JMS 2025; Vol 8(2):40-49
UDC:616.61-089.843-085.37.015
DOI: https://doi.org/10.55302/JMS258240ct

TACROLIMUS VARIABILITY FACTORS AND INTERPLAY WITH KIDNEY GRAFT FUNCTION

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Abstract:

Introduction: Aim of this study was to evaluate association of tacrolimus variable pharmacokinetic with graft function and survival, in the one-year follow-up period.

Materials and methods: A prospective study was conducted in 32 kidney transplanted patients. Intra-individual variability of CV>30% was defined as high. Due to concentration/dose ratio (Co/D) patients were divided into slow and fast metabolizers. The clinical outcome on graft function was estimated as change in the eGFR in the 1-year follow up period.

Results: CV > 30 % was found in 12.5% and low Co/D in 15.6% of patients. Both fast metabolizers and high IPV group patients showed higher creatinine after 6 and 12 months and lower eGFR, but the difference didn't reach statistical significance (135.11 ± 50.89 vs. 158.29 ± 68.33, p=0.499; 126.36 ± 45.60 vs.150.80 ± 58.60, p=0.322) (p=0.582, 0.703, 0,547, 0.652), respectively. The reduction ratio of eGFR ranged from -64 to +40%, mean -1.26 ± 20.60%. Fast metabolizers did not show reduction ratio >20% more frequently than others (3(15%) vs. 0(0%), p=0.585), as also and high tacrolimus variability (3(14.3%) vs. 0(0%), p= 0.657), respectively. In the predictive model age remained the sole factor of tacrolimus variability (β=1.18, CI:1.015-1.379, p=0.031).

Conclusion: Further follow up is needed on tacrolimus variability and kidney graft function.

Key words: kidney transplant, tacrolimus, variability, graft outcome.

Introduction

Since the day it was discovered, tacrolimus is accounted as key part of the standard immunosuppressive protocol in the treatment of kidney transplant patients, presenting as a first line choice therapy, way ahead other calcineurin inhibitors [1,2].

It's physical and chemical features, especially high lipophilic profile and water insolubility, in great measure contribute to its high bioavailability. Although it is recognized as very potent and efficient calcineurin inhibitor, it's narrow therapeutic index and high variable pharmacokinetic features, pose serious challenges for the clinicians in its everyday usage in kidney transplant patients [3].

Experiences demonstrated that the daily dose required for obtaining the optimum blood concentration of this medication can significantly vary between two different patients, but also in one same patient followed during some period of time.

This narrow therapeutic index means very thin window between the effective doses and those that cause adverse toxic effects. This demands mandatory individual continuous therapeutic monitoring of patients on therapy with tacrolimus in order to achieve and maintain an adequate blood concentration of tacrolimus and by that securing a good balance between the risk of organ rejection and toxicity [4,5].

This is achieved by measuring tacrolimus (TAC) blood concentration early in the morning, directly before taking the next prescribed dose of the medication, according to which adjustment of the therapeutic regime is made if it is required. The inappropriate dosing of tacrolimus in concentrations higher than needed can lead to acute or chronic nephrotoxicity, as well as to others numerous side effects and complications, emphasizing diabetes, infections and malignancies as one of the most frequent [6].

On contrary of previous mentioned, sub dosing of the medication can cause immunologically mediated rejection and subsequent graft loss. Although it seems that there is a strong correlation between high blood TAC concentrations and nephrotoxicity, there are studies that show that the nephrotoxic effects can be registered even in low daily doses and levels of TAC blood concentrations (4-6ng/ml) indicating presence of other factors important for experiencing potential harm of its use [4].

The high intra-individual variability of TAC that has wide range (4-89%) is associated with poor outcome and shorter graft survival.

There are a lot of published studies that analyse possible factors associated with high TAC intra/inter-individual variability and they indicate that demographic variables (gender, age, BMI), concomitant use of certain food, gastrointestinal and liver conditions, genetic polymorphisms, low serum albumin and hematocrit, therapy adherence, circadian rhythm, potential gut microbiota changes and drug interactions have significant influence on it [7-10].

Older age (above 65) thanks to aging process and reduction of clearance capacities can predispose to extended half-life and expose to TAC, as well as higher prevalence of TAC-mediated toxicity [7].

Aim of this study was to evaluate the incidence and association of tacrolimus variable pharmacokinetic with graft function and survival, in the one-year follow-up period

Materials and methods

We performed an observational, prospective, one-center study conducted in 32 kidney transplanted patients. Patients with age 18-65, clinically stabile after the third month of transplantation and on standard immunosuppressive protocol (TAC, MMF and steroids), were included in the study.

All patients had signed informed consent consistent with Helsinki principles. Patients on therapy with other calneurin inhibitor, poor adherence to therapy and incomplete medical documentation were excluded from the study.

The follow up period was 12 months. In order to access the intra and inter-patient TAC variability, we analysed TAC blood concentrations and daily TAC prescription for each patient separately, starting after the third month after transplantation in multiple time points accordingly to the parameter of interest.

All patients included in the study were on therapy with immediate-release tacrolimus formulation and the measurement of TAC blood concentration was performed early in the morning, just a short time before taking the next dose. A chemiluminescent analysis (CMIA) in whole blood sample with ARCHICTEC I System was used for quantitative measurement of TAC concentration in all patients.

The initial dosing of TAC was based on patient's body weight (0.1-0.2mg/Kg BW) divided in two single doses for every 12 hours, and after that was adjusted according to TAC blood concentration in every measurement for every patient separately.

The desired blood concentration of TAC according to latest KDIGO guidelines was 5-8ng/ml [11]. TAC values less than 2ng/ml or above 10ng/ml were repeated or counted as invalid.

The inter-individual variability was observed by defining the metabolic profile of the examined patients through calculation of the concentration/dose ratio of TAC (C0/D =TAC blood concentration/TAC daily dose in milligrams), which recognized as surrogate marker for TAC bio-variability and significant predictive factor for poor outcome [12].

We analysed patient's C0/D in particular periods of time, on 3,6,9 and 12 months after transplantation and regarding obtained results patients were divided in two groups, slow and fast metabolizers. Those with C0/D<1.05 are in the fast metabolizer group and according to many published studies worldwide, there is strong connection between this metabolic profile and the genetic polymorphisms of CYP3A5 isoenzyme [13].

Intra-individual variability was defined as coefficient of variation (CV%) and it was calculated as ratio between the standard variation and mean TAC blood concentration {(SD/mean TAC through concentration) x100}, obtained from at least 6 consequent measured TAC blood concentrations after the third month, for each clinically stable kidney transplant patients. Regarding "American society for food

and drug administration" (FDA) criteria, patients with CV>30% for TAC are counted as patients with high variability for the examined medication [14].

As clinical outcome we determined the influence of Intra/inter-patient variability on graft function and survival in the 12month period of follow up, by following the changes in patient's estimated glomerular filtration rate (eGFR).

For the predictive analysis for TAC variability, we assessed the socio-demographic data (gender, age, body weight), clinical data (comorbidities like diabetes and hypertension, dialysis vintage before transplantation, factors associated with the transplantation itself (living/nonliving), laboratory findings (serum albumin, hemoglobin and hematocrit) and pharmacology history for usage of calcium channel blockers and azole antifungal drugs.

Patients' data for kidney disease, comorbidities, dialysis modality and vintage, data connected with the transplantation itself, the choice and dosage of the immunosuppressive and other therapy, were obtained from the medical history of the patients as well as the electronic health system (Moj Termin).

Laboratory findings included in the analysis were part of patient's routines check-up.

For statistical analysis we used SPSS version 27. Descriptive analysis, median, standard deviation, X2 test, t-test, Chi square and Fisher test were used for presentation and comparison of the obtained data. By using multivariate logistic regression analysis, we determined the predictive factors for TAC variability. A C0/D<1.05ng/ml/mg and CV>30% indicated high inter/intra-patient variability for TAC.

The reduction ratio of eGFR on 6 and 12 months after transplantation in all 4 groups of patients (C0/D above and below 1.05 and CV above and below 30%) was used to determinate the clinical outcome of variability on graft function and survival.

Results

Demographic, clinical, dialysis and laboratory parameters of the examined group are presented in Table 1. The mean age of patients was 37 and there were more men than women (62% vs. 38%).

Almost 10% of examined population had diabetes and hypertension was noted in two thirds of them. Most of the patients have been on chronic hemodialysis program before transplantation (87%) and there was no much difference between the number of patients with living donor transplantation and number from deceased donor (56%).

Regarding nutritive laboratory indicators, medium hemoglobin, hematocrit and serum albumin, values in referent ranges were observed for all of them. 12.5% percent of examined population have been on therapy with antifungal-azoles and 25% were taking calcium channel blockers.

An increased mean values of serum creatinine of around 130umol/L were noted on the sixth and twelve months of the examination.

Table 1. Demographic, clinical, dialysis and laboratory data.

N=32	Mean value ± SD N (%)
Age (years)	37.88 ± 11.78
Male (%)	20 (62.5)
Hypertension (%)	24 (75)
Diabetes Mellitus (%)	3 (9.4)
Preemptive transplantation (%)	4 (12.5)
Dialysis vintage (months)	46.28 ± 60.05
haemodialysis (%)	28 (87.5)
peritoneal dialysis(%)	2 (6.3)
Deceased donor transplantation (%)	18 (56.3)
Body weight (Kg)	69.84 ± 13.15
Hemoglobin(g/l)	133.16 ± 19.38
Hematocrit	0.39 ± 0.051
Serum albumin (g/l)	46.09 ± 3.98
Ca channel blockers (%)	8 (25)
Azoles -antifingal drugs(%)	4 (12.5)
Creatinine 6м (μmol/l)	138.74 ± 53.52
Creatinine 12м (μmol/l)	131.45 ± 48.45

Estimated glomerular filtration rate and tacrolimus associated parameters are presented in **Table 2**. On the sixth month the medium eGFR was 60 ml/min/1.73m^2 with slightly improvement and increase of eGFR to 63 ml/min/1.73m^2 observed on the twelve month, but in the same time with change in the distribuition in patients followed by fall in the mediana to 59 or 58ml/min /1.73m^2.

Tacrolimus blood concentration were in range between 5.28 to 9.37 and mean value of 6.79ng/ml with higher variability expressed through standard deviation from 0.73 to 4.01. Calculated coeficient of variation (CV) >30% was found in 12.5% of study patients, while the fast metabolic profile with C0/D<1.05 was discovered in 15.6% of examined population.

Table 2. Estimated glomerular filtration rate and tacrolimus associated parameters

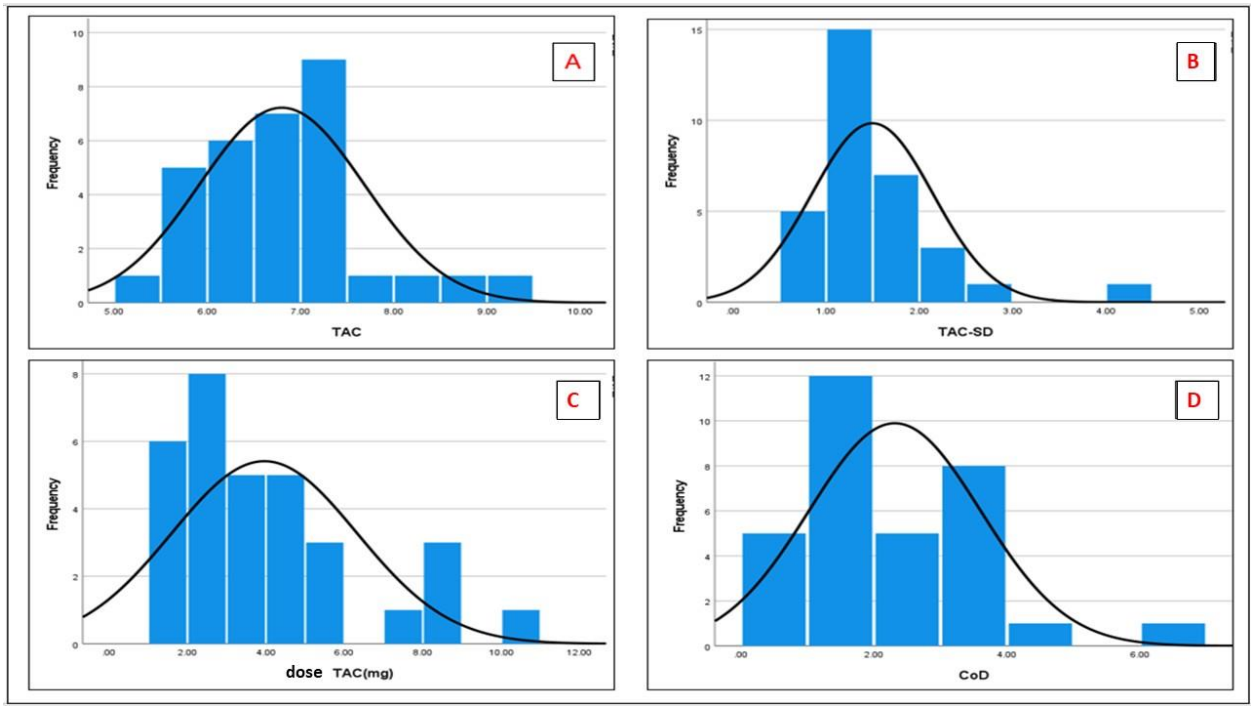
N=32	Mean, value ± SD N (%)	Median	Range (min-max)
eGFR 6 month (ml/min/1.73m^2)	60.78 ± 22.64	59.50	25 – 125
eGFR 12 month (ml/min/1.73m^2)	63.12 ± 24.44	58.50	27 - 125
TAC (ng/ml)	6.79 ± 0.834	6.6	5.28 – 9.37
TAC- SD	1.49 ± 0.64	1.31	0.73 – 4.01
Mean dose TAC (mg)	3.95 ± 2.35	3.22	1.39 – 10.37
Mean dose TAC - SD	0.47 ± 0.40	0.38	0 – 1.90
CV (%)	21.50 ± 7.15	20.35	10.33- 42.81
CV > 30 %	4 (12.5)		
Co/D (ng/ml/mg)	2.31 ± 1,29	1.85	0.62 – 6.31
Co/D < 1.05	5 (15.6)		

Abbreviations: eGFR – Estimated glomerular filtration rate according to CKD EPI formula, TAC – mean value of tacrolimus blood concentration, TAC- SD – standard deviation of mean value of tacrolimus blood concentration, CV – coeficient of variation of tacrolimus {(SD/mean TAC through concentration) x100}, Co/D – ratio of TAC blood concentration/TAC daily dose in milligrams

In **Picture 1** there is graphic presentation of tacrolimus depended variables regarding line of distribution of frequency. It is evident that there is distortion of the line towards the higher values of tacrolimus blood concentration (Picture 1-part A), which means more patients with higher values of TAC in blood that overcome recommended ranges (5-8ng/ml).

High variability of TAC blood concentration can be also observed through the standard deviation which indicates distortion of the line on the right side and very clearly presents separation from the median (Picture1 – part B).

It is especially visible the large group of patients that require higher daily dose of tacrolimus (Picture 1 – part C), which completely gives explanation for the high number of patients with fast metabolic profile and Co/D < 1.05ng/ml/mg (Picture 1 – part D).



Picture 1. Graphic presentation of tacrolimus depended variables regarding line of distribution of frequency: A – Mean TAC blood concentration (TAC ng/ml), B – standard deviation of TAC blood concentration, C – mean daily dose of TAC, D – TAC blood concentration/TAC daily dose Co/D (ng/ml/mg).

Clinical outcomes and change of kidney function on 6 -and 12 -month regarding Co/D can be seen in **Table 3**.

Group of patients stratified as fast metabolizers of tacrolimus, although statistically insignificant, they did demonstrated increase of serum creatinine on sixth and twelve months in the follow up period (135.11 ± 50.89 vs. 158.29 ± 68.33, p=0.499; 126.36 ± 45.60 vs.150.80 ± 58.60, p=0.322), respectively. Also, there was bigger decrease of eGFR in the fast metabolizer group of patients, even though it did not reach statisticle significance (p=0.394, 0.322), respectively. The presence of eGFR below 60ml/min/1.73m^2 in both time points compared for patients with Co/D<1.05 was insignificant (p=0.626, 0.633).

Predictive analysis for tacrolimus intra-patient variability defined as CV>30%, presented age (p=0.031) and concomitant use of Calcium channel blockers (0.5) as statisticali significant/borderline significant factors of prediction of variability in the univariant model, respectively. In the multivariate predictive model of analysis, age remain strongest factor of prediction of TAC variablitiy (β=1.18, CI 1.015-1.379, p=0.031), with significant clinical aplicability of the model in 45% patients (R^2=0.450).

Table 3. Comparation of kidney function on 6 and 12 months regarding Co/D

Kidney function	Co/D > 1.05 Mean value ± SD N (%)	Co/D < 1.05 Mean value ± SD N (%)	p
Creatinine 6 month (μmol/l)	135.11 ± 50.89	158.29 ± 68.33	0.499
Creatinine 12 month (μmol/l)	126.36 ± 45.60	150.80 ± 58.60	0.322
eGFR 6 month (ml/min/1.73m^2)	61.94 ± 22.52	54.00 ± 24.82	0.394
eGFR 12 month (ml/min/1.73m^2)	64.94 ± 24.82	58.40 ± 24.32	0.322
eGFR 6 month < 60ml/min/1.73m^2	13 (51.9%)	3 (50%)	0.626
eGFR12 month < 60ml/min/1.73m^2	19 (47.4%)	5 (60%)	0.633

Abbreviations: eGFR- estimated glomerular filtration rate, Co/D- ratio of TAC blood concentration/TAC daily dose in milligrams

Change of kidney function and clinical outcome on 6 and 12 months in respect of coefficient of variation (CV) are presented in **Table 4**.

Patients with high CV had higher values of serum creatinine and lower estimated glomerular filtration rate in both time points 6 and 12 month, but the difference did not reach statistical significance (p=0.582, 0.703, 0,547, 0.652), respectively.

The percentage of patients with eGFR below 60ml/min/1.73m^2 in both time frames was bigger in high variability (CV>30%) for tacrolimus group of patients, but again without statistical significance (4 (75%) vs. 28 (46%), p=0.300; 28 (46%) vs. 20 (45%), p=0.323), respectively.

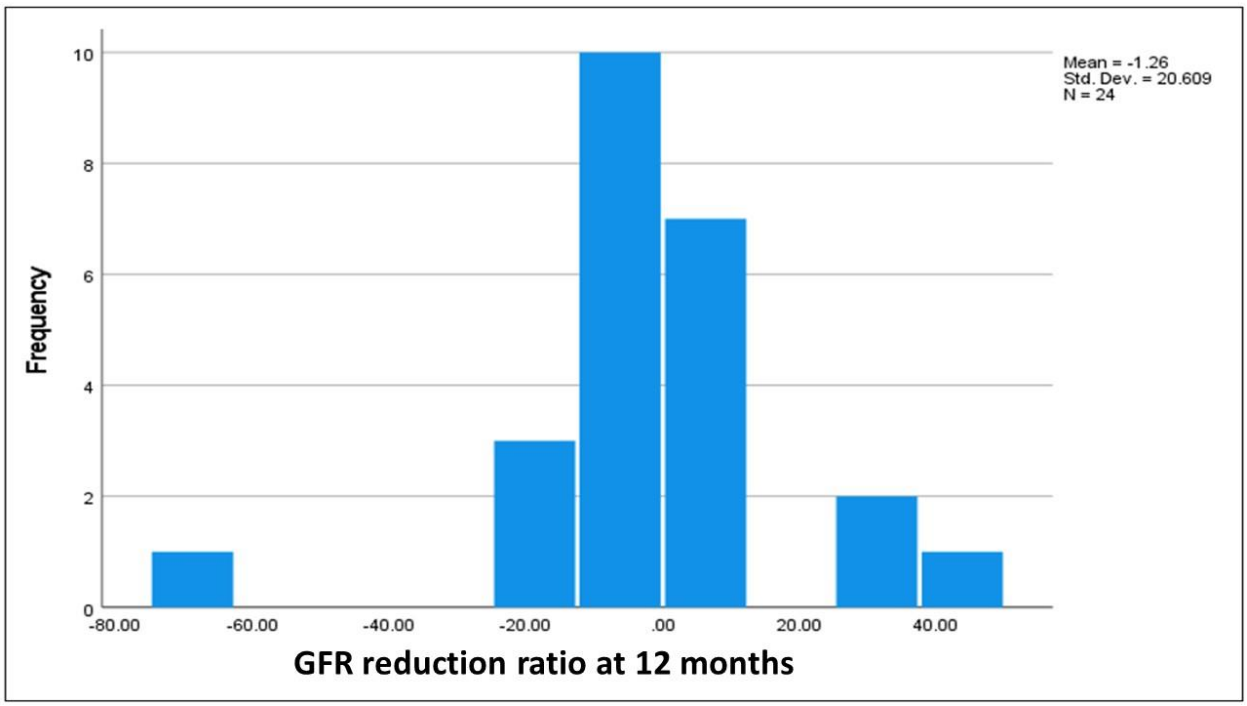
Table 4. Comparation of kidney function on 6 and 12 month regarding coefficient of variation (CV%)

Kidney function	CV > 30% Mean value ± SD N (%)	CV < 30% Mean value ± SD N (%)	p
Creatinine 6 month (μmol/l)	140.92 ± 58.83	123.33 ± 45	0.582
Creatinine 12 month (μmol/l)	133.20 ± 50.96	122.75 ± 39.84	0.703
eGFR 6 month (ml/min/1.73m^2)	56.00 ± 16.87	61.46 ± 23.51	0.547
eGFR 12 month (ml/min/1.73m^2)	56.50 ± 17.22	64.50 ± 25.79	0.652
eGFR 6 month < 60ml/min/1.73m^2	4 (75%)	28 (46%)	0.300
eGFR12 month < 60ml/min/1.73m^2	28 (46%)	20 (45%)	0.323

Abbreviations: eGFR- estimated glomerular filtration rate

The reduction ratio rate of eGFR of the whole group of patients after 12 months can be seen on **Picture 2**. As it is shown the range is very wide and varies from –64 to +40% with mean value of -1.26 ± 20.60%, that significantly deviate from mediana by –2.72%.

Concerning fast metabolizers group with Co/D<1.05, the reduction ratio rate of eGFR was not above 20% and did not differ in great measure from slow metabolizer group of patients with Co/D.>1.05, (3 (15%) vs. 0 (0%), p=0.585), respectively. In respect of intra-patient variabilty analysis, patients with high coeficient of variability (CV>30%), also did not demonstrate reduction rate ratio of eGFR greater than 20% compared to CV<30% group of patients (3(14.3%) vs. 0(0%), p= 0.657),respectively.



Picture 2. Reduction ratio of eGFR on 12 months

Discussion

Our study evaluates 32 clinically stabile kidney transplant patients in a follow-up period of twelve months. The percentage of patients with high intra-patient (12.5%) and high inter-patient (15.6%) variability is consistent with our previous experiences from another examined group of transplanted patients (15), as well as with other published studies so far [4,16,17].

As predictive factors for variability, published literature emphasize age, dialysis vintage, drug interactions and genetic polymorphisms as most important [18-20].

Our results confirmed age as powerful predictive factor for TAC variability, clinically applicable in 45 % of study population, while the concomitant use of TAC with calcium channel blockers had borderline statistical significance in the univariate predictive analysis, which was lost in the multivariate analysis at the end.

Regular monitoring of tacrolimus blood concentrations provides prompt and adequate correction of daily doses if needed, but even with good compliance in these patients our results established high variability with wide range of values in daily doses and tacrolimus blood concentrations.

Given the known for examined predictive factors of variability in our study so far, this raises the question and urges the need for further analysis of genetic factors as an answer and possible explanation for this high variability [20].

We analysed the influence of the registered tacrolimus variability on graft function on 6 and 12 months, after the three-month period of stabilisation of graft function after transplantation.

Although all patients were clinically stable, the analysis demonstrated fall in the eGFR in some degree, especially in those group of patients with high variability, which compares with published literature so far [13,19,20].

The fact that this fall in eGFR did not reach statistical significance we explain by the limitation of our study, the small number of population and short follow-up period of only one year. Even though, according to actual guidelines, a reduction of kidney function (eGFR) above 20% for period of two years is counted as an indicator for graft function impairment [21].

Beside the limitation effect of our results due to the 12-month follow-up period, they certainly did point out the bigger (for now statistically insignificant) difference and more frequent and progressive fall in eGFR in patients with high variability, for both fast metabolizers with C0/D<1.05 and those with high coefficient of variability (CV>30%).

Conclusion

Tacrolimus variability was registered in over 10% of patients with transplanted kidney.
The reduction rate of kidney function followed through eGFR after period of 12 months was more frequent and bigger in these patients.

We need more studies in larger group of patients, for longer period of time, in order to determine the real and precise effects of variability on graft function, as well as to evaluate potentially modifying factors that influence variability in an effort to preserve kidney function and graft survival.

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