Long-term Glomerular Filtration Rate and Kidney Disease: Improving Global Outcomes Stage Stability After Conversion to Once-Daily Tacrolimus in Kidney Transplant Recipients

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ABSTRACT

Close monitoring of estimated glomerular filtration rate (eGFR) is important for early recognition of worsening renal function to prevent further deterioration. Safe conversion from twice-daily tacrolimus (TD-Tac) to once-daily tacrolimus (OD-Tac) has been reported, but the effects on eGFR are contrasting.

The aim of our study is to evaluate long-term stability of eGFR after 1:1 conversion from TD-Tac to OD-Tac and the effects on serum cytokine blood levels. Forty-six consecutive kidney transplant recipients treated with TD-Tac 3 to 5 years post-transplant, with stable renal function, were enrolled in the study (2009–2011). Clinical and biochemical parameters were evaluated for 12 months before conversion up to 6 years after conversion. The patients served as their own controls. A panel of cytokines was evaluated repeatedly during the first year after conversion. Mean values of eGFR were not different long-term after conversion ($P = .11$) compared with baseline, and the majority of patients remained stable on Kidney Disease: Improving Global Outcomes stage during the study period; eGFR was stable in 30.0% after 5 years, decreased > 1 mL/min/1.73 m^2/y in 13.3%, and improved > 1 mL/min/1.73 m^2/y in 56.7%. Cytokine levels and C-reactive protein did not show any significant deterioration. Metabolic parameters were stable during the 6 years of follow-up. OD-Tac therapy can preserve an effective immunosuppressive state together with a safe profile of eGFR.

KIDNEY transplant recipients are at increased risk for decline of estimated glomerular filtration rate (eGFR) with development and progression of chronic kidney disease (CKD) [1]. Observational evidence acknowledges the importance of closely monitoring eGFR because interventional opportunities would exist for early recognition of worsening in eGFR values after kidney transplant [2–6]. Drug toxicity, in particular calcineurin inhibitor (CNI) immunosuppressive therapy, together with classical risk factors, including endocrine and metabolic complications, cardiovascular disease, and infections, are recognized as factors responsible for progression of CKD. Most causes of CKD are irreversible with a lifelong course and treatment aims at slowing progression to kidney failure [1].

The importance of determining the rate of decline in kidney function over time is to identify rates that exceed the normal range. A population-based cohort study suggested a mean decline in eGFR approximately between 0.3 to 1 mL/min/1.73 m^2/y in healthy adults and an average decline

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Table 1. Patient Characteristics at Time of Conversion From Twice-Daily Tacrolimus to Once-Daily Tacrolimus

	Total No. of Patients = 43
Age, median (IQR), y	55 (47–61)
Male sex, No. (%)	30 (69.8)
Etiology of ESRD, No. (%)	
Chronic GN	20 (46.5)
Genetic disease	4 (9.3)
FSGS	3 (7.0)
ADPKD	3 (7.0)
Angiosclerosis	4 (9.3)
Diabetic nephropathy	1 (2.3)
Other	8 (18.6)
Living donor, No. (%)	5 (11.6)
Years post-transplant, median (IQR)	7.1 [6.6–8.3]

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ESRD: end-stage renal disease; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; IQR, interquartile range.

of 2.65 mL/min/1.73 m^2/y in patients with impaired kidney function (G3-G5 Kidney Disease: Improving Global Outcomes [KDIGO] eGFR categories) [7,8]. Individuals who are progressing more rapidly (sustained decline in eGFR of more than 5 mL/min/1.73 m^2/y) [1] with increased morbidity and mortality should be targeted to slow their progression and associated adverse outcomes [9,10].

Kidney transplantation requires the use of immunosuppressive CNI nephrotoxic drugs, that require a fine balance between adequate immunosuppression to prevent rejection and excessive treatment leading to toxic, infectious, and neoplastic complications [11,12]. Once-daily tacrolimus (OD-Tac) (Advagraf®) [13] is the more recent oral formulation of the established immunosuppressive agent tacrolimus, which is administered twice daily. Evidence has supported a safe 1:1 conversion from twice-daily tacrolimus (TD-Tac) to OD-Tac in stable kidney transplant recipients, but the effects on eGFR are contrasting [14–16] because of CNI nephrotoxicity on one hand and chronic rejection on the other. Despite advances in immunosuppressive therapy, progressive renal dysfunction after kidney transplant due to chronic rejection remains the main cause of long-term graft loss (50%-80%) [17]. The intrapatient variability in tacrolimus plasma levels, more pronounced in cases of nonadherence to antirejection therapy, occurring especially in patients treated with TD-Tac, is one of the main causes of chronic rejection [18,19].

A protracted inflammatory state is considered the main cause of evolution toward interstitial fibrosis, tubular atrophy, and atherosclerosis that characterize CKD [20,21], with cytokines playing a crucial role [22,23]. Nevertheless, the inflammatory cytokine profile in these cases has not been clearly reported.

The aim of our study is to evaluate long-term eGFR and metabolic parameters in stable kidney transplant recipients after conversion from TD-Tac to OD-Tac (primary outcome) and to evaluate the effects on serum cytokine blood levels (secondary outcome).

MATERIALS AND METHODS

From January 2009 to February 2011, 46 consecutive kidney transplant recipients treated with TD-Tac were enrolled in the study. Eligibility criteria were age 18 to 70 years, stable renal function as evaluated by eGFR stability during the previous year, and a period of 3 to 5 years post transplant. They were converted from TD-Tac to OD-Tac at the same dosage (1 mg:1 mg). Standard additional immunosuppressive regimen included mycophenolate mofetil and less than 10 mg/d corticosteroids in all patients.

Tacrolimus trough levels, serum creatinine concentrations, glomerular filtration rate estimated by the 4-variable Modification of Diet in Renal Disease (MDRD) study equation [24,25], and clinical assessments were evaluated monthly for 12 months before conversion (T11-/T0), 15 days after conversion (T1+), monthly to 12 months (T2+/T12+), and every 3 months until 6 years after conversion (December 2017) (T2y/T6y).

Patients served as their own controls based on values before vs after conversion. Tacrolimus trough levels were maintained in the therapeutic range of 4 to 8 ng/mL, as measured by the Flex technique (Dimension XP and System Siemens, Erlangen, Germany) according to the product insert. The accuracy and precision were evaluated by the International Testing Scheme (D.W. Holt, St Georges Hospital Medical School, London, United Kingdom). Plasma samples were collected at baseline (T0) and at T1+, T6+, and T12+ to assess a panel of cytokines including interleukin (IL) 2, IL-4, IL-6, IL-8, IL-10, interferon-γ, tumor necrosis factor-α, and C-reactive protein (CRP). Commercially available multiplex bead-based immunoassay kits (Human 27-plex, Bio-Rad Laboratories, Hercules, Calif, United States) were used to measure IL-2 concentrations using assays performed according to the manufacturer instructions. Each sample was assayed in duplicate. Data were

Table 2. Interleukin Levels in the First 12 Months After Conversion From Twice-Daily Tacrolimus to Once-Daily Tacrolimus

	T0	T1+	T6+	T12+	*P* Value
IL-2	6.9 (4.1)	43.0 (69.2)	7.4 (4.4)	5.4 (1.3)	.36
IL-4	46.6 (14.8)	32.0 (15.4)	24.0 (14.0)	3 (0)	<.001
IL-6	96.4 (135.8)	90.5 (141.7)	14.2 (6.8)	4.3 (1.1)	.047
IL-8	111.1 (57.9)	96.5 (54.4)	80.3 (54.7)	51.8 (33.0)	.22
IL-10	8.5 (3.5)	17.5 (17.7)	8.7 (4.2)	11.9 (14.5)	.10
IFN-γ	900 (567)	1459 (1952)	402 (245)	20 (0.5)	.26
TNF-α	28.6 (11.2)	32.5 (59.1)	32.0 (12.1)	19.0 (0)	.28
CRP	3000 (1500)	2526 (1065)	4269 (1912)	2703 (1076)	.66

Results are expressed as mean (SD).
Abbreviations: IL, interleukin; IFN, interferon; TNF, tumor necrosis factor; CRP, C-reactive protein.

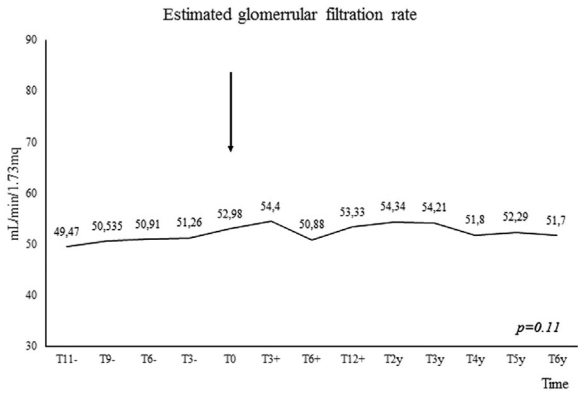

Fig 1. Estimated glomerular filtration rate during the study follow-up. Estimated glomerular filtration rate mean values as determined at 12 (T11-), 9 (T9-), 6 (T6-), and 3 (T3-) months before tacrolimus conversion, at time of conversion (T0), at 3 (T3+), 6 (T6+), and 12 (T12+) months after conversion, and yearly thereafter. Arrow shows conversion.

analyzed with Bio-Plex manager software, version 4.1.1 (Bio-Rad Laboratories). Values with a coefficient of variation > 12% were excluded from the final data analysis. The concentrations (pg/mL) of various analytes in the plasma samples were determined with the aid of standard curves generated in the multiplex assays. The Immunoturbidity Latex Test was performed for CRP measurements (with normal range, 100–6000 μg/L).

Primary outcome was to evaluate the stability of kidney transplant function after 6 years of follow-up following conversion. Deterioration of renal function was defined as a decrease of eGFR > than 1 mL/min/1.73 m^2/y, stability was defined as no variations of eGFR or variations ≤ 1 mL/min/1.73 m^2/y, and improving of renal function was defined as an increase of eGFR > 1 mL/min/1.73 m^2/y [1,8]. Secondary outcome was to assess signs of immunologic activation after conversion, as indicated by variation in inflammatory cytokine levels.

Statistical Techniques

We preliminarily performed a descriptive analysis consisting of percentages, averages, and standard deviations. Mean variations and total area of distributions were evaluated with the *t* test for independent or paired data as appropriate. The use of a parametric test, despite the limited data size, was justified by Kolmogorov-Smirnov tests. Time-dependent parameters were evaluated by general linear models. All tests were performed using SPSS system 24.0 (SPSS Inc, Chicago, Ill, United States) with $P < .05$ considered significant.

RESULTS

Forty-three patients completed the study during the first 3 years, 30 patients (70%) completed 5 years of follow-up, and 25 (58%) completed 6 years. Three patients started hemodialysis, 4 patients died, and the remaining 11 (25%) were lost at follow-up. Patients' baseline characteristics are reported in Table 1.

Tacrolimus trough level mean values showed a significant reduction during the first 2 determinations after conversion ($P < .03$) compared with pre-switch mean values and remained stable in the therapeutic range afterward. The evaluation of each patient showed stable tacrolimus trough levels after conversion in 36 of 43 (84%) patients, whereas a significant reduction of trough levels was recorded in 7 of 43 (16%) patients.

Administered daily dosage showed a significant increase after switch to OD-Tac in the whole group ($P = .003$; 95% CI, −0.42 to −0.086). Thirty (70%) patients needed a significant increase in the administered dosage, while 6 (14%) needed a decrease, and 7 (16%) needed no modifications in daily dosage. The majority of patients needed a daily dosage increase on the third trough level evaluation after conversion (T3+).

Serum creatinine and eGFR mean values were not significantly different during the year before and after conversion ($P = .25$ and $P = .75$, respectively). Serum creatinine mean value was 1.54 (SD, 0.55) mg/dL pre-switch and 1.61 (SD, 0.65 mg/dL) post-switch, while eGFR mean value was 51.24 (SD, 17.32) mL/min/1.73 m^2 before and 50.87 (SD, 18.82) mL/min/1.73 m^2 after conversion. Cytokine levels and CRP did not show any significant modification during 1 year of follow-up after conversion except for IL-4 and IL-6, which showed a significant reduction of levels (Table 2).

Renal function remained stable during 6 years of follow-up ($P = .11$) (Fig 1); eGFR was stable in 9 patients (30.0%) after 5 years, decreased > 1 mL/min/1.73 m^2/y in 4 (13.3%) patients, and improved more than 1 mL/min/1.73 m^2/y in 17 (56.7%) patients. Similar results were achieved after 6 years of follow-up: 10 (40.0%) patients maintained stable renal function, 5 patients (20.0%) decreased eGFR > 1 mL/min/1.73 m^2/y, and 10 (40.0%) patients improved their eGFR of > 1 mL/min/1.73 m^2/y. KDIGO classification of renal function is reported in Table 3. The majority of patients remained stable on KDIGO stage during the study period: 27 patients (63.0%) at T0, 26 (61.0%) at T12+, 15 (34.9%) at T2y, 22 (71.0%) at T3y, 25 (83.3%) at T4y, 19 (70.4%) at

Table 3. Distribution of Patient eGFR Categories Before and After Tacrolimus Conversion

KDIGO Stage (mL/min/1.73 m^2)	T11-	T0	T12+	T2y	T3y	T4y	T5y	T6y
1 (eGFR > 90)	2 (5.0)	2 (5.0)	1 (2.3)	3 (7.0)	-	-	-	-
2 (eGFR 60–89)	10 (23.3)	12 (27.9)	13 (30.2)	10 (23.3)	9 (29.0)	8 (26.7)	6 (21.4)	11 (44.0)
3 (eGFR 30–59)	13 (30.2)	14 (32.6)	11 (25.6)	22 (51.2)	17 (54.8)	15 (50.0)	18 (64.3)	10 (40.0)
4 (eGFR 15–29)	15 (34.9)	10 (23.3)	9 (20.9)	8 (18.6)	5 (16.1)	6 (20.0)	4 (14.3)	4 (16.0)
5 (eGFR < 15)	3 (7.0)	5 (11.6)	9 (20.9)	-	-	1 (3.3)	-	-

Results are expressed as No. (%).
Abbreviations: eGFR, estimated glomerular filtration rate; KDIGO, Kidney Disease: Improving Global Outcomes.

Table 4. Metabolic Parameters and Tacrolimus Levels Before and After Tacrolimus Conversion

	T11-	T0	T12+	T2y	T3y	T4y	T5y	T6y	*P* Value
Glycemia, mg/dL	89 (83–99)	92 (86–97)	96 (87–103)	98 (86–110)	86 (80–100)	90 (81–112)	91 (83–110)	83 (78–94)	.24
Total cholesterol, mg/dL	202 (182–230)	195 (177–211)	189 (177–200)	198 (176–220)	176 (159–203)	184 (161–217)	187 (162–219)	184 (164–211)	.35
HDL cholesterol, mg/dL	50 (40–64)	53 (45–61)	50 (45–55)	54 (48–60)	58 (50–64)	61 (43–69)	55 (44–70)	56 (47–68)	.25
Triglycerides, mg/dL	150 (111–204)	145 (105–184)	140 (96–184)	135 (88–182)	120 (75–170)	119 (100–158)	128 (90–200)	121 (99–194)	.39
Tacrolimus level, ng/dL	6.1 (4.2–7.5)	5.0 (4.4–5.6)	4.8 (4.3–5.4)	4.8 (4.0–5.7)	4.9 (4.1–5.4)	4.6 (3-7-6.2)	5.2 (3.8–6.3)	4.6 (4.1–6.4)	.72

Results are expressed as median (interquartile range).
Abbreviation: HDL, high-density lipoprotein.

T5y, and 19 (79.2%) at T6y. Only a minority of patients presented deterioration of KDIGO stage: 11 (25.6%) at T12+, 6 (14.0%) at T2y, 7 (22.6%) at T3y, 4 (13.3%) at T4y, 5 (18.5%) at T5y, and 2 (8.0%) at T6y.

Metabolic parameters, in particular glycemia ($P = .24$), total cholesterol ($P = .35$), and triglycerides ($P = .39$), were stable during the entire study period (Table 4).

DISCUSSION

Kidney transplant recipients are defined as having CKD, irrespective of the level of GFR or presence of markers of kidney damage. Kidney transplant recipients have an increased risk of mortality and kidney outcomes compared with the general population and they require specialized medical management [1,26]. Regular monitoring of eGFR may promote earlier detection of kidney function decline. Several studies have demonstrated a variability in the rates of decline in eGFR suggesting progression rates of approximately 0.3 to 1 mL/min/1.73m^2/y among healthy adults and rates 2 to 3 times higher in individuals with impaired kidney function [7,27]. In these patients, data from the Modification of Diet in Renal Disease study showed that the average rate of decline in eGFR ranged from 2.3 to 4.5 mL/min/1.73m^2/y, depending on the baseline eGFR [28]. A more recent study of patients with G3-G5 KDIGO eGFR categories (eGFR < 60 mL/min/1.73m^2) showed a mean decline in eGFR of 2.65 mL/min/1.73m^2/y [8]. The importance of determining the rate of decline in kidney function over time is to identify rates which exceed the normal range, in particular individuals who are progressing more rapidly (sustained decline in eGFR of > 5 mL/min/1.73 m^2/y) [1], characterized by increased morbidity and mortality [9,10].

In stable kidney transplant recipients treated with CNI and converted from TD-Tac to OD-Tac at the same dosage, the variability of eGFR is contrasting. Some studies showed no significant changes in the mean eGFR 12 months after conversion [14]. Other studies demonstrated a significant kidney graft function improvement with an increase in eGFR from 24 months to < 5 years after conversion [15,16].

In our study we showed that stable kidney transplant recipients, converted from TD-Tac to OD-Tac at the same dosage (1 mg:1 mg), are characterized by a stability of eGFR during 6 years of follow-up. In addition, the majority of patients remained stable on KDIGO stage during the study period, in agreement with the role of baseline eGFR.

Numerous studies demonstrate the role of cytokines in chronic allograft rejection [21,22].

T cells and the related cytokines, such as IL-2 and interferon-γ, appear to be activated post-transplantation, and therefore monitoring antirejection will be mainly oriented toward the monitoring of these molecules.

Tacrolimus is a highly potent immunosuppressive agent whose activity, demonstrated in vitro and in vivo, is to inhibit the phosphatase activity of calcineurin in a dosage dependent manner. This action leads to reduced production of a range of cytokines, in particular IL-2, which are

involved in the immune reaction activated after kidney transplant and in transplant rejection [29].

The inflammatory cytokines represent the specific target of immunosuppressive therapy, and high plasma levels may indicate systemic activation of the immunologic response associated with allograft rejection [23]. In our study, we monitored a panel of cytokines during the first year after conversion showing no significant modification and no increase of inflammatory state. Even better, we observed a significant reduction of IL-4 and IL-6 levels. These results confirm the efficacy of immunosuppression and represent a surrgate marker of good compliance to OD-Tac therapy.

High tacrolimus intrapatient variability, especially with TD-Tac, has been demonstrated to predict accelerated development and progression of CKD in stable renal transplant recipients. This factor is considered as a potentially modifiable risk factor for poor allograft long-term outcome [30].

Several studies have described a safe conversion from TD-Tac to OD-Tac regimen in stable kidney transplant recipients. A slow release of tacrolimus may show a safer profile by avoiding toxic peak concentrations [31] related to lower allograft rejection as well as toxicity rates [29], in particular a better control of glycemic metabolism. Therefore, OD-Tac may minimize the adverse effects of tacrolimus immunosuppressive therapy on metabolic complications and cardiovascular disease. In our study we confirm preliminary results in which we observed a long-term stability of metabolic parameters, in particular glycemia, total cholesterol, and triglycerides, during the entire study period [29].

The main limitation of our study was the loss of patients during long-term follow-up.

CONCLUSIONS

In our study we confirmed a safe conversion from TD-Tac to OD-Tac regimen in stable kidney transplant recipients, according to literature. Furthermore, we demonstrated a stability in the long-term eGFR and KDIGO stages. Therefore, OD-Tac therapy can preserve an effective immunosuppressive state together with a safer profile of eGFR. We suggest, as demonstrated in literature, that this effect is related to avoidance of toxic peak concentrations with a better control of metabolic parameters and possibly improved adherence to therapy.

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