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Chronic Allograft Nephropathy Score Before Sirolimus Rescue Predicts Allograft Function in Renal Transplant Patients

A. Basu, J.L. Falcone, H.P. Tan, D. Hassan, I. Dvorchik, K. Bahri, N. Thai, P.S. Randhawa, A. Marcos, T.E. Starzl, and R. Shapiro

ABSTRACT

Chronic allograft nephropathy (CAN) is a major indication for initiation of sirolimus (SRL) in renal transplantation (TX) to prevent deterioration of renal function. We evaluated whether the CAN score at time of sirolimus rescue (SRL-R) predicts renal allograft function. CAN score is the sum of the following 4 categories: glomerulopathy (cg, 0–3), interstitial fibrosis (ci, 0–3), tubular atrophy (ct, 0–3), and vasculopathy (cv, 0–3). This is a retrospective cohort study of renal transplant recipients from July 2001 to March 2004. Immunosuppression consisted of preconditioning with rabbit anti-thymocyte globulin or alemtuzumab and maintenance with tacrolimus (TAC) monotherapy with spaced weaning, if applicable, SRL-R was achieved by conversion from TAC, or by addition to reduced doses of TAC. Ninety patients received SRL. Thirty-three of these patients met the inclusion criteria of the following: (1) receipt of SRL for >6 months, and (2) follow-up of ≥6 months. There were 16 patients in the low-CAN (0–4) group and 17 patients in the high-CAN (>4) group. Cockcroft-Gault (C-G) glomerular filtration rate (GFR) was calculated at SRL-R and at 1, 3, 6, and 12 months. The ΔGFR was significantly better in the low-CAN group at 1, 3, and 6 months. A trend toward an improved ΔGFR was present at 12 months in the low-CAN group ($P = .16$). CAN scoring at the time of SRL-R predicts recovery of renal allograft function (as measured using ΔGFR), and should be used in preference to biochemical markers (Cr and C-G GFR), which may not be reliable predictors.

THE MOST common causes of late renal allograft loss are chronic renal allograft dysfunction (CRAD) and death with a functioning graft (DWFG).[1] A major cause of CRAD is chronic allograft nephropathy (CAN).

CAN is progressive long-term condition characterized by interstitial fibrosis, tubular atrophy, vascular occlusive changes, and glomerulosclerosis.[2,3] There are 2 distinct phases of injury: an initial phase and a later phase. Each phase represents cumulative and progressive damage from both immunologic and nonimmunologic etiologies.[4] Chronic changes are scored for the glomerulopathy (cg), interstitial fibrosis (ct), tubular atrophy (ct), and vasculopathy (cv). It is possible to apply relative weightings and calculate overall severity scores (such as the CAN score) using this coding system.[2] In the Banff classification, the following histological patterns have been defined: CAN (a): interstitial fibrosis, tubular atrophy and/or loss, glomerulopathy, and mesangial matrix increase (grades 1–3a); CAN (b): interstitial fibrosis, tubular atrophy and/or loss, together with typical vascular lesions and mononuclear infiltrates (grades 1–3b); and calcineurin inhibitor (CNI) nephrotoxicity: hyaline changes particularly in the afferent arterioles of the glomerulus and vacuolation of tubular epithelial cells.[3]

The CNIs Tacrolimus (TAC) and cyclosporine (CsA) have been mainstays of chronic immunosuppression because of a decreased incidence of acute rejection and also improved allograft and patient survival rates in recent decades.[1] CNI drugs cause progressive renal injury related to direct nephrotoxicity; the continued use of CNI agents

From the Thomas E. Starzl Transplantation Institute, Pittsburgh, Pennsylvania, USA.

Support provided by Thomas E Starzl Transplantation Institute.

Address reprint requests to A. Basu, Thomas E Starzl Transplantation Institute, MUH 7 South, 3459 Fifth Avenue, Pittsburgh, PA 15213.

0041-1345/07/$–see front matter
doi:10.1016/j.transproceed.2006.10.017

contributes to the development of CAN.[5] There is a high incidence of CAN in patients using Tacrolimus as maintenance immunosuppression — 67% at 2 years after renal transplantation.[6] Thus, although there has been a dramatic increase in kidney graft survival with TAC, CNI-related nephrotoxicity may compromise long-term outcomes.[7] The reduction or withdrawal of CNI in combination with alternative immunosuppressive regimens such as sirolimus (SRL) has become a popular practice in renal transplantation.

SRL is a macrocyclic lactone antibiotic produced by *Streptomyces hygroscopicus*. It inhibits the mammalian target of rapamycin (mTOR), a kinase in costimulatory and cytokine-driven pathways of T-cell regulation.[8–10] SRL lacks the acute and chronic nephrotoxic profile of the CNIs. Because it inhibits growth-factor–induced proliferation of fibroblasts, endothelial cells, hepatocytes, and smooth muscle cells, it offers an important alternative to CNI drugs to prevent CAN.[11]

The reduction or withdrawal of CNI in combination with sirolimus rescue (SRL-R) has been a growing topic of interest. Improvements in renal function have been shown in patients with CAN (b) and CNI nephrotoxicity with SRL-R therapy. Allograft function was less likely to improve for patients with CAN (a) or serum creatinine level >400 μmol/L.[12]

At the American Transplant Congress (ATC) in 2003, we presented the University of Pittsburgh Medical Center's (UPMC) experience with SRL-R in kidney and kidney/pancreas transplant recipients.[13] Other clinical studies have tried to identify independent factors that predict successful conversion from TAC to SRL in patients with CAN.[14,15]

Among the factors that predict success in conversion from TAC to SRL in CAN are serum creatinine level at the time of conversion,[14] and degree of CAN, as assessed using the CAN score at the time of SRL conversion.

No clinical studies have evaluated the allograft outcomes in antibody preconditioned patients with regard to SRL-R from TAC-based immunosuppression. The purpose of this current retrospective study was to determine if the CAN score at the time of SRL-R predicts subsequent renal allograft function. Changes in calculated glomerular filtration rate (GFR) were evaluated in patients who had received antibody preconditioning with rabbit anti-thymocyte globulin or alemtuzumab to see if CAN score[2,3] at SRL-R was a significant predictor of subsequent renal allograft function.

PATIENTS AND METHODS

This retrospective cohort study used the in-house database, Electronic Data Interface for Transplantation (EDIT), at the Thomas E. Starzl Transplantation Institute at the University of Pittsburgh Medical Center. Evaluation of all the renal allograft recipients from July 1, 2001 to March 31, 2004 yielded 190 patients diagnosed with CAN based on renal biopsy specimen. Of these 190 patients with CAN, 90 had received SRL-R from TAC. From this patient population, 33 satisfied the inclusion criteria of the following: (1) no extra-renal transplant, (2) receipt of SRL for at least 6 months, and (3) follow-up maintenance of at least 6 months. Patients satisfying all inclusion criteria were divided into 2 groups based on the biopsy-reported CAN scoring done by a blinded pathologist using the 1997 Banff classification system. CAN score was calculated as the sum of the following 4 categories: glomerulopathy (eg. 0–3), interstitial fibrosis (ci, 0–3), tubular atrophy (ct, 0–3), and vasculopathy (cv, 0–3). Of these 33 patients, 16 were in the low-CAN score group with a score ≤4, and 17 were in the high-CAN score group with a score >4. Review was made of the clinical course, including laboratory values, dosage and trough levels of immunosuppressive agents, and steroid and antibody treatment of rejection episodes. Data collection was approved by the Institutional Review Board (IRB) of the UPMC.

Immunosuppressive preconditioning had been achieved with either Thymoglobulin 5 mg/kg (Sangstat, Fremont, Calif, United States (n = 30) or Campath-1 H 30 mg (Berlex, Montvale, NJ, United States) (n = 3) preoperatively followed by TAC monotherapy, with target trough levels of around 10 ng/mL.[16–18] The IRB of the University of Pittsburgh judged the above immunosuppressive regimen to be within the boundaries of historically based standard treatment, not needing formal IRB approval. The Presbyterian University Hospital Innovative Practices Committee and the Pharmacy and Therapeutics Committee both approved the protocol.

After biopsy-reported CAN scoring, SRL conversion was accomplished either by converting (n = 19) from TAC to SRL or by an addition (n = 14) of SRL to tapered doses of TAC. Eight patients in the low-CAN score group had TAC abruptly changed to SRL, whereas the remaining 8 patients in the low-CAN score group had SRL added to tapered doses of TAC; the other patients in each category belonged to the high CAN score group. SRL levels were maintained at a trough serum level of 5–10 ng/mL either as monotherapy or when used with TAC. TAC levels were maintained at a trough level between 5 and 10 ng/mL when used with SRL. Spaced weaning was not carried out for patients on SRL monotherapy. Patients with acute rejection based on biopsy specimen were treated with corticosteroids and mycophenolate mofetil (MMF) as indicated (low-CAN, n = 3; high-CAN, n = 4).

Serum creatinine level was determined for each patient at the time of SRL-R. Cockcroft-Gault glomerular filtration rate (GFR) was calculated for each patient at the time of SRL-R and at 1, 3, 6, and 12 months after SRL-R. The low-CAN and high-CAN groups were compared for significant differences in GFR changes with Mann-Whitney *U* tests using SPSS 12.0 software (Chicago, Ill, United States), defining statistical significance as $P < .05$. The dynamics of change in the GFR in the low-CAN score and high-CAN score groups were evaluated using the Wilcoxon signed rank test using SPSS 12.0 software, defining statistical significance as $P < .05$.

RESULTS

The characteristics of the study population (n = 33) at the time before SRL-R are shown in Table 1. The median age in the low-CAN and high-CAN groups was 50.5 years and 50.0 years, respectively. The low-CAN group was 56% male and the high-CAN group was 59% male with no age or gender differences between CAN groups. The median GFR for the low-CAN and high-CAN groups were 33.0 mL/min and 33.3 mL/min, respectively ($P = .51$). The time from transplantation to the initiation of SRL-R and the serum creatinine values at the time of SRL-R were not

Table 1. Patient Information Before Initiation of SRL-R

	Low-CAN Group (n = 16)	High-CAN Group (n = 17)	P
CAN score	Median, 3.5	Median, 6.0	
Age (y)	50.5	50.0	.66
Male:female ratio	9:7	10:7	
Time from transplantation to SRL-R (d)	208	372	.12
Cockcroft-Gault GFR (mL/min)	33.0	33.3	.51
SCr at SRL-R (mg/dL)	2.9	2.5	.36

Abbreviation: SCr, serum creatinine.
Note: All values are expressed as median.

statistically significant between the groups ($P = .12$ and $P = .36$, respectively).

The changes in GFR after SRL-R for patients in the low-CAN and high-CAN groups are shown in Table 2. For patients in the low-CAN group, the average GFR increased significantly from baseline at 1, 3, and 6 months after SRL-R ($P = .006$, $P = .002$, and $P = .004$, respectively) (Wilcoxon signed rank test). The net change in GFR in this group was +2.8 mL/min at 12 months; however, this positive change was not statistically significant ($P = .21$). In the high-CAN group, there was no significant change in GFR at 1, 3, 6, or 12 months from initiation of SRL-R ($P = .78$, $P = .49$, $P = .6$, and $P = .61$, respectively) (Wilcoxon signed rank test).

At the time of SRL-R, there was no difference in GFR values between the groups. However, statistically significant changes in GFR dynamics did occur between the 2 patient groups at 1, 3, and 6 months ($P = .008$, $P = .001$, and $P = .004$, respectively) (Mann-Whitney *U* test). At 12 months, the change in GFR was not significantly better in the low-CAN score group ($P = .16$).

There was 1 patient death each in the low-CAN group and the high-CAN group during the 1-year follow-up period (Table 2). The patient in the low-CAN group died of cardiovascular causes with a failing allograft during the eighth month following SRL-R. The patient in the high-CAN group suffered from bipolar disorder and died during the 12th month after SRL-R with a functioning allograft.

Table 2. Effect of SRL-R on Change (Δ) in GFR in Patients in the Low-CAN and High-CAN Groups (Mann-Whitney *U* Test)

	Low-CAN Group	High-CAN Group	P
Cockcroft-Gault GFR at SRL-R (mL/min)	33.0	33.3	.51
ΔGFR 1 mo after SRL-R (mL/min)	+11.6	+2.05	.008
ΔGFR 3 mo after SRL-R (mL/min)	+9.1	−1.4	.001
ΔGFR 6 mo after SRL-R (mL/min)	+10.2	−0.05	.004
ΔGFR 12 mo after SRL-R (mL/min)	+2.8 (n=15)	−2.75 (n=16)	.16

Note: All values are expressed as median.

Table 3. Patient and Graft Survival Rates and Serum Creatinine Levels 1 Year After SRL-R

	Low-CAN Group	High-CAN Group
Patient survival	94%	94%
Graft survival	87.5%	94%
Death-censored graft survival	93.3%	100%
Median SCr (mg/dL)	3.0	2.85

Patient survival, graft survival, and serum creatinine level 1 year after SRL-R are shown in Table 3, and are not significantly different. One graft was lost in the low-CAN group in a 75-year-old woman after she developed pulmonary aspergillosis in the ninth month following SRL-R.

DISCUSSION

CAN is a major determinant of CRAD leading to worsening allograft function and allograft loss after renal transplantation. Traditional CNI-based therapies are being reevaluated because they cause direct nephrotoxicity and worsen CAN. The mammalian target of rapamycin (mTOR) inhibitor SRL has been recently investigated as a subsequent substitution therapy for CNI therapy. Because of its lack of direct nephrotoxicity, low side effect profile, antitumor activity, and effectiveness.[8] SRL-R is an intensely studied area in renal transplantation, using the initial benefits of TAC while reducing long-term exposure using SRL.

Previous comparisons of renal transplant recipients with CAN (n = 51) receiving SRL-R with a historical control group (n = 292) receiving SRL-R did not show any differences in patient survival or allograft survival during a period of 5 years of follow-up (unpublished data from the University of Pittsburgh). This result suggests that patients receiving SRL-R had no benefit of therapy, but raised the possibility that many of these patients had undergone SRL-R when CAN was too advanced. The current study suggests that a cohort of patients with a CAN score ≤4 will benefit from SRL-R as measured by significantly improved GFR measurements for up to 6 months of follow-up after SRL-R.

This study shows that CAN scoring at the time of SRL-R can predict recovery of renal allograft function as measured using changes in GFR. Statistical significance for improved GFR was reached at 1, 3, and 6 months after SRL-R in the low-CAN group compared with the high-CAN group. For patients within the low-CAN group, the mean GFR increased significantly at 1, 3, and 6 months after SRL-R; GFR improvement did not reach statistical significance after 1 year of follow-up ($P = .21$), although a net change of +2.8 mL/min was seen. Within the high-CAN group, improvement in GFR did not occur. In spite of these differences, there were no differences in patient or graft survival or quality of kidney function.

Factors predicting the success of SRL-R are still under investigation for different patient groups. Previous studies have shown that serum creatinine level <2.8 mg/dL, degree of CAN, and proteinuria <800 mg/d can predict renal

allograft function after SRL-R.[14,15,19] In the report by Diekmann et al,[15] although proteinuria was the only significant independent factor, responders had a significantly lower grade of CAN and significantly lower grade of vascular fibrous intimal thickening than nonresponders.

This analysis specifically demonstrates that patients with a CAN score ≤4 can benefit from SRL-R. In this study, patients in the low-CAN group had a median serum creatinine level of 2.9 mg/dL, higher than the significant level shown in the study by Egidi et al.[14] Patients in the high-CAN group had a median serum creatinine level of 2.5 mg/dL. Differences were not significant between the groups ($P = .36$). Serum creatinine level may not be a reliable predictor of recovery of renal allograft function in these patients.

Proteinuria was not systemically assessed at the time of SRL-R in the patient groups in this study. This information could have provided additional insight about the value of proteinuria in predicting SRL-R success, and is a limitation of this study.

Sankaranarayanan et al[19] showed that the Chronic Allograft Damage Index (CADI) score at the time of conversion from CNI to SRL was a reliable predictor of renal outcome. The CADI consists of the 6 histological changes characteristic of chronic rejection, ie, interstitial inflammation and fibrosis, glomerular sclerosis and mesangial matrix increase, vascular intimal proliferation, and tubular atrophy.[20] The Banff schema and CADI score provide equivalent information.[3] The change in GFR was significantly worse in the high-CADI (median, 7.5) group compared with the low-CADI (median, 3) group at 3, 6, and 12 months following conversion.[19] In this study, there were 15 patients in the low-CADI score group and 16 patients in the high-CADI score group.[19] GFR, however, was calculated using the MDRD formula,[19] unlike the Cockcroft-Gault formula in our study.

The small number of patients (n = 33) in this study makes it less powerful to determine smaller differences between groups. However, even given the low power of this study, there were statistically significant changes in GFR not only within the low-CAN group, but also between groups at 1, 3, and 6 months after SRL-R. The changes in GFR were not significant between the low-CAN and high-CAN groups at 12 months, although a trend toward an improvement in change in GFR was noted for the low-CAN group. Likewise, the retrospective nature of this study is suboptimal. Variations in the antibody used for preconditioning and differences in the SRL-R protocol decrease the validity of the study design. However, this analysis was performed in an unselected group of patients of varying ethnicity with prior kidney transplants and with varying number of comorbidities.

Two kinds of protocols exist for switching from CNI-based to SRL-based immunosuppression. In the protocols with an overlap period between CNI and SRL therapy, there exists the potential for excessive initial immunosuppression and the need for careful therapeutic drug monitoring.[8] The immediate conversion to SRL has the advantage of simplicity and avoids potential overimmunosuppression.[21] In the protocols with immediate conversion, the possibility of acute rejection might be increased, if the time to achieve target SRL levels is prolonged.

In a single-center, randomized controlled trial. 40 renal transplant recipients between 6 months and 8 years posttransplantation were randomly assigned to remain on their CNI (CsA or TAC) or to switch to SRL.[21] The principal inclusion criteria in this study was suboptimal renal function, defined as serum creatinine level between 120 and 400 μmol/L (1.36–4.52 mg/dL). Two patients never took their study drugs, leaving 19 patients in each group. At 12 months, there was a significant change in GFR following conversion to SRL (12.9 mL/min, 95% confidence interval, 6.1–19.7; $P < .001$). No patient in either group experienced an acute rejection episode. The availability of SRL allows substitution for CNI therapy with a nonnephrotoxic agent and potentially avoids risk of acute rejection.[21] No attempt was made in this study to quantify the burden of CAN prior to switching to SRL.[21]

We did not do protocol kidney transplant biopsies in our study. Such biopsies could help in documenting the progression of CAN in the low-CAN and high-CAN score groups during the period of follow-up. The Rapamune Maintenance Regimen Trial showed that early CsA withdrawal from a SRL-CsA-steroid (ST) regimen improved renal function and histology.[22] Protocol mandated biopsies were done at engraftment and at 12 and 36 months after transplantation. All 6 componenets of the CADI score were lower in the patients in the SRL-ST group, who had undergone early CsA withdrawal. Inflammation and tubular atrophy scores decreased significantly in the SRL-ST groups between 12 and 36 months.[22]

In summary, the presence of a CAN score ≤4 was predictive of improved GFR at 1, 3, and 6 months after SRL-R compared with a group of patients with a CAN score >4. CAN scoring may be a more reliable predictor of allograft function rather than serum creatinine level or Cockcroft-Gault GFR measurements. Prospective SRL-R trials should be done to confirm the conclusions of this study.

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