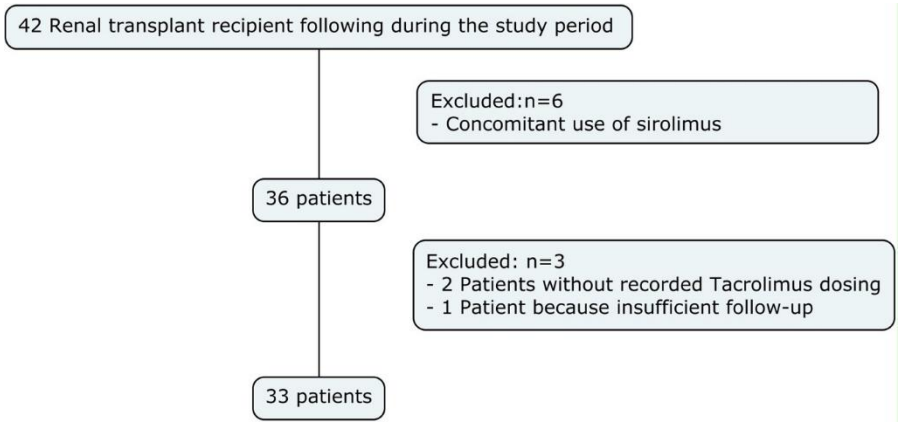

Figure 1. Flow diagram of the patient selections process.

Characteristic n = 33	
Demographics and Anthropometry	
Age at Transplantation, (years) [Median, IQR 25-75]	**10.5** [3.7 - 12.6]
Gender Male, n % Female, n %	 16 (48%) 17 (52%)
Follow up (years) [Median, IQR 25-75]	**9.21** [3.7 – 10]
Weight over all CL/F estimations (kg) [Median, IQR 25-75, Minimum and Maximum]	**39.0** [28.8 – 50.0, 6.30, 101.2]
Height over all estimations (cm) [Median, IQR 25-75, Minimum and Maximum]	**138.1** [123.0 – 154.4,84.9, 175.2]
Pharmacological parameters	
Tac levels n [Median, Min - Max]	**82** [10 – 264]
Tac AUC (ng*h/mL) [Median, IQR 25-75]	**133** [100 – 174]
CL/F [L/ h] [Median, IQR 25-75]	**28.8** [19.08 –42.8]
CL/F/kg [L/h/kg] [Median, IQR 25-75]	**0.502** [0.332 –0.787]
Biochemical Parameters	
Cystatin C [mg/L] [Median, IQR 25-75]	**1.24** [1.01 -1.59]
Creatinine [umol/L] [Median, IQR 25-75]	**74** [52 - 104]
Cystatin C eGFR [mL/min/1.73m^2] [Median, IQR 25-75]	**73** [54 – 90.7]
Albumin [g/L] [Median, IQR 25-75]	**43** [40 - 45]
Hemoglobin [g/L] [Mean ± standard deviation]	**110.6** ± 18.36
Total cholesterol [mmol/L] [Median, IQR 25-75]	**3.86** [3.27 – 4.43]
HDL Cholesterol [mmol/L] [Median, IQR 25-75]	**1.31** [1.06 – 1.56]
Triglycerides [mmol/L] [Median, IQR 25-75]	**1.32** [0.91 – 1.81]

Apart the age at transplantation and the number of Tacrolimus levels, the values above represent the average of the average for each patient. One patient never received a triglyceride measurement. Table Glossary: Tac= Tacrolimus, CL/F=Apparent Clearance, eGFR=estimated cystatin C GFR. We did not calculate how many patients had anemia because of the age-dependency of hemoglobin in children. No patient experienced post-transplant erthrocytosis.

Table 1: Patient characteristics.

Development of the formula for estimation of cl/f

One hundred twenty-two PK profiles were available for the correlation analysis between the AUC and the trough level. Median tacrolimus trough level was 7.8 ng/mL (interquartile range [25-75%]: 5.575-9.825 ng/mL), and median tacrolimus AUC was 130.5 ng*h/mL (interquartile range [25-75%]: 99.5-126.6 ng*h/mL). We plotted the AUC against the trough level (C_0). The Pearson correlation coefficient between tacrolimus AUC and C_0 was 0.745 ($p < 0.0001$). As such, there was a significant linear regression between the AUC and the trough level. Based on that regression analysis, we determined a formula for the AUC based on the trough level, which reads:

$$AUC=11.8118 * C_0+40.56 \text{ (Formula 1)}$$

The Pearson correlation coefficient was 0.745, $p<0.0001$. In Figure 2, the correlation between the measured AUC and formula 1 is showing a reasonable correlation. While neither the tacrolimus trough levels nor the tacrolimus AUC were normally distributed, we elected to plot the linear regression line. The line can be described $Y= 0.8388*X + 28.03$, r^2 0.5550, $p< 0.0001$. The AUC can be calculated from the trough level as AUC = 11.81*C0 + 40.56. (Formula 1)

We then tested the agreement between the estimated AUC based on the trough level using the formula 1. The mean bias was +2.874%±24.17%, with a 95% limit of agreement from -44.51 to +50.26. Most of the values were within +30% and -30%, which implies a reasonable agreement. Mean bias was 2.874% with 95% limits of agreement from – 44.51 to 50.26% (Figure 3). Based on these findings, we calculated the estimated CL/F using formula 1.

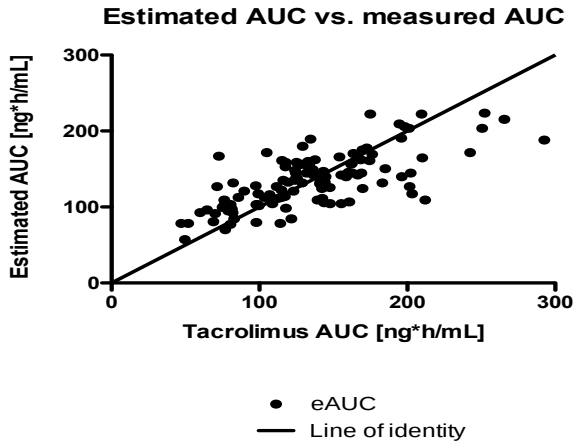

Figure 2. The relationship between tacrolimus levels and tacrolimus AUC in 122 PK profiles of 33 pediatric renal transplant recipients.

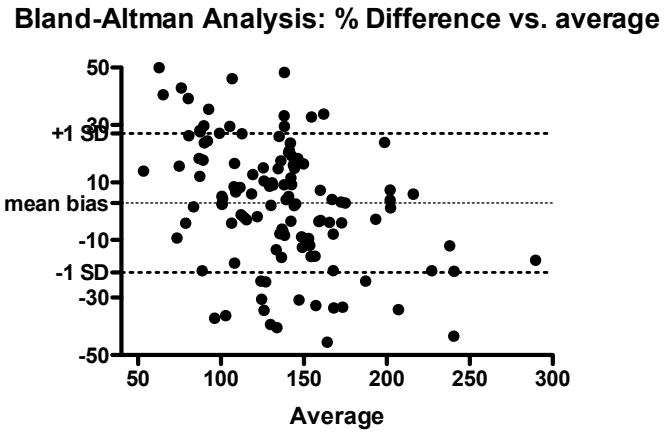

Figure 3. Bland- Altman analysis between measured AUC based on the full PK profiles and the estimated AUC based on the trough levels using formula 1. Mean bias was 2.874% with 95% limits of agreement from – 44.51 to 50.26%.

Analysis of estimated auc, cl/f and CL/F/kg

We could calculate 1,914 estimated tacrolimus CL/F. The median tacrolimus trough level was 6.9 ng/mL. The median estimated AUC was 133 ng*h/mL, with a 25th percentile of 100 and a 75th percentile of 174 ng*h/mL. Median CL/F was 28.8 L/h with a range from 2.85 – 163.8 L/h. The 25th percentile was 19.08 L/h, and the 75th percentile was 42.8 L/h. As the average CL/F in adult renal transplant patients had been reported as 21.5 L/h, [2] we compared our findings using a one-sample t-test and found that our median CL/F was not significantly different. One of the few previous pediatric studies by Andrews et al. reported a mean clearance of 50.5 L/h, [15] which was significantly higher in both the one sample t-test and the Wilcoxon signed rank test when compared to our findings. For the CL/F/kg the results were as follows: There were 1,776 measurements with concomitant weight. Median CL/F/kg was 0.502 L/h/kg with a range from 0.073 – 4.05 L/kg. The 25th percentile was 0.332 L/h/kg, and the 75th percentile was 0.787 L/h/kg.

We then performed correlation studies. The results are provided in table 2 for the CL/F. In the univariate analysis, we found some moderate and some weak, but significant correlations of the estimated CL/F with hemoglobin (n{number of observations}=1,257, r=-0.3767, p<0.0001), HDL-cholesterol (n=236, r-0.3973, p<0.0001), creatinine (n=1,394, r=-0.2002, p<0.0001), total cholesterol (n=373, r=-0.1821, p=0.0004), albumin (n=1,090, r=-0.08911, p=0.0032), and cystatin C (n=490, r=0.08877, p=0.0495). There was no significant correlation with triglycerides and eGFR.

Parameter	Creat	CysC	eGFR	Hgb	Albumin	Chol	Trig	HDL
Number of XY Pairs	1394	490	490	1257	1090	373	360	236
Spearman r	-0.2002	0.08877	-0.08791	-0.3767	-0.08911	-0.1821	0.1027	-0.3973
95% confidence interval	-0.2515 to -0.1477	-0.002454 to 0.1785	-0.1777 to 0.003323	-0.4245 to -0.3267	-0.1494 to -0.02812	-0.2813 to -0.07902	-0.003779 to 0.2068	-0.5025 to -0.2805
P value (two-tailed)	< 0.0001	0.0495	0.0518	< 0.0001	0.0032	0.0004	0.0516	< 0.0001

Creat=creatinine, CysC=cystatin C, eGFR=estimated glomerular filtration rate, Hgb=hemoglobin, Chol=cholesterol, Trig=triglycerides, HDL=high Density

Table 2: Correlation analysis of the main biomarkers for GFR, eGFR, albumin, hemoglobin and lipid parameters with CL/F based on dose/kg. All were significant using univariate analysis, except eGFR and triglycerides.

Finally, we performed mixed effect multivariate regression analysis for CL/F [L/h] on all available observations. Only hemoglobin and cholesterol (Table 3) remained significant. All eGFR markers no longer were significant.

Equation	Obs	Parms	RMSE	R^2	F	P
CL/F/wt	288	5	0.34972	0.114	9.1005	0.000

CL/F/wt	Coef.	Std. Err.	t	P>t	[95% Conf. Interval]	
hemoglobin	-0.0076509	0.0014574	-5.25	0.000	-0.0105196	-0.0047822
creatinine	-0.0001756	0.00015	-1.17	0.243	-0.0004708	0.0001197
cholesterol	-0.0679386	0.0222953	-3.05	0.003	-0.1118243	-0.024053
albumin	0.0119771	0.0065007	1.84	0.066	-0.0008187	0.024773
_cons	1.176326	0.3279986	3.59	0.000	0.5306992	1.821952

Parms=Parameters, RMSE=Root Mean Squared Error

Table 3: Mixed model multivariate regression analysis of the main parameters that were significant in the univariate approach affecting apparent Tacrolimus Clearance [L/h], but excluding HDL because of the low number of observations: Hemoglobin and cholesterol remained significant. We did not try to put the post-transplant time into the model.

Discussion

In this retrospective cohort study, we had 3,904 tacrolimus trough levels and multiple PK profiles of 33 renal transplant recipients, for which we could calculate 1,914 CL/F based on the trough levels. The median CL/F was 0.5 L/h/kg or 28.84 L/h, similar to that found in adults [2]. The average CL/F in adult renal transplant patients had been reported as 21.5 L/h [2]. Our results were lower than those from one of the few previous pediatric studies performed by Andrews et al., who reported a mean clearance of 50.5 L/h [15]. As the clearance often is not normally distributed, one possible explanation for the difference may be due an overestimation by reporting the mean. Furthermore; this difference may be attributable to 40% of *CYP 3A5* expressers in that cohort [15]. In our cohort, we had only one identified *CYP 3A5* expresser who was only tested because of the excessive requirements of up to 26 mg of tacrolimus per day, and most patients have not been tested. For stem cell transplant recipients, Przepiorka et al. found a CL/F for patients < 6 years to be 0.159±0.083 L/h/kg, for 6 to 12 years old of 0.109±0.053 L/h/kg and for > 12 years of 0.104±0.068 L/h/kg using continuous intravenous tacrolimus. These values were significantly lower than the one observed in our study. The intravenous application explains the lower CL/F as bioavailability is lower in oral administration likely caused by pre-systemic metabolism [16-18]. Given that the average oral bioavailability of tacrolimus is between 4 and 89%, our values fit those of Przepiorka [18]. The Bland &Altman analysis revealed a reasonable agreement, suggesting that our estimation of CL/F based on the trough level may be a reasonably accurate estimation of the apparent clearance.

Using univariate correlation analysis, estimated tacrolimus CL/F was moderately correlated with hemoglobin, HDL-cholesterol, and weakly with correlated with total cholesterol, creatinine, albumin and cystatin C. There was no significant correlation with triglycerides and eGFR. Using mixed model regression multivariate analysis, CL/F was still independently affected by hemoglobin and cholesterol. We are unaware of any pediatric study that analyzed the relationship between CL/F and hemoglobin or cholesterol.

To understand these findings, we need to review the pharmacokinetic characteristics of tacrolimus. Bioavailability of tacrolimus after oral administration varies between 4 and 89% and is most likely caused by pre-systemic metabolism in gut wall and liver [16]. Tacrolimus is highly bound to red blood cells, 1-α- acid glycoprotein and albumin [16]. Eventually, tacrolimus is eliminated via metabolism by CYP-3A enzymes in both the liver and gut wall [16]. It has been demonstrated that *CYP3A5* expressers require at least a 1.5-fold higher tacrolimus dose compared to *CYP3A5* non-expresser [15]. Renal elimination is negligible as <1% of an intravenous dose is excreted in urine in unchanged forms [16].

The impact of hemoglobin has been previously described in adult patients only [19]. The higher the level of hemoglobin, the lower the clearance of tacrolimus [19]. Binding of tacrolimus to erythrocytes restricts its availability for metabolism [20] de Jonge, et al. demonstrated that after kidney transplantation the continued increase in hematocrit explained 23.6% of the further decline in mean tacrolimus CL/F [19].

Anemia after kidney transplantation shows a biphasic pattern. Early after the surgical procedure many transplant patients are anemic. Hemoglobin levels increase with the restoration of kidney function during the first 3 months. But even despite a good a stable graft function anemia can be present in 10 – 42% of patients after the first post-transplant year [21]. These findings can explain the higher clearance of tacrolimus found in post kidney transplant patients related with anemia despite normal eGFR [21].

We also found a relationship between total cholesterol, HDL-cholesterol and tacrolimus CL/F. Zahir et al. described in adults patients that an increase in cholesterol is associated with lower unbound tacrolimus level and a lower CL/F [4]. Our findings are in agreement with Zahir's observations. Tacrolimus mainly associates with soluble proteins and also with HDL, LDL, and VLDL. The association of tacrolimus with HDL is almost 30% [4]. Zahir et al. hypothesized that tacrolimus is covalently bound to HDL which decreases its CL/F [4]. It has been reported that over the post transplant period there is an increase in HDL and VLDL fractions in renal transplant patients receiving tacrolimus [4]. We are unaware of any description of this relationship in pediatric patients.

Our study has several limitations. The retrospective nature of the study forms a significant limitation. However, such a large number of tacrolimus CL/F measurements would not be easily obtained in

prospective studies. The lack of complete pharmacokinetic profiles for every time point is a shortcoming as is the fact that we estimated CL/F from trough levels. However, the number of 122 full PK profiles compares favorably with other studies, and the Bland & Altman analysis suggests a reasonable accuracy of our calculations. As this was not a planned study, there is wide interpatient variability of the number studies regarding albumin, hemoglobin, eGFR and lipid studies in these patients. Nonetheless, the large number of CL/F measurements over a long follow-up forms a considerable strength. The findings may also not be generalizable because of the low number of *CYP 3A5* expressers in our population. Lastly, as shown by de Jonge, tacrolimus CL/F increases over time over the first 12 months after transplantation, possibly due to a decrease of the *CYP3A4* activity [19]. Another explanation could be the concomitant change of the steroid drug interaction [7] or developmental changes [8]. The study was not designed to look specifically at the ongoing temporal trends over the much longer follow-up period in our study. In the first year, hemoglobin typically increases but then decreases again over time as renal function worsens.[22] In some studies hemoglobin may drop again even if renal function is normal [21].

Conclusion

The current study highlights the moderate impact of anemia, HDL and total cholesterol on the tacrolimus CL/F in pediatric renal transplant patients. While lower hemoglobin increases the tacrolimus CL/F, higher cholesterol decreases the tacrolimus CL/F. Transplant physicians should carefully monitor and recognize the high risk of anemia in renal transplant patients and its effects on tacrolimus CL/F. Another important factor that should be observed by physicians include total and HDL cholesterol levels as higher levels decreased estimated tacrolimus CL/F in our study. Most importantly, renal function will decline over time after transplantation, and with worsening eGFR, hemoglobin will decline [3]. As anemia is often undertreated in these patients, the increased clearance of tacrolimus may further add to shorter allograft survival as anemia may be associated with tacrolimus underexposure. We recommend checking tacrolimus levels rigorously in the setting of anemia and possibly considering increasing the Tacrolimus dose or treating the anemia.

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Conflict of Interest

All authors had no relationships or circumstances that present a potential conflict of interest.

Author Contributions

GF conceived the study, applied for the ethics submission, was involved in all aspects of the paper generation and revised each draft, performed the statistical analysis, and coordinated all coauthors' activities. EY, GF and CIRC performed the statistical analysis, interpreted the data and wrote the first draft. CIRC and EY collected and interpreted the data. MM, ACAE and ALAG provided critical input into all aspects of the design and execution of the study and participated in all phases of the paper writing. All authors participated in revising the manuscript critically for important intellectual content and approved the final version to be submitted to the journal.

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