OPEN

Early Kidney Allograft Dysfunction (Threatened Allograft): Comparative Effectiveness of Continuing Versus Discontinuation of Tacrolimus and Use of Sirolimus to Prevent Graft Failure: A Retrospective Patient-Centered Outcome Study

Ravinder K. Wali, MD,[1] Heather A. Prentice, PhD,[1] Venkata Reddivari, MD,[2] Geroge Baffoe-Bonnie, MD,[2] Cinthia I. Drachenberg, MD,[3] John C. Pappadimitriou, MD,[3] Emilio Ramos, MD,[2] Matthew Cooper, MD,[4] Johann Jonsson, MD,[1] Stephen Bartlett, MD,[4] and Matthew R. Weir, MD[2]

Background. Due to lack of treatment options for early acute allograft dysfunction in the presence of tubular-interstitial injury without histological features of rejection, kidney transplant recipients are often treated with sirolimus-based therapy to prevent cumulative calcineurin inhibitor exposure and to prevent premature graft failure. **Methods.** We analyzed transplant recipients treated with sirolimus-based (n = 220) compared with continued tacrolimus-based (n = 276) immunosuppression in recipients of early-onset graft dysfunction (threatened allograft) with the use of propensity score-based inverse probability treatment weighted models to balance for potential confounding by indication between 2 nonrandomized groups. **Results.** Weighted odds for death-censored graft failure (odds ratio [OR], 1.20; 95% confidence interval [95% CI], 0.66-2.19, $P = 0.555$) was similar in the 2 groups, but a trend for increased risk of greater than 50% loss in estimated glomerular filtration rate from baseline in sirolimus group (OR, 1.90; 95% CI, 0.96-3.76; $P = 0.067$) compared with tacrolimus group. Sirloimus group compared with tacrolimus group had increased risk for death with functioning graft (OR, 2.01; 95% CI, 1.29-3.14; $P = 0.002$) as well as increased risk of late death (death after graft failure while on dialysis) (OR, 2.39; 95% CI, 1.59-3.59; $P < 0.001$). Analysis of subgroups based on the absence or presence of T cell–mediated rejection or tubulointerstitial inflammation in the index biopsy, or the use of different types of induction agents, and all subgroups had increased risk of death with functioning graft and late death if exposed to sirolimus-based therapy. **Conclusions.** Use of sirolimus compared with tacrolimus in recipients with early allograft dysfunction during the first year of transplant may not prevent worsening of allograft function and could potentially lead to poor survival along with increased risk of late death.

(*Transplantation Direct* 2016;2: e98; doi: 10.1097/TXD.0000000000000585. Published online 11 August 2016.)

Among the recipients of solid organ transplants, renal transplant patients have the highest 1-year graft and patient survival.[1,2] However, the rate of renal allograft loss after the first year has only marginally improved during the past decade.[3-6] Although a variety of factors[7] are involved, the degree of allograft function during the first year of transplant is a potent predictor of long-term graft survival[8,9] as well as patient survival.[10,11]

Persistence of allograft dysfunction after treatment of acute rejection[12-14] or unexplained suboptimal graft function[12] remains a challenging problem, especially if the biopsy findings demonstrate the predominant features of tubulo-interstitial inflammation (TII) (composite of any combination of tubulointerstitial inflammation, tubular-calcification/degeneration, and vacuolization), or interstitial fibrosis (IF)/tubular atrophy (TA) with or without interstitial inflammation.[13,15-22] El-Zoghby et al[14] demonstrated that in nearly one third of cases with new-onset allograft dysfunction early after transplantation had TII. Surveillance biopsy data indicates that development and progression of TII is an active process and progresses over time,[23,24] leading to progressive decline in allograft function.[23,25] Interstitial injury on protocol biopsies, even in the absence of allograft dysfunction, is a potent risk factor for future graft loss.[26,27]

Allograft dysfunction in the presence of nonspecific TII or IF/TA is often assumed to be due to cumulative exposure to calcineurin inhibitors (CNIs),[28-30] although there are no clinical or specific histological characteristics[31] to make a definitive diagnosis of CNI-related injury.[14,32,33] Notwithstanding these controversies, diverse approaches have been adopted during the past decade to abrogate these patterns of presumed CNI effects. For example, CNI dose reduction,[34] discontinuation of CNI,[35] or replacement of CNI with mammalian target of rapamycin inhibitors (mTORIs) either in the early,[36-38] or later years[39] after transplant have been attempted with inconsistent results. These studies invariably included recipients with stable graft function at the time of study enrollment. Consequently, these studies are not informative for the safety and efficacy of using mTORIs in recipients with new-onset allograft dysfunction, specifically during the first year of transplantation.

The hypothesis that continuation of CNIs can be associated with worsening renal function specifically in renal transplant recipients with TII and IF/TA and early allograft dysfunction requires validation. To test this hypothesis, we used single-center data with well-established and robust clinical practices for tracking of the longitudinal data in patients with new-onset acute allograft dysfunction, who either continued to be maintained on tacrolimus- based or were converted to use sirolimus-based therapy.

Based on the recent observations[21,32,40] that worsening allograft function can be due to different patterns of injury in the allograft, we limited this analysis only to those without other concomitant features of microcirculatory inflammatory changes in the index biopsies,[21] presence of other pathologies including absence of proteinuria, and absence of preexisting or de novo anti-HLA antibodies (Figure 1).

MATERIALS AND METHODS

Patients and Immunosuppression Therapy

Patients aged 18 years or older who underwent kidney allograft biopsy after completing the first 90 days of follow-up for unexplained new-onset allograft dysfunction (threatened allograft), defined by any of the following: (a) greater than 20% increase in baseline creatinine, (b) recipients in whom baseline creatinine levels continued to remain suboptimal (mean serum creatinine >2.0 mg/dL), (c) recipients with
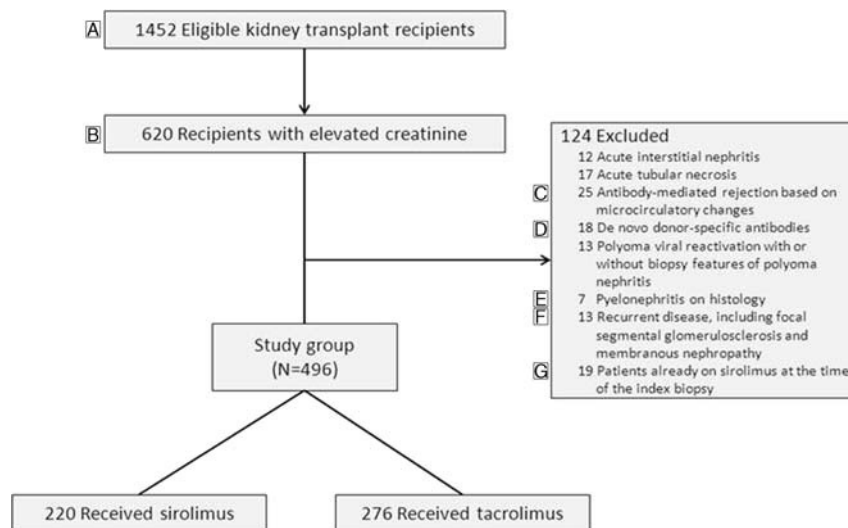
FIGURE 1. Flow diagram of the study groups: description of the cohort that underwent allograft biopsy following discharge after transplantation and the details of the recipients that were excluded from this study analysis. A, Recipients of kidney transplants with cytotoxicity and cytometric flow crossmatch negative at the time of transplantation and without pre-existing anti-HLA antibodies. B, Recipients underwent biopsy for either an increase in serum creatinine or serum creatinine continued to remain elevated > 2 mg/dL. C, Index biopsy showed histological features of microcirculatory inflammation (peritubular capillaritis (PTC > 1), glomerulitis (G > 1), without or with C4d positive staining. D, Recipients with de novo anti-HLA antibodies at the time of index biopsy. E, Histological features were consistent with acute pyelonephritis and were treated with antibiotics. F, Recipients who showed features of different glomerular diseases (focal segmental glomerulosclerosis (n = 5), membranous disease (n = 3), membranoproliferative disease (n = 3), IgA nephropathy (n = 2). G, Recipients who are already on sirolimus based therapy at the time of index biopsy.

elevated serum creatinine from the baseline and without histological features of acute rejection (T or B cell, and C4d negative by immunofluorescence) and without circulating anti-HLA antibodies and without proteinuria, were included in this analysis.

From January 2002 to December 2006, a total of 1452 patients who had negative cytotoxicity and cytofluometric crossmatches and without preexistent anti-HLA antibodies were discharged with functioning allografts after transplantation. Immunosuppression consisted of induction therapy with 3 doses of solumedrol along with either basiliximab or a single dose of alemtuzumab for first time transplants. Thymoglobulin induction (4-6 mg/kg as tolerated) was used for retransplant candidates. All patients were discharged on a combination of tacrolimus and mycophenolate mofetil (MMF)-based therapy, and most patients were discharged without maintenance steroids (steroid-free). The goal for 12-hour tacrolimus trough level was 6 to 8 ng/mL, and MMF dose was 1 g to 2 g/d. Dose adjustments of either tacrolimus or MMF were made for untoward events as deemed necessary during the follow-up period. Six hundred twenty (42.6%) of these patients underwent ultrasound-guided allograft biopsies (index biopsy) for the evaluation of acute allograft dysfunction. Patients (n = 124) were excluded from this analysis for various reasons including those with proteinuria at the time of index biopsy (Figure 1).

Histological Analysis

Two renal pathologists analyzed the allograft biopsies according to the Banff criteria[41] longitudinally and conveyed the first-read results to the treating physician. Each treating physician among a group of 6 transplant physicians independently developed the plan of treatment based on center-specific practices, temporal trends in serum creatinine levels in each patient, appropriate treatment for BPAR based on Banff criteria, and to either continue the baseline IS with tacrolimus and MMF or to eliminate tacrolimus and use a combination of sirolimus and MMF-based therapy. With a goal 12-hour tacrolimus trough level (6-8 ng/mL) and 24-hour sirolimus trough level (6-8 ng/mL) with an aim to keep MMF dose range (1 g to 2 g/d) with dose adjustments based on individual tolerability. Patients with BPAR in the index biopsy (n = 174) were now on maintenance steroid therapy with prednisone (5-10 mg/d) during the remaining follow-up period. Rebiopsy of the allograft was performed in both groups of patients for 20% or more increase in the serum creatinine levels from the baseline (time of index biopsy) during the follow-up period. All patients were followed up longitudinally, and for this analysis, data were censored after completion of 36 months of follow-up from the time of index biopsy. The exposure to sirolimus was performed based on the model of Newer-User Study design[42] and intention-to-treat analysis to define the patient-centered outcomes, data were analyzed based on the original assignment of either continuation of tacrolimus or replacement with sirolimus therapy at the time of index biopsy.

Outcomes

Primary outcome was a composite of: (a) BPAR during the follow-up period, (b) death-censored allograft failure (GF), (c) death with functioning graft (DWFG), (d) 50% or greater reduction in estimated glomerular filtration rate (eGFR) at 12 months (censored for GF and DWFG). Secondary outcomes included individual components of the primary outcome, such as BPAR during the follow-up, GF, and DWFG. We also studied death after graft loss (late death) defined as death within the first year after initiation of dialysis after graft failure. Additionally, graft function by eGFR was analyzed in 2 different ways: (a) 50% or greater reduction in eGFR at 12, 24, and 36 months from the time of index biopsy and (b) delta eGFR from baseline at the time of index biopsy to 12, 24, and 36 months. Both eGFR outcomes were assessed with or without last observation carried forward for sensitivity analysis. Based on our a priori hypothesis, GF and DWFG were explored in different strata's of eGFR at the time of index biopsy.

Covariates

Different sets of covariates were chosen for the adjusted models that included:

a. Patient characteristics: age, 60 years or older (yes/no), sex (male/female), race/ethnicity (white/black/other), diabetes mellitus status (yes/no), hypertension (yes/no).

b. Transplant-specific characteristics: type of transplant (living donor/deceased donor), retransplants (yes/no), delayed graft function (yes/no), type of induction therapy (basiliximab/thymoglobulin/alemtuzumab, time to index biopsy (≤6 months/> 6 months), acute rejection on index biopsy (yes/no), IF/TA (yes/no), TII (yes/no), arterial sclerosis or hyalinosis (yes/no)}, glomerular partial sclerosis (yes/no), periglomerular fibrosis (yes/no). Estimated GFR at the time of index biopsy (continuously and dichotomously as <30/≥30 mL/min).

Statistical Methods

All statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, NC), and individual plots were generated using GraphPad Prism (GraphPad Software, Inc). A P value less than 0.05 was considered statistically significant. Quantitative variables were expressed as mean ± standard deviation or medians (interquartile range) where appropriate; categorical variables were expressed as numbers and frequencies. Differences between study groups were assessed primarily by Student t test and Wilcoxon-Mann-Whitney test for normally distributed and non-normal quantitative variables, respectively, or χ^2 and Fisher exact test for categorical variables.

We aimed to test the primary hypothesis that continued use of tacrolimus in patients with new-onset allograft function during first year of transplant would result in accelerated graft loss and death. Analytical approaches included univariable and multivariable weighted logistic regression models for dichotomous outcomes and weighted Cox proportional-hazards models for time-dependent outcomes. Both weighted logistic and Cox proportional hazards multivariable models included all the covariates as described in the covariate section. Backward and stepwise selection procedures were used for identifying independent associations in reduced multivariable models. Proportional hazards assumptions were tested for final Cox proportional hazards models by generating a time-dependent covariate created for the model. Estimated Kaplan-Meier curves and log-rank tests were used to compare baseline time from index biopsy to the outcome of interest between the 2 groups.

Due to the nature of treatment assignment, bias in treatment selection by indication was a concern.[43] To allow for an unbiased comparison between the 2 treatment groups in regression models for the primary and secondary outcomes of interest, inverse probability of treatment weighting (IPTW) using propensity scoring was applied to account for potential confounding by indication between the 2 nonrandomized treatment groups.[44,45] Upon invoking IPTW, the distribution of preclinical factors may be balanced across the nonrandomized treatment groups, ensuring preclinical factors are independent of treatment, and there is no confounding by indication. Propensity scores (PS) for the probability of receiving tacrolimus or sirolimus treatment based on the patient's given preclinical factors were estimated for each individual patient using a multivariable logistic regression model including: age $\geq$ 60, sex, race/ethnicity, diabetes mellitus status, type of induction therapy, and acute rejection on index biopsy. After generation of individual estimated PS, distributions for both groups were compared to ensure balance across the 2 groups (data not shown). To implement IPTW in weighted regression models, each patient was weighted by the inverse probability of receiving the treatment they actually received based on their individual estimated PS.

Exploratory subgroup analyses were also performed to assess the univariate association between treatment groups and the primary and secondary outcomes after stratification according to the aforementioned covariates and also included eGFR at 6, 12, 24, and 36 months after index biopsy (dichotomized as <30/$\geq$30 mL/min). Logistic models were used for dichotomous outcomes and Cox proportional-hazards models were used for time-dependent outcomes. A 2-way interaction term was included in regression models to also test if any difference in the primary and secondary outcomes between the 2 groups depended on the presence of the parameter of interest. Forest plots were generated to display results from these stratified subgroup analyses. Estimated measures above null value indicated increased risk for the outcome.

In the secondary analyses, mean eGFR and changes in eGFR were also evaluated longitudinally at 12, 24, and 36 months as compared with the baseline at index biopsy. Estimated Kaplan-Meier curves for GF, DWFG, and late death were stratified by baseline eGFR less than 30 versus 30 mL/min or greater at the time of index biopsy.

RESULTS

Demographics of Recipients and Corresponding Donors

A total of 496 recipients were included in data analysis, sirolimus and mycophonolic acid (sirolimus group, n = 220) and continuation of tacrolimus and mycophenolic acid (tacrolimus group, n = 276). Steroid treatment was added in those who were treated for BPAR at the time of index biopsy (36.4% vs 34.1%, P = 0.593), respectively.

Baseline demographics of the recipients and their corresponding donors are described in detail in Tables 1 and 2, respectively. Among the recipients' characteristics, both groups were fairly well balanced (Table 1) except patients in the tacrolimus group were significantly older (P = 0.023) and had a significantly increased proportion with diabetes mellitus (P = 0.007). Interestingly, significantly different types of

TABLE 1.

Recipients' demographics, transplant-related characteristics, and index biopsy histology findings in the overall study population

Variable	Sirolimus (N = 220)	Tacrolimus (N = 276)	P
Age $\geq$ 60 y, n (%)	54 (24.6)	71 (25.7)	0.7642
Male sex, n (%)	137 (62.3)	177 (64.1)	0.6695
Ethnicity, n (%)			0.2699
Black	108 (49.1)	125 (45.3)	
White	101 (45.9)	143 (51.8)	
Other/unknown	11 (5.0)	8 (2.9)	
Diabetes mellitus, n (%)	89 (40.5)	145 (52.7)	0.0067
Hypertension, n (%)	19 (8.6)	19 (6.9)	0.4740
Missing	0	1	
Retransplant, n (%)	19 (8.6)	30 (10.9)	0.4086
Induction, n (%)			0.0052
Simulect	94 (42.7)	135 (48.9)	
Thymoglogublin	32 (14.6)	60 (21.7)	
Alemtuzumab	94 (42.7)	81 (29.4)	
Delayed graft function, n (%)	58 (26.4)	87 (31.5)	0.2100
Time to index biopsy, mo			
Mean $\pm$ SD	10.4 $\pm$ 15.5	10.5 $\pm$ 16.9	0.9229
Median (IQR)	3.8 (0.5-12.9)	1.0 (0.5-12.4)	
Histological characteristics of index biopsy			
Acute rejection (any grade)	80 (36.4)	94 (34.1)	0.5925
Acute tubular necrosis, n (%)	53 (24.1)	77 (27.9)	0.3384
Arterial hyalinosis or sclerosis, n (%)	128 (58.2)	112 (40.6)	0.0001
Glomerular partial sclerosis, n (%)	6 (2.7)	39 (14.2)	<0.0001
Interstitial fibrosis (IF/TA), n (%)	179 (81.4)	171 (62.0)	<0.0001
Periglomerular fibrosis, n (%)	4 (1.9)	23 (8.4)	0.0046
Transplant glomerulopathy	17 (7.7)	12 (4.4)	0.1157
Tubulointerstitial injury,[a] n (%)	109 (49.6)	68 (24.7)	<0.0001
eGFR, mean $\pm$ SD			
At biopsy	31.7 $\pm$ 22.6	32.6 $\pm$ 20.3	0.6677
At 6 mo[b]	35.4 $\pm$ 21.9	44.9 $\pm$ 19.3	<0.0001
At 12 mo[c]	35.0 $\pm$ 21.7	44.8 $\pm$ 19.5	<0.0001
At 24 mo[d]	36.1 $\pm$ 21.9	43.8 $\pm$ 20.8	0.0005
At 36 mo[e]	38.7 $\pm$ 22.0	44.2 $\pm$ 19.9	0.0179

[a] Tubulointerstitial injury: any combination of tubular calcifications, degeneration, or striped cortical fibrosis.

[b] Sirolimus, n = 212, and tacrolimus, n = 251.

[c] Sirolimus, n = 184, and tacrolimus, n = 243.

[d] Sirolimus, n = 171, and tacrolimus, n = 226.

[e] Sirolimus, n = 132, and tacrolimus, n = 208.

induction agents were used in the groups. Variations in the use of induction agents at the time of transplantation could not be explained based on the available recipient and donor characteristics except it could be related to temporal trends in the use of induction agents as we transitioned from basiliximab-based to alemtuzumab-based therapy during this period. The number of living donors and retransplants was uniformly distributed in the 2 groups (Table 1).

Time (in months) to index biopsy (P = 0.923) as demonstrated in Table 1, and proportions of different Banff grades of BPAR (P = 0.661) (data not shown) were similar in the 2 groups. Histological characteristics of index biopsy (Table 1) showed that an increased number of patients in the sirolimus group had IF/TA (81.4% vs 62.0%, P < 0.001), TII (49.6% vs 24.7%, P < 0.001), and arterial hyalinosis or

TABLE 2.

Donor characteristics of the overall study population

Variable	Sirolimus (N = 220)	Tacrolimus (N = 276)	P
Age: mean ± SD, y	43.9 ± 15.9	41.8 ± 16.1	0.1479
Male sex, n (%)	102 (47.4)	144 (52.9)	0.2283
Ethnicity, n (%)			0.2391
Black	55 (25.0)	52 (18.8)	
White	140 (63.6)	193 (69.9)	
Other/unknown	25 (11.4)	31 (11.2)	
Hypertension >10 y, n (%)	51 (23.6)	62 (22.6)	0.7972
Living donor, n (%)	73 (33.2)	94 (34.1)	0.8376
Creatinine: mean ± SD, mg/dL	1.1 ± 1.4	1.0 ± 0.4	0.3942
Cold ischemia time, h			
Mean ± SD	20.8 ± 16.4	20.7 ± 16.5	0.9714
Median (IQR)	23.0 (2.0-35.0)	22.0 (2.0-34.0)	
ECD, n (%)	64 (29)	88 (32)	0.821

ECD, extended criteria donor.

sclerosis (58.2% vs 40.6%, $P < 0.001$). However, glomerular partial sclerosis (2.7% vs 14.2%, $P < 0.001$) and periglomerular fibrosis (1.9% vs. 8.4%, 0.002) were more common in the tacrolimus group. Baseline eGFR was similar in the 2 groups ($P = 0.668$). Corresponding donor characteristics were similar in the 2 groups including the recipients of extended criteria donor kidneys (Table 2).

Primary Composite Outcome

The weighted odds for the primary composite outcome were significantly increased in the sirolimus-based group when compared with the tacrolimus-based group (odds ratio [OR], 1.93; 95% confidence interval [95% CI], 1.32-2.83; $P = 0.001$) (Table 3). Unadjusted rates were 686.4 per 1000 person-years (95% CI, 625.1-747.7) versus 525.4 per 1000 person-years (95% CI, 466.5-584.3). Among various factors in the weighted model (Table S1, SDC, http://links.lww.com/TXD/A23), delayed graft function was the only additional independent risk factor. A trend toward increased risk for primary outcome in those assigned to sirolimus persisted in different subgroups of recipients (Figure S1, SDC, http://links.lww.com/TXD/A23). A significant association was observed in 2-way interactions analyses for sirolimus group with diabetes mellitus (interaction, $P = 0.013$).

Secondary Outcomes

During the follow-up period of 36 months after the index biopsy, an increased number of participants in the sirolimus group versus tacrolimus group required rebiopsy (57% vs 42%, $P < 0.001$). The weighted odds for BPAR were significantly increased in the sirolimus group when compared with the tacrolimus group (OR, 1.67; 95% CI, 1.16-2.41; $P = 0.006$) (Table 3). Unadjusted rates were 554.5 per 1000 person-years (95% CI, 488.9-620.2) versus 413.0 per 1000 person-years (95% CI, 355.0-471.1); (long rank, $P = 0.832$; Figure 2A). Similar grades of BPAR were noted in both groups (data not shown). Delayed graft function remained the only independent risk factor in weighted multivariable models to predict BPAR during the follow-up (Table S2, SDC, http://links.lww.com/TXD/A23). The observed interaction with diabetes mellitus remained significant (interaction, $P = 0.010$) (Figure S2, SDC, http://links.lww.com/TXD/A23).

Contrary to primary composite outcome as well as BPAR, the weighted odds for death-censored GF was similar in the 2 groups (OR, 1.20; 95% CI, 0.66-2.19; $P = 0.555$) (Table 3). Unadjusted rates were 118.2 per 1000 person-years (95% CI, 75.5-160.8) in the sirolimus group versus 108.7 per 1000 person-years (95% CI, 72.0-145.4) in the tacrolimus group (log rank, $P = 0.183$; Figure 2B). Presence of periglomerular fibrosis or transplant glomerulopahty in the index biopsy significantly increased the risk of graft failure in the adjusted multivariable model (Table S3, SDC, http://links.lww.com/TXD/A23). Analysis of GF in different subgroups demonstrated no significant associations (Figure S3, SDC, http://links.lww.com/TXD/A23).

Increased odds for DWFG was significant in the sirolimus group (OR, 2.01; 95% CI, 1.29-3.14; $P = 0.002$) (Table 3). Unadjusted rates for the sirolimus group were 250.0 per 1000 person-years (95% CI, 192.8-307.2) compared with 144.9 per 1000 person-years (95% CI, 103.4-186.5) in the tacrolimus group (long rank, $P = 0.027$; Figure 2C). Assignment to sirolimus was the only significant factor in the multivariable model (Table S4, SDC, http://links.lww.com/TXD/A23). Increased risk of DWFG persisted in all the subgroups treated with sirolimus (Figure S4, SDC, http://links.lww.com/TXD/A23).

The sirolimus group had increased odds for late death compared with tacrolimus group (OR, 2.39; 95% CI, 1.59-3.59; $P < 0.001$) (Table 3). An increased number of patients 100.0 per 1000 person-years (95% CI, 60.4-139.6)

TABLE 3.

Primary and secondary outcomes in the sirolimus and tacrolimus groups, unweighted and inverse propensity score weighted models

Outcome	Sirolimus (N = 220)	Tacrolimus (N = 276)	Unweighted OR (95% CI)	P	Weighted[a] OR (95% CI)	P
Primary composite[b]	151 (68.6)	145 (52.5)	1.98 (1.37-2.86)	0.0003	1.93 (1.32-2.83)	0.0008
Follow-up acute cellular rejection	122 (55.5)	114 (41.3)	1.77 (1.24-2.53)	0.0018	1.67 (1.16-2.41)	0.0062
Graft failure	26 (11.8)	30 (10.9)	1.10 (0.63-1.92)	0.7402	1.20 (0.66-2.19)	0.5554
Death with functioning graft	55 (25.0)	40 (14.5)	1.97 (1.25-3.09)	0.0034	2.01 (1.29-3.14)	0.0021
≥50% eGFR decrease at 12 mo	23 (12.5)	18 (7.4)	1.79 (0.93-3.42)	0.0800	1.90 (0.96-3.76)	0.0668
Late death[c]	77 (35.0)	52 (18.8)	2.32 (1.54-3.49)	<0.0001	2.39 (1.59-3.59)	<0.0001

[a] Models weighted using an inverse propensity score weighted regression model.

[b] Composite of follow-up acute cellular rejection, graft failure (death censored), death with function graft, and ≥50% eGFR decrease at 12 months.

[c] Late death is defined as death after graft loss and after stopping sirolimus/tacrolimus-based therapy while on dialysis.
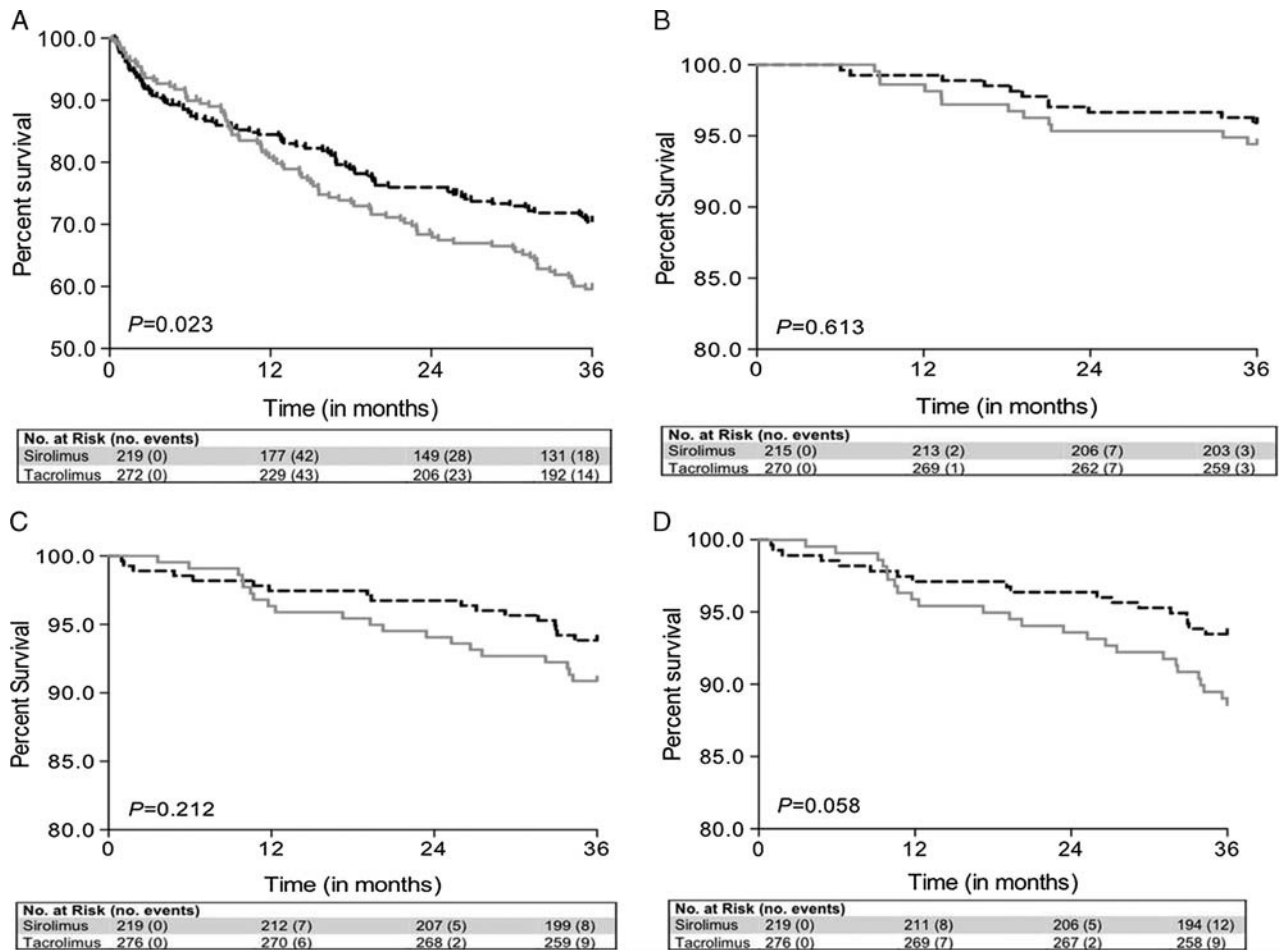
FIGURE 2. Kaplan-Meier survival curves for time to: (A) follow-up acute cellular rejection, (B) graft failure (death censored), (C) death with functioning graft, and (D) overall death. Individual curves represent the estimated percent survival over time since the index biopsy. Gray lines represent the sirolimus group and dotted black lines represent the tacrolimus group.

in the sirolimus group had late death versus 43.5 per 1000 person-years (95% CI, 19.4-67.5) in the tacrolimus-treated group (long rank, $P < 0.001$; Figure 2D). Group treatment remained the only independent factor in the multivariable model (Table S5, SDC, http://links.lww.com/TXD/A23). All the subgroups studied had increased risk of death while on dialysis if exposed to sirolimus-based therapy rather than tacrolimus-based therapy before graft failure (Figure S5, SDC, http://links.lww.com/TXD/A23).

Reduction ($\geq$50%) in eGFR at 12 months compared with baseline trended toward being higher in the sirolimus-based group versus tacrolimus-based group (OR, 1.90; 95% CI, 0.96-3.76; $P = 0.067$); unadjusted rates were 125.0 per 1000 person-years (95% CI, 77.2-172.8 and 74.4 per 1000 person-years (95% CI, 41.3-107.4), respectively. This risk plateaued at other periods (24 and 36 months) during the follow-up. Overall plots of eGFR over time trended to be lower in the sirolimus group versus tacrolimus group after censoring for deaths with function as well as death-censored graft failure (Figure 3A), or when analyzed with last observation carried forwards (Figure 3B), or with missing data through follow-up (Figure 3C). Furthermore, changes in eGFR (delta eGFR) at 12, 24, and 36 months suggested increased loss of eGFR in the sirolimus group without or with censoring (Figures 4A-C).

Baseline eGFR at the time of index biopsy was an important determinant for future outcomes. Kaplan-Meier plots for BPAR during follow-up (Figure 5A), DWFG (Figure 5C), and late death (Figure 5D) demonstrated that except for the death-censored GF (Figure 5B), tacrolimus groups with either eGFR of 30 or greater, or less than 30 mL/min at the time of index biopsy (black lines) had significantly lower event rates compared with the corresponding eGFR stratas in the sirolimus-based groups (Figures 5A, C, and D), whereas death-censored GF was similar in both groups and all eGFR stratas ($P = 0.351$) (Figure 5B).

Subgroup Analysis

In addition to the use of PS-based IPTW modeling for bias, we reexplored the impact of selection bias that may have developed at the time of interpretation of index biopsy findings by analysis of the primary and secondary outcomes in the subgroups defined by the absence or presence in the index biopsy of: (a) BPAR (because these patients were on maintenance steroids after treatment for T cell mediated rejections) (Table 4A), (b) IF/TA (Table 4B), (c) TII (Table 4C), and (d) the use of different induction agents (Table 4D-F) at the time of transplantation. The results continued to remain significant for both primary and secondary outcomes in the
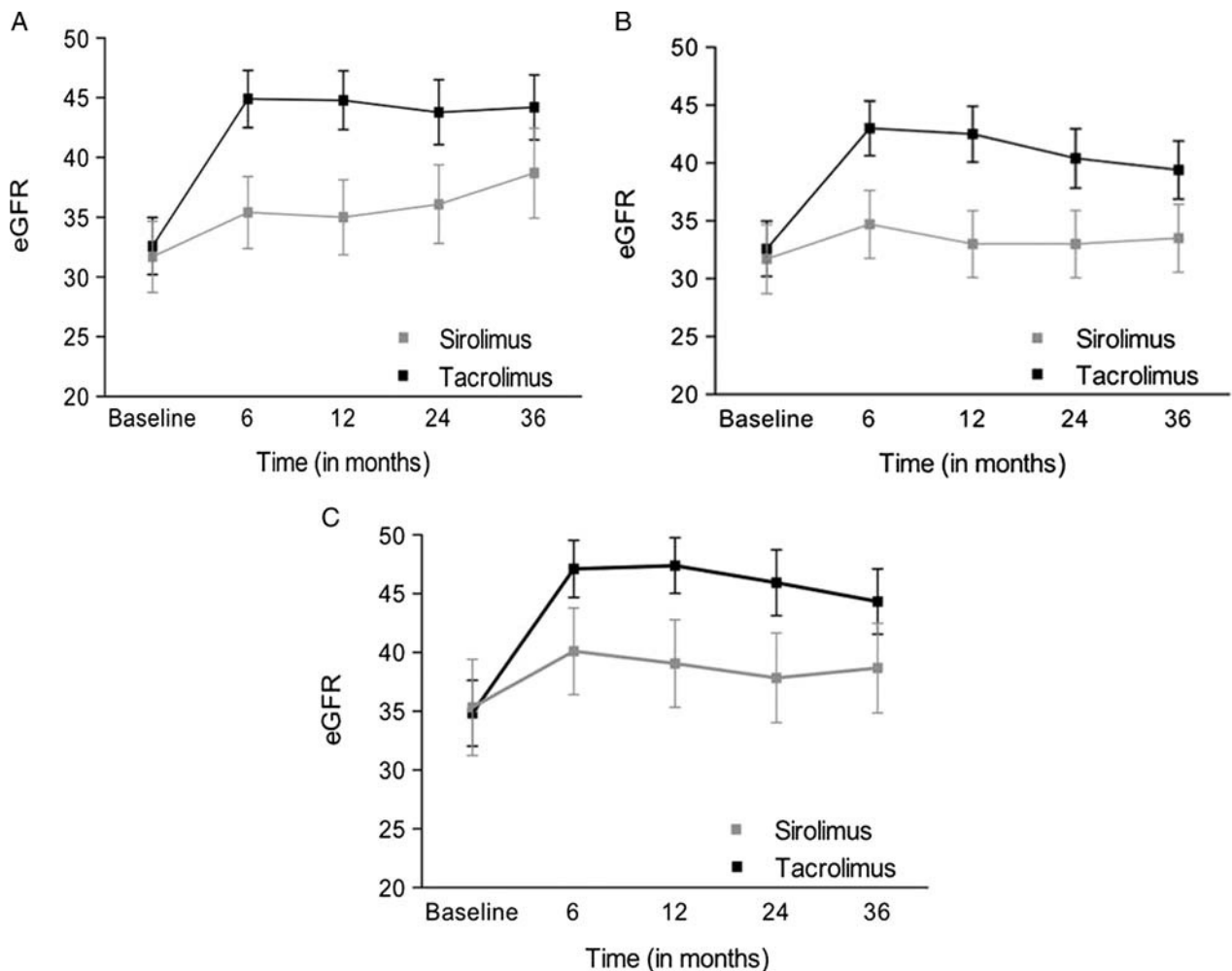
FIGURE 3. eGFR since baseline: (A) after censoring for death and graft failure, (B) with the last eGFR values carried forwards, and (C) after excluding individuals with missing data due to death or graft loss. Points show the mean at each time point and lines represent the 95% confidence interval.

sirolimus-treated subgroups and unchanged from the overall results (Table 4A to D).

A post hoc power analysis based on the primary composite outcome with 68.8% (sirolimus group) and 52.5% (tacrolimus group), an α of 0.05 and total sample size of 496 patients demonstrated an observed power of 95.6%.

DISCUSSION

This retrospective and pragmatic patient-centered outcome study in a large cohort of kidney transplant recipients with acute allograft dysfunction (threatened allograft) during the first year after transplant demonstrated that discontinuation of tacrolimus neither prevented graft failure nor improved the eGFR during 36 months of follow-up. More importantly, we observed that use of sirolimus in these patients increased the risk of death while on sirolimus-based treatment. The risk of increased mortality continued to persist even after discontinuation of sirolimus therapy immediately after the onset of graft failure. Additionally, discontinuation of tacrolimus increased the need for rebiopsy with an increased trend for BPAR. A significantly increased number of patients in the sirolimus-based group had 50% or greater decrease in eGFR from baseline during the first

12 months compared with the tacrolimus-based group. This is an important cause for concern, and it raises the possibility that increased risk of death may be related to the rapid decay in graft function after conversion to sirolimus- based therapy or perhaps a direct effect of the drug. Despite the fact that the tacrolimus group had a greater number of older patients as well as patients with diabetes mellitus, both the primary endpoint and DWFG were significantly higher in the sirolimus group. The risk of these adverse outcomes in the sirolimus-treated group persisted upon PS IPTW analyses. Whereas the sirolimus groups had significantly an increased number of biopsies with moderate-to- severe TII at the time of index biopsy, the rate of graft loss did not differ between the subgroups when analyzed on the basis of absence or presence of these histological characteristics in the index biopsies. Similarly, the rate of graft loss did not differ among the prespecified subgroups (low- or high-immunological risk) that were analyzed by forest plots methods.

Within the limitations of residual confounding and intervening physician biases, these data of hard endpoints support the notion that continuation of tacrolimus in recipients with early-onset declining graft function and histological evidence of TII on index biopsy does not culminate in accelerated graft failure. On the contrary, our data suggest that sirolimus-based
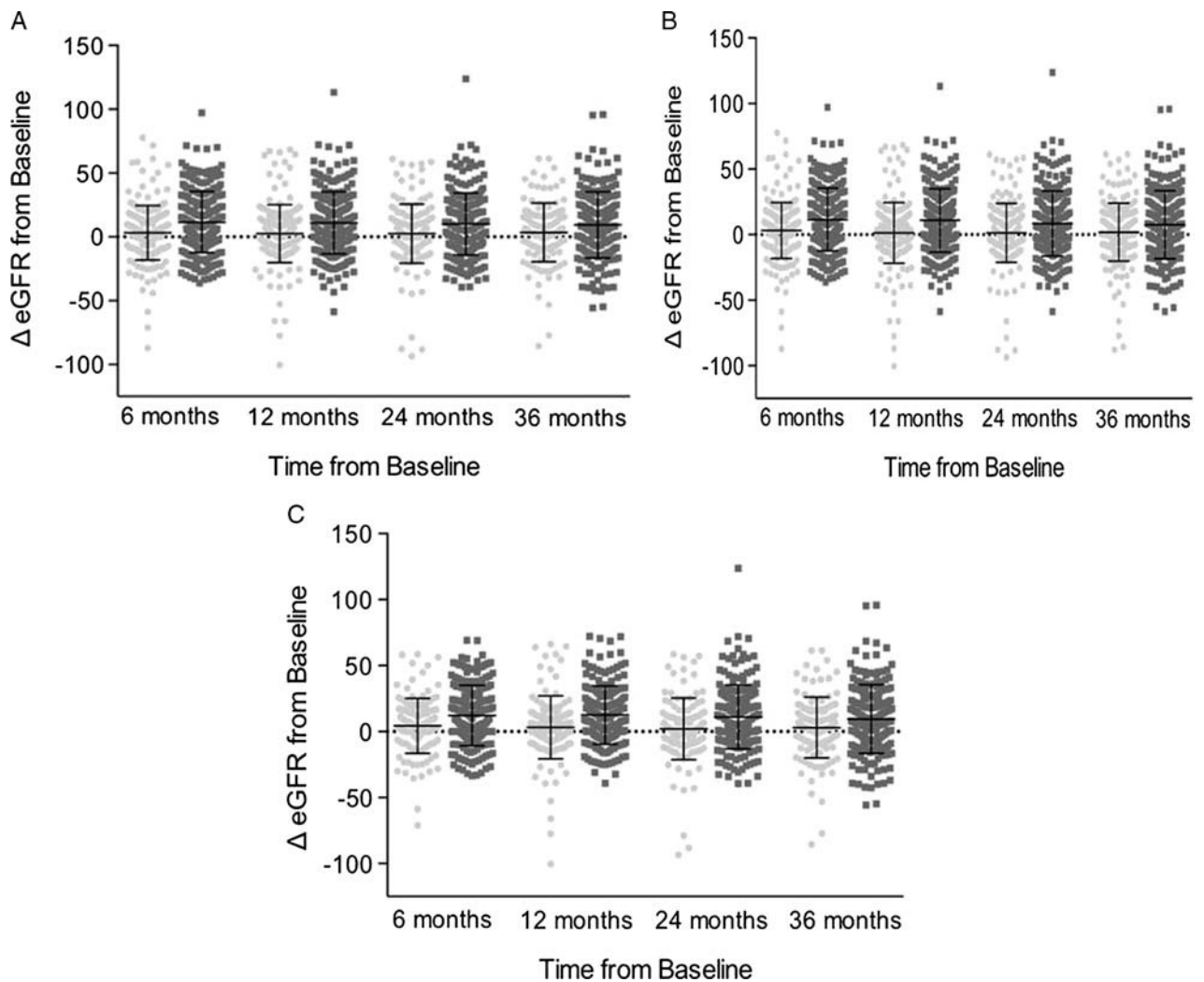
FIGURE 4. Change in eGFR from the baseline eGFR measurement at index biopsy and follow-up at 12, 24, and 36 months (A) after censoring for death and graft failure and (B) with the last eGFR values carried forwards, and (C) after excluding individuals with missing data due to death or graft loss. Gray dots represent the sirolimus group and black squares represent the tacrolimus group. Red lines represent the mean and standard deviation in change in eGFR from baseline eGFR at each time point.

therapy in such patients could be a contributing factor for increased risk of death with function (DWF) as well as late death.

The persistence of acute allograft dysfunction after early acute rejection is less common due to low incidence of BPAR during the first year of transplant, particularly after induction with alemtuzumab-based therapy.[46] Early-onset graft dysfunction is perhaps different in its pathophysiology compared with late-onset allograft dysfunction.[40] In the absence of microcirculatory abnormalities in the allograft biopsy as well as lack of anti-HLA antibodies, new-onset allograft dysfunction during the first year of transplantation presents a clinical challenge simply due to lack of interventions to preserve allograft function, specifically if the allograft biopsy reveals the features of TII.

One of the challenging aspects of current immunosuppression therapy is an ongoing debate about the impact of CNI toxicity, particularly in those recipients who have new-onset allograft dysfunction in the presence of prominent histological features of TII, IF/TA, and nonspecific inflammation.[12,18,47] The role of CNIs in the development of tubular injury continues to be debated.[13,20,24,25,28,31,48,49] Calcineurin inhibitor-sparing

approaches[35,50] as well as conversion to mTORIs early after transplantation in patients with stable allograft[36-38] have reported variable results. Our study results are consistent with other studies[51,52] that the use of mTORI can be associated with increased mortality. However, it is worthwhile to bear in mind that sirolimus use in healthy mice models has been associated with increasing lifespan by postponing cancer deaths, retarding mechanisms of aging, or both.[53]

It can be argued that these unfavorable results with the use of sirolimus in patients with a threatened allograft are perhaps due to protopathic bias[43] because the patients assigned to sirolimus had significantly more severe degree of tubular injury compared with the comparator group. However, the mortality risk remained unchanged in the IPTW adjusted models. Additionally, subgroup analysis based on the presence or absence of tubular damage at the index biopsy continued to show that those assigned to sirolimus were at increased risk of mortality outcomes. Because we did not demonstrate a significant impact on the allograft function in the tacrolimus group during the period of follow-up, it is possible that perhaps tacrolimus as a prototype of CNI may
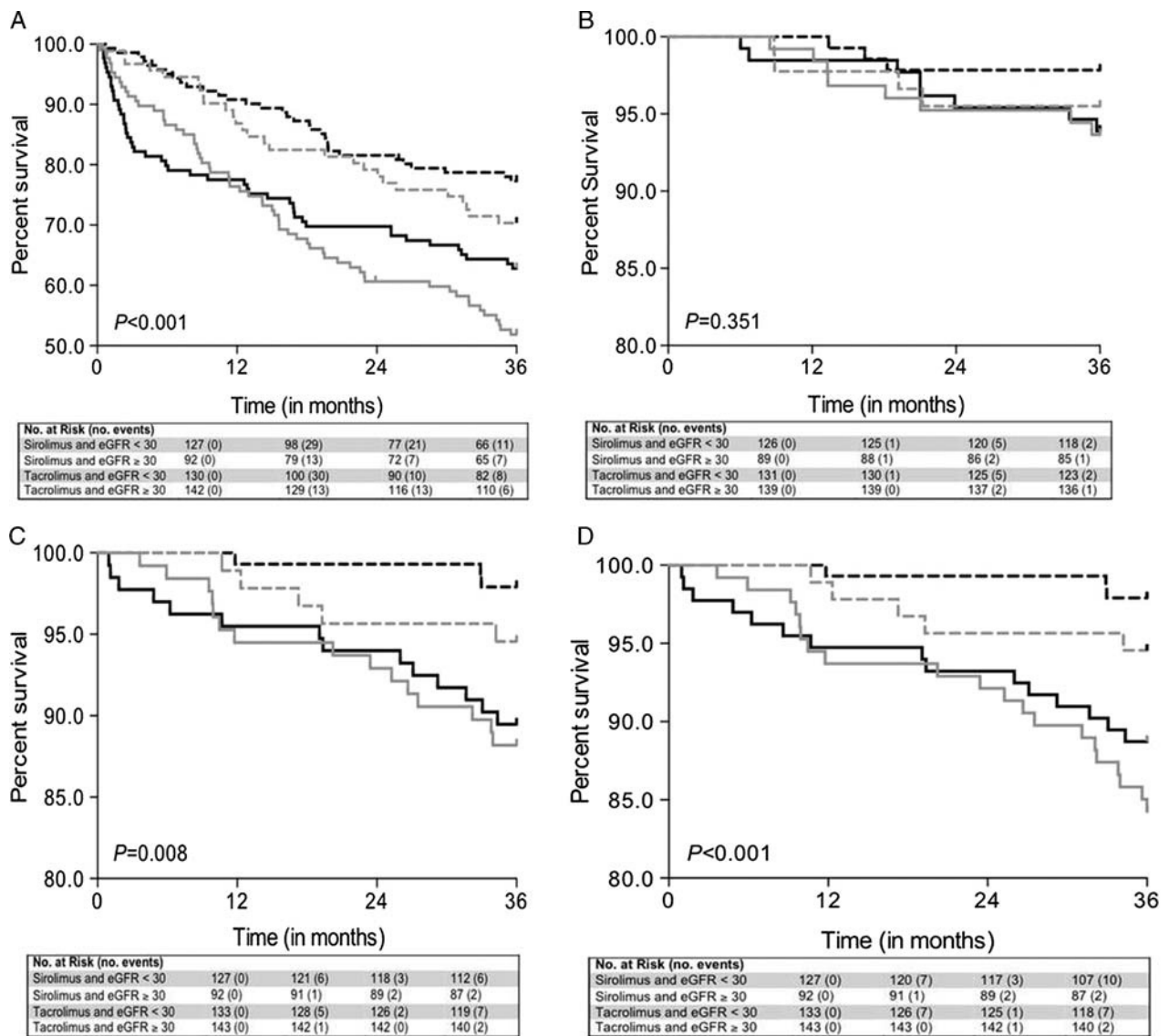
FIGURE 5. Kaplan-Meier survival curves stratified by baseline eGFR less than 30 versus 30 or greater. Individuals were stratified according to their baseline eGFR (<30 or ≥ 30) at the time of index biopsy and group assignments (sirolimus or tacrolimus) for (A) follow-up acute cellular rejection, (B) graft loss (death censored), (C) death with function graft, and (D) overall death. Individual curves represent the estimated percent survival over time since index biopsy. Gray lines represent the sirolimus group and black lines represent the tacrolimus group. Solid lines represent those with a baseline eGFR less than 30 and dotted lines represent those with a baseline eGFR of 30 or greater.

not adversely affect the graft function in those recipients with biopsy findings showing predominantly TII as has been illustrated by other investigators.[28,31,49,54]

Death after graft loss (late-death) is not often studied endpoint in the transplant patients. Recent registry data demonstrated that all-cause mortality after graft failure increases significantly.[55] Similarly, we observed that exposure to sirolimus was associated with increased mortality during the first year after graft failure while on dialysis, whether it is a simple association or cause-effect phenomenon cannot be answered in this data analysis. It should, however, be a cause for concern until more definitive data are available, that exposure to sirolimus increases the risk for death even after its discontinuation. This risk may be even stronger than we reported because we only captured this outcome data for the first year after graft failure.

Our study is not without limitations. One of the major limitations is that it is a retrospective single-center study. Other limitations of this analysis are the lack of data for the events that resulted in deaths either while on sirolimus therapy or after discontinuation of sirolimus while on dialysis. Also, we did not capture the data for the development and progression of proteinuria if any while on sirolimus-based therapy. Selection bias at the time of assignment to sirolimus due to physician preferences, recipient demographics, histological characteristics of the index biopsy, and other unmeasured confounders may have influenced these results. That is perhaps less likely given the details of this analysis and the consistency of results in the subgroups studied. Additionally, we evaluated other important outcomes, such as late death.

These data suggest, contrary to the general belief, that continuation of tacrolimus in patients with a threatened allograft does not portend a bad prognosis either for the graft or patient survival. Although retrospective studies offer considerable insights into the relationship between exposure and outcomes, causal inferences must be made with extreme

TABLE 4.

Primary and secondary outcomes for sirolimus therapy after stratification by potential confounders (tacrolimus group as the reference group).

Outcome	Present		Absent	
	OR (95% CI)	P	OR (95% CI)	P
(A) Acute cellular rejection on index biopsy	(N = 174)		(N = 322)	
Primary composite[a]	2.64 (1.38-5.05)	0.0033	1.70 (1.08-2.68)	0.0216
Follow-up acute cellular rejection	1.96 (1.07-3.59)	0.0298	1.69 (1.08-2.63)	0.0209
Graft failure	0.98 (0.40-2.40)	0.9581	1.18 (0.58-2.40)	0.6563
Death with functioning graft	1.50 (0.74-3.07)	0.2645	2.33 (1.29-4.21)	0.0049
≥50% eGFR decrease at 12 mo	1.52 (0.55-4.20)	0.4182	1.99 (0.85-4.65)	0.1122
Late death[b]	2.28 (1.20-4.35)	0.0124	2.34 (1.37-3.99)	0.0019
(B) IF/TA	(N = 350)		(N = 146)	
Primary composite[a]	1.75 (1.13-2.70)	0.0116	2.78 (1.26-6.12)	0.0112
Follow-up acute cellular rejection	1.48 (0.97-2.26)	0.0672	3.13 (1.47-6.68)	0.0031
Graft failure	0.85 (0.45-1.62)	0.6265	2.08 (0.67-6.41)	0.2031
Death with functioning graft	1.74 (1.00-3.01)	0.0492	3.11 (1.34-7.25)	0.0085
≥50% eGFR decrease at 12 mo	1.29 (0.63-2.64)	0.4927	4.29 (0.90-20.31)	0.0668
Late death[b]	1.93 (1.19-3.15)	0.0083	4.17 (1.88-9.26)	0.0004
(C) Tubulointerstitial injury[c]	(N = 177)		(N = 319)	
Primary Composite[a]	2.02 (1.06-3.82)	0.0317	2.21 (1.36-3.59)	0.0013
Follow-up acute cellular rejection	1.39 (0.74-2.61)	0.3108	2.14 (1.35-3.38)	0.0012
Graft failure	0.51 (0.17-1.47)	0.2117	1.75 (0.90-3.40)	0.1013
Death with functioning graft	3.38 (1.21-9.41)	0.0199	1.88 (1.09-3.24)	0.0028
≥50% eGFR decrease at 12 mo	1.19 (0.41-3.49)	0.7461	2.34 (1.01-5.43)	0.0486
Late death[b]	3.14 (1.34-7.36)	0.0084	2.35 (1.43-3.87)	0.0008
(D) Simulect	(N = 229)		(N = 267)	
Primary composite[a]	2.64 (1.50-4.66)	0.0008	1.59 (0.97-2.61)	0.0642
Follow-up acute cellular rejection	1.76 (1.03-2.99)	0.0378	1.75 (1.08-2.84)	0.0240
Graft failure	1.81 (0.77-4.23)	0.1718	0.74 (0.35-1.57)	0.4290
Death with functioning graft	2.29 (1.22-4.31)	0.0102	1.78 (0.92-3.43)	0.0861
≥50% eGFR decrease at 12 mo	1.96 (0.72-5.32)	0.1886	1.63 (0.69-3.85)	0.2613
Late death[b]	2.83 (1.58-5.07)	0.0005	2.03 (1.13-3.63)	0.0176
(E) Thymoglogublin	(N = 92)		(N = 404)	
Primary composite[a]	1.00 (0.42-2.36)	1.0000	2.24 (1.48-3.39)	0.0001
Follow-up acute cellular rejection	1.15 (0.49-2.73)	0.7449	1.92 (1.29-2.86)	0.0012
Graft failure	1.57 (0.39-6.32)	0.5243	1.01 (0.55-1.86)	0.9681
Death with functioning graft	2.04 (0.54-7.64)	0.2916	1.87 (1.15-3.05)	0.0112
≥50% eGFR decrease at 12 mo	1.85 (0.11-30.74)	0.6692	1.70 (0.87-3.32)	0.1227
Late death[b]	1.40 (0.41-4.83)	0.5924	2.36 (1.52-3.67)	0.0001
(F) Alemtuzumab	(N = 175)		(N = 321)	
Primary composite[a]	1.79 (0.96-3.35)	0.0663	2.01 (1.26-3.21)	0.0034
Follow-up acute cellular rejection	2.03 (1.11-3.70)	0.0220	1.57 (1.00-2.46)	0.0520
Graft failure	0.51 (0.21-1.24)	0.1372	1.75 (0.85-3.60)	0.1313
Death with functioning graft	1.51 (0.70-3.24)	0.2962	2.30 (1.31-4.05)	0.0039
≥50% eGFR decrease at 12 mo	1.32 (0.53-3.30)	0.5514	1.97 (0.77-5.02)	0.1572
Late death[b]	1.94 (0.98-3.85)	0.0570	2.54 (1.52-4.26)	0.0004

[a] Composite of follow-up acute cellular rejection, graft failure (death-censored), death with function graft, and ≥50% eGFR decrease at 12 months.

[b] Late death is defined as death after graft loss and after stopping sirolimus based therapy while on dialysis.

[c] Tubulointerstitial injury: any combination of tubular calcifications, degeneration, or striped cortical fibrosis.

degree of caution. The results of this data should be interpreted in light of the weaknesses as well as limitations of data analysis. Furthermore, the results of this data analysis do not apply to the use of other mTORI such as everolimus. Notwithstanding these limitations, these results are perhaps generalizable due to lack of rigid inclusion and exclusion criteria, center-specific consistency in treatment algorithm, a large cohort of patients, emphasis on hard outcomes, and intermediate duration of follow-up.

There are significant gaps in our understanding about the immunopathological basis of TII in the development of early allograft dysfunction and the impact of TII in the absence of microcirculatory inflammation. Whether such recipients could benefit by other interventions, such as replacement of tacrolimus with belatacept (under investigation: NCT01820572), or the use of low dose tacrolimus and sirolomus as has been demonstrated in recipients of kidney-pancreas transplants,[56] or the use of mesenchymal

stem cells that may facilitate the processes of tissue repair due to their known anti-inflammatory and antifibrotic effects (under investigation: NCT 00659620), are evolving paradigms. In conclusion, the results of this data analysis bode poorly for the use of sirolimus with respect to lowering the risk of graft loss and death in recipients with threatened allografts during the first year of transplant.

ACKNOWLEDGMENT

REFERENCES

1. Hariharan S, Johnson CP, Bresnahan BA, et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med*. 2000;342:605–612.

2. Rana A, Gruessner A, Agopian VG, et al. SUrvival benefit of solid-organ transplant in the United States. *JAMA Surgery*. 2015;150:252–259.

3. Gill JS, Tonelli M. Penny wise, pound foolish? Coverage limits on immunosuppression after kidney transplantation. *N Engl J Med*. 2012;366:586–589.

4. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant*. 2011;11:450–462.

5. Lodhi SA, Lamb KE, Meier-Kriesche HU. Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success. *Am J Transplant*. 2011;11:1226–1235.

6. Meier-Kriesche HU. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant*. 2004;4:378–383.

7. Pascual M, Theruvath T, Kawai T, et al. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med*. 2002;346:580–590.

8. Hariharan S, McBride MA, Cherikh WS, et al. Post-transplant renal function in the first year predicts long-term kidney transplant survival. *Kidney Int*. 2002;62:311–318.

9. First MR. Renal function as a predictor of long-term graft survival in renal transplant patients. *Nephrol Dial Transplant*. 2003;18(Suppl 1):i3–i6.

10. Fellstrom B, Jardine AG, Soveri I, et al. Renal dysfunction is a strong and independent risk factor for mortality and cardiovascular complications in renal transplantation. *Am J Transplant*. 2005;5:1986–1991.

11. Meier-Kriesche HU. Decreased renal function is a strong risk factor for cardiovascular death after renal transplantation. *Transplantation*. 2003;75:1291–1295.

12. El Ters M, Grande JP, Keddis MT, et al. Kidney allograft survival after acute rejection, the value of follow-up biopsies. *Am J Transplant*. 2013;13:2334–2341.

13. Gago M, Cornell LD, Kremers WK, et al. Kidney allograft inflammation and fibrosis, causes and consequences. *Am J Transplant*. 2012;12:1199–1207.

14. El-Zoghby ZM, Stegall MD, Lager DJ, et al. Identifying specific causes of kidney allograft loss. *Am J Transplant*. 2009;9:527–535.

15. Einecke G, Reeve J, Sis B, et al. A molecular classifier for predicting future graft loss in late kidney transplant biopsies. *J Clin Invest*. 2010;120:1862–1872.

16. Famulski KS, Reeve J, de Freitas DG, et al. Kidney transplants with progressing chronic diseases express high levels of acute kidney injury transcripts. *Am J Transplant*. 2013;13:634–644.

17. Israni AK, Leduc R, Jacobson PA, et al. Inflammation in the setting of chronic allograft dysfunction post-kidney transplant: phenotype and genotype. *Clin Transplant*. 2013;27:348–358.

18. Mannon RB, Matas AJ, Grande J, et al. Inflammation in areas of tubular atrophy in kidney allograft biopsies: a potent predictor of allograft failure. *Am J Transplant*. 2010;10:2066–2073.

19. Mengel M, Reeve J, Bunnag S, et al. Scoring total inflammation is superior to the current Banff inflammation score in predicting outcome and the degree of molecular disturbance in renal allografts. *Am J Transplant*. 2009;9:1859–1867.

20. Park WD, Griffin MD, Cornell LD, et al. Fibrosis with inflammation at one year predicts transplant functional decline. *J Am Soc Nephrol*. 2010;21:1987–1997.

21. Sis B, Einecke G, Chang J, et al. Cluster analysis of lesions in nonselected kidney transplant biopsies: microcirculation changes, tubulointerstitial inflammation and scarring. *Am J Transplant*. 2010;10:421–430.

22. Torres IB, Moreso F, Sarro E, et al. The Interplay between inflammation and fibrosis in kidney transplantation. *BioMed Res Int*. 2014;2014:750602.

23. Cosio FG, Grande JP, Wadei H, et al. Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant*. 2005;5:2464–2472.

24. Rush DN, Cockfield SM, Nickerson PW, et al. Factors associated with progression of interstitial fibrosis in renal transplant patients receiving tacrolimus and mycophenolate mofetil. *Transplantation*. 2009;88:897–903.

25. Cosio FG, Grande JP, Larson TS, et al. Kidney allograft fibrosis and atrophy early after living donor transplantation. *Am J Transplant*. 2005;5:1130–1136.

26. Seron D, Moreso F, Bover J, et al. Early protocol renal allograft biopsies and graft outcome. *Kidney Int*. 1997;51:310–316.

27. Yilmaz S, Tomlanovich S, Mathew T, et al. Protocol core needle biopsy and histologic Chronic Allograft Damage Index (CADI) as surrogate end point for long-term graft survival in multicenter studies. *J Am Soc Nephrol*. 2003;14:773–779.

28. Matas AJ. Chronic progressive calcineurin nephrotoxicity: an overstated concept. *Am J Transplant*. 2011;11:687–692.

29. Myers BD, Ross J, Newton L, et al. Cyclosporine-associated chronic nephropathy. *N Engl J Med*. 1984;311:699–705.

30. Nankivell BJ, Borrows RJ, Fung CL, et al. Calcineurin inhibitor nephrotoxicity: longitudinal assessment by protocol histology. *Transplantation*. 2004;78:557–565.

31. Snanoudj R, Royal V, Elie C, et al. Specificity of histological markers of long-term CNI nephrotoxicity in kidney-transplant recipients under low-dose cyclosporine therapy. *Am J Transplant*. 2011;11:2635–2646.

32. Matas AJ, Leduc R, Rush D, et al. Histopathologic clusters differentiate subgroups within the nonspecific diagnoses of CAN or CR: preliminary data from the DeKAF study. *Am J Transplant*. 2010;10:315–323.

33. Naesens M, Lerut E, Damme BV, et al. Tacrolimus exposure and evolution of renal allograft histology in the first year after transplantation. *Am J Transplant*. 2007;7:2114–2123.

34. Weir MR. Late calcineurin inhibitor withdrawal as a strategy to prevent graft loss in patients with suboptimal kidney transplant function. *Am J Nephrol*. 2004;24:379–386.

35. Dudley C, Pohanka E, Riad H, et al. Mycophenolate Mofetil Creeping Creatinine Study G. Mycophenolate mofetil substitution for cyclosporine a in renal transplant recipients with chronic progressive allograft dysfunction: the "creeping creatinine" study. *Transplantation*. 2005;79:466–475.

36. Holdaas H, Rostaing L, Seron D, et al. Conversion of long-term kidney transplant recipients from calcineurin inhibitor therapy to everolimus: a randomized, multicenter, 24-month study. *Transplantation*. 2011;92:410–418.

37. Lebranchu Y, Thierry A, Thervet E, et al. Efficacy and safety of early cyclosporine conversion to sirolimus with continued MMF-four-year results of the postconcept study. *Am J Transplant*. 2011;11:1665–1675.

38. Weir MR, Mulgaonkar S, Chan L, et al. Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled spare-the-nephron trial. *Kidney Int*. 2011;79:897–907.

39. Schena FP, Pascoe MD, Alberu J, et al. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. *Transplantation*. 2009;87:233–242.

40. Gourishankar S, Leduc R, Connett J, et al. Pathological and clinical characterization of the 'troubled transplant': data from the DeKAF study. *Am J Transplant*. 2010;10:324–330.

41. Racusen LC, Halloran PF, Solez K. Banff 2003 meeting report: new diagnostic insights and standards. *Am J Transplant*. 2004;4:1562–1566.

42. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. *Am J Epidemiol*. 2003;158:915–920.

43. Salas M, Hofman A, Stricker BH. Confounding by indication: an example of variation in the use of epidemiologic terminology. *Am J Epidemiol*. 1999;149:981–983.

44. Cole SR, Hernan MA. Adjusted survival curves with inverse probability weights. *Comput Methods Programs Biomed*. 2004;75:45–49.

45. Lunceford JK, Davidian M. Stratification and weighting via the propensity score in estimation of causal treatment effects: a comparative study. *Stat Med*. 2004;23:2937–2960.

46. Hanaway M, Woodle E, Mulgaonkar S, et al. Alemtuzumab induction in renal transplantation. *N Engl J Med*. 2011;364:1909–1919.

47. Dahle DO, Mjoen G, Oqvist B, et al. Inflammation-associated graft loss in renal transplant recipients. *Nephrol Dial Transplant*. 2011;26: 3756–3761.

48. Baboolal K, Jones GA, Janezic A, et al. Molecular and structural consequences of early renal allograft injury. *Kidney Int*. 2002;61:686–696.

49. Stegall MD, Park WD, Larson TS, et al. The histology of solitary renal allografts at 1 and 5 years after transplantation. *Am J Transplant*. 2011;11: 698–707.

50. Weir MR. Long-term impact of discontinued or reduced calcineurin inhibitor in patients with chronic allograft nephropathy. *Kidney Int*. 2001;59: 1567–1573.

51. Cortazar F, Molnar MZ, Isakova T, et al. Clinical outcomes in kidney transplant recipients receiving long-term therapy with inhibitors of the mammalian target of rapamycin. *Am J Transplant*. 2012;12:379–387.

52. Isakova T, Xie H, Messinger S, et al. Inhibitors of mTOR and risks of allograft failure and mortality in kidney transplantation. *Am J Transplant*. 2013;13:100–110.

53. Harrison DE, Strong R, Sharp ZD, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. 2009;460:392–395.

54. Gaston RS. Chronic calcineurin inhibitor nephrotoxicity: reflections on an evolving paradigm. *Clin J Am Soc Nephrol*. 2009;4:2029–2034.

55. Kaplan B, Meier-Kriesche HU. Death after graft loss: an important late study endpoint in kidney transplantation. *Am J Transplant*. 2002;2: 970–974.

56. Ciancio G, Sageshima J, Chen L, et al. Advantage of rapamycin over mycophenolate mofetil when used with tacrolimus for simultaneous pancreas kidney transplants: randomized, single-center trial at 10 years. *Am J Transplant*. 2012;12:3363–3376.