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Early or Late Calcineurin Inhibitor Withdrawal and Mycophenolate Mofetil-Based Immunosuppression to Prevent Graft Loss in Patients with Suboptimal Kidney Transplant Function

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Abstract

The rate of graft loss in kidney transplant recipients after the first year of transplantation has remained unchanged despite the short-term gains achieved with the recent advances in immunosuppression therapy. Progressive allograft dysfunction after transplantation is an important risk factor for future graft failure. The most common histologic finding in graft biopsies of patients with progressive loss of graft function in the absence of known causes is the development of interstitial fibrosis and tubular atrophy, not otherwise specified, defined in the past as chronic allograft nephropathy. Nearly one-third of recipients of kidney transplants demonstrate de novo interstitial fibrosis and tubular atrophy not otherwise specified by three months after transplantation in the absence of changes in serum creatinine values. The pathogenesis of interstitial fibrosis and tubular atrophy following kidney transplantation is complex. One of the critical mechanisms for the onset and progression of interstitial injury is the result of epithelial-mesenchymal transition due to time-dependent immunologic and nonimmunologic graft injury. Markers of epithelial-mesenchymal transition can be found in more than 40% of allograft biopsies by the third month after transplantation. Calcineurin inhibitors have the potential of inducing epithelial-mesenchymal transition, and their toxicity could perpetuate tubular injury and interstitial inflammation, leading to interstitial fibrosis and tubular atrophy. These interstitial changes progress despite reductions in calcineurin inhibitor dose.

Early detection of interstitial fibrosis and tubular atrophy not otherwise specified may indicate the need for calcineurin inhibitor withdrawal. The calcineurin inhibitor can be replaced with anti-metabolites such as mycophenolate mofetil in those who are on azathioprine-based maintenance therapy. If patients are already on mycophenolate mofetil-based therapy, conversion from calcineurin inhibitor- to sirolimus-based therapy may be another option to prevent further deterioration of allograft function. Conversion therapy can improve graft function and may prolong graft survival.

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To prevent progressive nephron loss, every effort should be made to detect interstitial fibrosis and tubular atrophy by allograft biopsy before measurable changes in serum creatinine level. Once the serum creatinine has increased, often the histologic damage is irreversible. As a result, conversion to calcineurin inhibitor-sparing regimens in patients with advanced graft dysfunction with estimated glomerular filtration rate of less than 30 ml/min/1.73 m^2 may not be as beneficial as earlier conversion therapy. **(Trends in Transplant. 2009;1:13-27)**

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Key words

Chronic allograft nephropathy. Interstitial fibrosis and tubular atrophy of not otherwise specified. Calcineurin inhibitors. Mammalian target of rapamycin inhibitors. Mycophenolate mofetil. Epithelial to mesenchymal transition. Graft failure. Acute rejection.

Introduction

During the first year after kidney transplantation, the primary goal is to use maintenance immunosuppression to prevent acute cellular and antibody mediated rejection. In fact, during the past decade, due to significant advances in immunosuppression therapy, the rate of acute cellular rejection during the first year after transplantation has decreased to less than 20%, a significant achievement in short-term gains. These gains have not so far translated into long-term gains since the absolute rate of graft loss either due to patient death (death with a functioning graft) or censored after patient death has not improved significantly despite these advances. In addition, long-term deterioration in allograft function with a consequent increase in cardiovascular disease remains a daunting challenge.

Worsening allograft function that develops in the absence of histologic features of other well-defined causes is usually defined as chronic allograft nephropathy (CAN). It was also referred to as chronic rejection by the Banff 1997 classification. Often the histologic features of CAN are nonspecific[1] and could be due to a combination of factors such as donor-related nephron loss, posttransplant hypertension, and diabetes, or as a consequence of ischemia-reperfusion injury, drug toxicity, and viral infections such as reactivation of polyomavirus or cytomegalovirus. However, the most prominent histologic features of CAN are interstitial fibrosis and tubular damage. The terminology of CAN was redefined by the recent Banff meeting, and has been labeled as chronic interstitial fibrosis and tubular atrophy, not otherwise specified (IF/TA NOS)[2].

Prevalence of interstitial fibrosis and tubular atrophy, not otherwise specified

Analysis of the data from the centers that have performed protocol biopsies during the past ten years would suggest that IF/TA NOS is an early event following successful transplantation regardless of the origin of the kidney, i.e. deceased donor or living donor kidney transplants[3].

Protocol biopsies have demonstrated that IF/TA NOS can be detected in 25% of allografts at three months[4], 40% of grafts at two years[5] and almost 99% of grafts at 10 years[6]. Similarly, histologic features at three months have also been associated with long-term graft survival. Serón, et al. demonstrated that protocol biopsies at three months in patients with stable serum creatinine could predict the 10-year graft survival. The CAN score and not acute rejection correlated with long-term prognosis; patients with no CAN had a 10-year graft survival of 95.4% compared with 82.3% in patients with interstitial fibrosis, and 41.3% in patients with combination of both interstitial and tubular lesions[7].

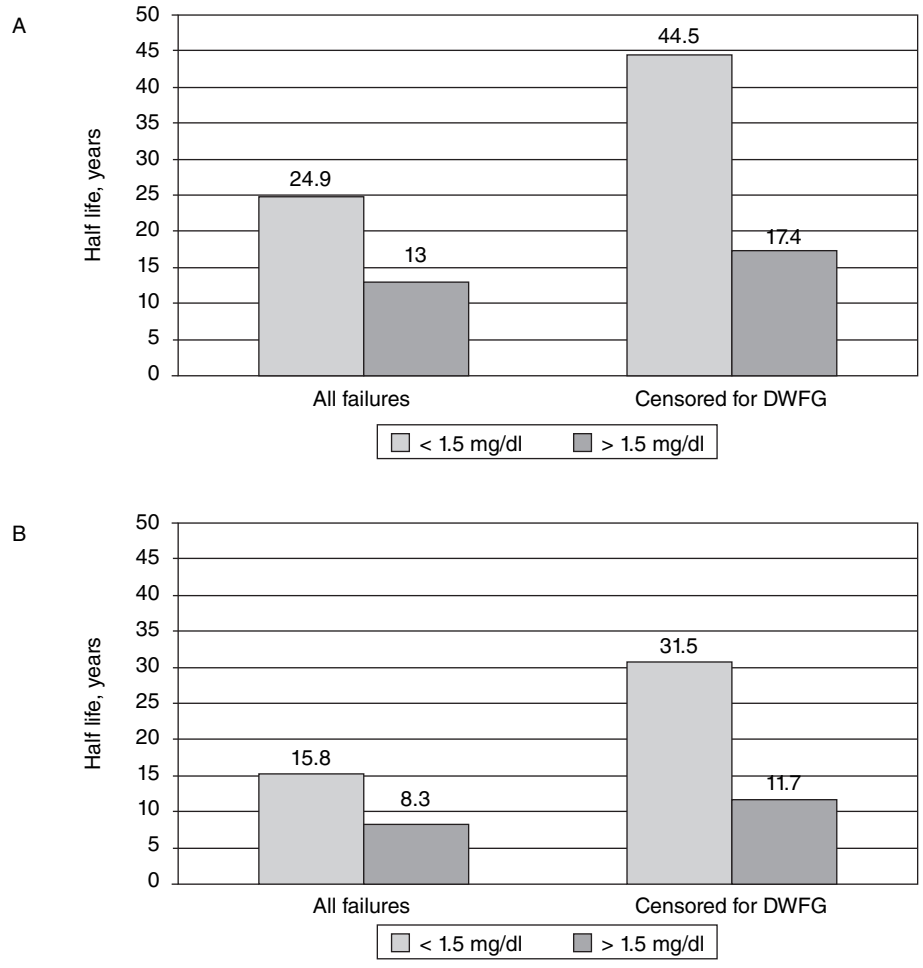

Figure 1. *Projected graft half-life in recipients of deceased and living donor kidney transplants based on serum creatinine values at 12 months posttransplant. Projected median kidney graft half-life in years, for living donor **(A)**, and deceased donor **(B)** kidney grafts based on the serum creatinine values (mg/dl) at one year posttransplant without and with censoring for death with functioning graft (DWFG). Modified and reproduced with permission from Siddiqi, et al.*[12]

Similarly, early tubulointerstitial damage at three months profoundly influenced graft survival beyond 10 years[8], and changes in IF/TA over time from the baseline biopsies at three months could also predict the graft survival[9].

Freeze, et al. described that IF/TA are more prominent features of chronic graft damage than vascular changes. When the biopsies were scored according to Banff 97 criteria, 48% of biopsies had CAN grade II, and 7.5% had CAN grade III. Arterial wall thickening was present in 66% of the late biopsies. The Banff CAN score and serum creatinine levels were independent predictors of future graft survival. Intriguingly, the presence or absence of arterial wall thickening had no prognostic impact[10].

In addition to histologic features, surrogate markers of graft function during the first six months after transplantation also predicts graft as well as patient survival. In the United Network for Organ Sharing (UNOS) data set of recipients of kidney transplants with a functioning graft at one year (n = 85,135), nearly half of the recipients (n = 41,299; 48.5%) had serum creatinine

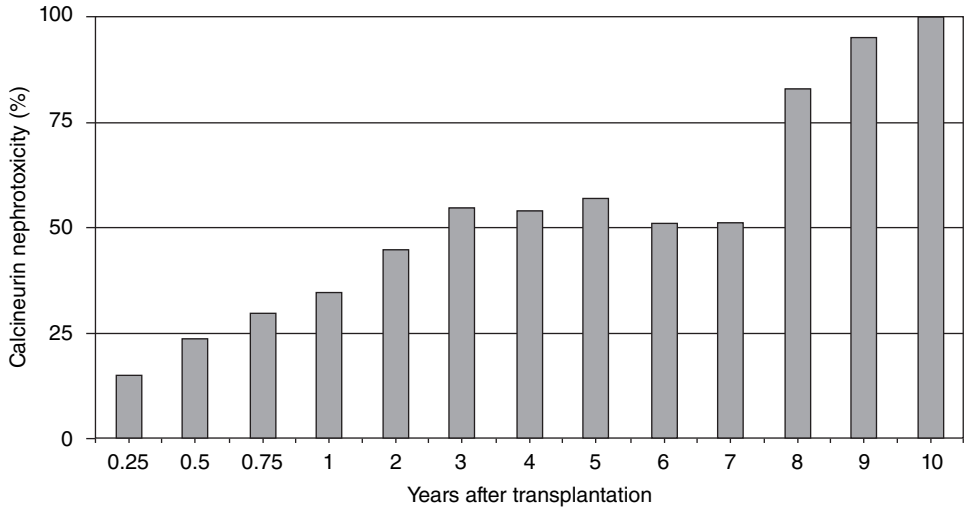

Figure 2. *Prevalence of calcineurin inhibitor toxicity in recipients of kidney and kidney-pancreas transplants. Point prevalence of histologically defined calcineurin-inhibitor nephrotoxicity during the follow-up period on yearly biopsies after first year of transplantation. Modified and reproduced with permission from Nankivell, et al.*[6]

> 1.5 mg/dl. Among these recipients, the graft half-life was reduced by more than 50% compared to those with serum creatinine ≤ 1.5 mg/dl at one year posttransplantation. This accelerated graft failure was similar in the recipients of either living or deceased donor kidneys (Fig. 1). Furthermor these differences in graft survival persisted even after censoring for death with functioning graft[11,12] (Fig. 2).

Therefore, the cumulative evidence would suggest that the presence of IF/TA of any degree during the first year posttransplant, even in the absence of detectable changes in the graft function as measured by either serum creatinine or estimated/measured glomerular filtration rate (GFR), are independently associated with immediate and long-term graft survival[13-15].

Novel insights into the molecular mechanisms of interstitial fibrosis and tubular atrophy, not otherwise specified

Tubulointerstitial fibrosis is an important hallmark of future deterioration of renal function in any type of kidney disease[16]. Epithelial-to-mesenchymal transition (EMT) is the result of injury to epithelial cells, which is responsible for the initiation and progression of interstitial fibrosis in different types of kidney disease. An interesting and novel study by Strutz, et al.[17] showed that as a result of injury to tubular epithelial cells, these cells have the potential to express fibroblast markers, which is the harbinger of EMT. The majority of renal tubules in adult kidney, other than the collecting duct, are developmentally derived from the metanephric mesenchyme through mesenchymal to epithelial trans-differentiation[18]. The healthy kidney either lacks or has a sparse population of fibroblasts compared to other organs, with a change in this pattern during different pathologic conditions.

The active process of EMT in the kidney correlates with the expression of a novel cytokine, S100A4. It is the human homolog of mouse fibroblast-specific protein-1$^+$, a mesenchymal marker[19]. Increased expression of fibroblast-specific protein-1$^+$ is associated with changes in the epithelial phenotype to mesenchymal phenotype. Fibroblast-specific protein-1$^+$ expression is induced early by transforming growth factor-β1 (TGF-β1) and epithelial growth factor. These two cytokines are considered as

potent stimuli for inducing EMT in the allograft[20]. Following tubular injury, fibroblast-specific protein-1$^+$ epithelial cells cross the damaged tubular basement membrane and accumulate in the interstitium of the kidney[21]. While in the interstitium, these damaged epithelial cells lose their epithelial markers and, under the influence of different cytokines, they change their phenotype to fibroblasts[21]. During the process of renal fibrogenesis, one-third of new fibroblasts are derived from local EMT. Twenty percent are derived from the bone marrow stem cells, and the remaining numbers develop from the local proliferation of EMT-derived fibroblasts[22]. These findings reinforce the notion that fibrogenesis is a local epithelial event. Among the network of cytokines, profibrotic TGF-β1 is the main driving force to maintain the phenomenon of EMT. The TGF-β1 initiates as well as propagates the pathway of EMT process in allograft fibrosis[23].

Long-term calcineurin inhibitor (CNI), either cyclosporine (CsA) or tacrolimus, use is considered to be tubulotoxic through renal vasoconstriction effects and the ability of the CNI to induce EMT. Experimental evidence has substantiated the longstanding notion that CNI use is associated with IF/TA. *In vitro* culture studies of human renal tubular epithelial cells in the presence of CsA have consistently demonstrated that CsA has the potential of inducing EMT[24,25].

Recently, it was demonstrated that biopsies from kidney transplants with allograft nephropathy showed a significant increase in S100A4, a marker of EMT, along with an increase in CD8 lymphocytes. Comparison of implantation biopsies with the biopsies obtained after the onset of allograft nephropathy with IF/TA demonstrated increased expression of S100A4 and other markers of EMT. EMT markers in biopsies with IF/TA was markedly increased compared to protocol biopsies in patients with stable graft function or minor degrees of interstitial fibrosis[20].

One of the interesting features of EMT is its reversibility. The reversibility is partly determined by the surviving cells that are able to repopulate the injured tubules with new functional epithelia. The plasticity of tubular epithelial cells is considered to be a unique phenomenon that is regulated by several growth factors. Major regulators of renal epithelial cell plasticity are two multifunctional growth factors, bone morphogenetic protein-7 (BMP-7) and TGF-β1. While TGF-β1 promotes EMT, BMP-7 reverses EMT by directly counteracting TGF-β1. The antagonistic actions of these two cytokines are important regulators of the repair process in injured kidneys. Due to plasticity of epithelial cell, it could be modulated to reverse the process of IF/TA[26,27].

Preclinical studies of novel therapeutic strategies such as hepatocyte growth factor[26] or BMP-7[27] for blocking TGF-β1 and other signaling involved in the regulation of EMT, could lead to clinical applications to slow or arrest nephron loss due to IF/TA NOS.

As we wait for these preclinical experimental studies to reach the clinical arena, strategies to slow or arrest the progression of IF/TA and block the EMT pathway could include careful minimization of CNI, or replacement of CNI with other immunosuppressants such as mammalian target of rapamycin (mTOR) inhibitors and potent anti-metabolites such as mycophenolate mofetil (MMF). The following two studies demonstrate that at present, the use of sirolimus can block the EMT pathways in recipients of kidney transplants.

Stallone, et al. evaluated the histologic and clinical effects of sirolimus on biopsy proven CAN. They used confocal microscopy to estimate the magnitude of alpha-smooth muscle actin protein expression as a marker of fibroblast activation in the biopsies of kidney transplant recipients with CAN at baseline and again at 24 months. These patients were randomized to 40% CNI reduction plus MMF (group I; 50 patients) or immediate CNI withdrawal using sirolimus with MMF (group II; 34 patients). At the end of 24 months, graft survival was significantly better in the group II patients. There was a significant decrease in the level of alpha-smooth muscle actin expression in the allograft biopsies of group II patients. On the contrary, CAN grading worsened significantly along with an increase in alpha-smooth muscle actin expression at the interstitial and vascular level in the biopsies of group 1 patients, who were maintained on minimized doses of CNI. Hence, this study, albeit small, indicated that the progression in IF/TA can be modified with the use of sirolimus therapy and discontinuation of CNI[28] (Figs. 3 and 4).

Pontrelli, et.al.[29] performed morphometric analysis of kidney biopsies in patients with CAN

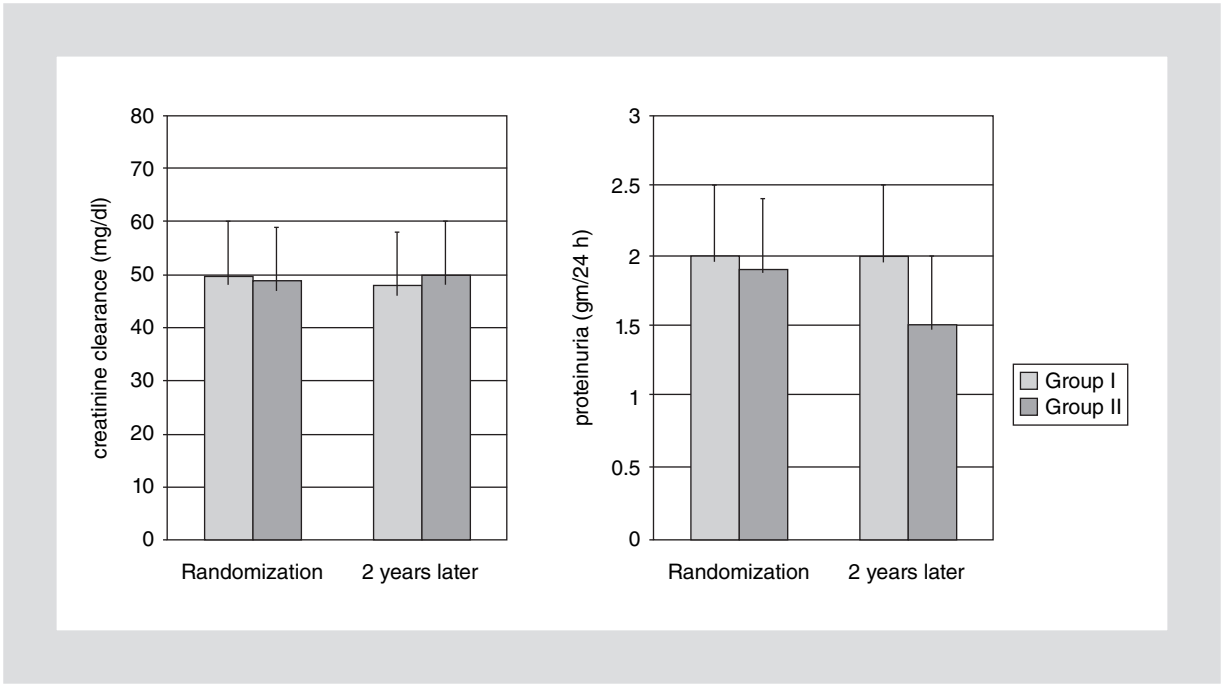

Figure 3. *Changes in creatinine clearance (mg/ml/24 hours) and 24-hour proteinuria in patients with chronic allograft nephropathy while maintained on calcineurin inhibitor therapy and after conversion to sirolimus-based therapy. (A) Creatinine clearance in ml/min (CrCl by Nankivell formula): Group I: (50.8±22.9 vs. 47.8±17.6) vs. Group II: (50.1±19.3 vs. 53.1±21.5). (B) Proteinuria (g/24 hours) at baseline and at two years: Group I (0.75±0.43 vs. 0.92±0.52) vs. Group II (0.83±0.19 vs. 1.2±0.69). Modified and reproduced with permission from Stallone, et al.[28]*

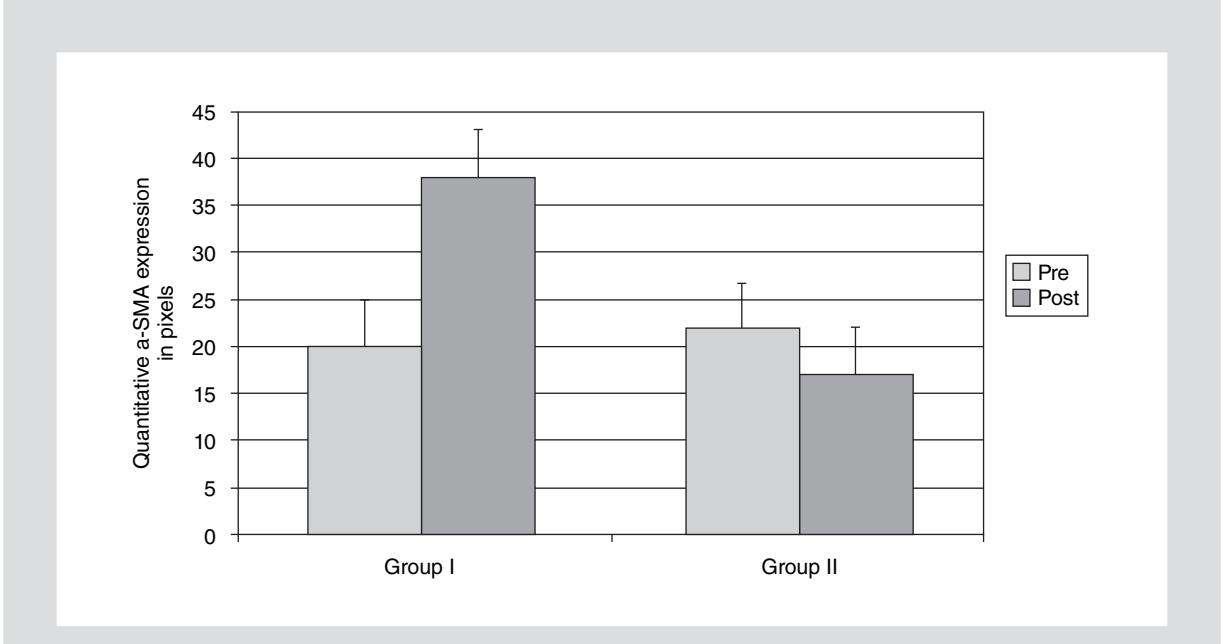

Figure 4. *Markers of epithelial-mesenchymal transition in graft biopsies while on calcineurin inhibitor (CNI) therapy and after conversion to sirolimus-based therapy. Quantification of alpha-smooth muscle actin (α-SMA) expression in the interstitial areas in the allograft biopsies in Group 1 (maintained on CNI-based therapy) and Group II (converted from CNI- to sirolimus-based therapy) at the time of enrollment and after two years of follow-up. Alpha-SMA expression is an indirect marker of epithelial-mesenchymal transition. Modified and reproduced with permission from Stallone, et al.[28]*

before and after conversion to sirolimus therapy. Six patients remained on CNI therapy, and in other 12 patients CNI was converted to sirolimus therapy. Morphometric analysis and gene expression of plasminogen activator inhibitor-1 was performed at baseline and again at the end of 24 months in both groups of patients. The conversion from CNI to sirolimus was associated with a significant regression of glomerulosclerosis and a significant reduction in the rate of progression in fibrogenesis. The degree of interstitial fibrosis increased only 26% from baseline in the conversion group, compared to an increase of 112% in those patients who remained on CNI therapy. Sirolimus use was associated with a significant decrease in glomerular and tubulointerstitial plasminogen activator inhibitor-1 expression in the kidney biopsy tissues in the conversion group. In addition, *in vitro* study showed that plasminogen activator inhibitor-1 gene expression in the cultured renal proximal tubular cells decreased on exposure to sirolimus. These observations indicate that sirolimus has the potential of decreasing plasminogen activator inhibitor-1 expression, which in turn prevents extracellular matrix deposition that can prevent progression of interstitial fibrosis.

Another therapeutic intervention that has been demonstrated to reduce fibrogenesis in the kidney is the use of MMF-based therapy. Besides the clinical studies that demonstrate that MMF can prevent acute rejection in the recipients of kidney allografts, MMF also has the potential of preventing progression of interstitial fibrosis and proteinuria in a model of chronic scarring[30-32].

Strategies to prevent the progression of graft dysfunction due to interstitial fibrosis and tubular atrophy (CAN)

The use of CNI is considered critical during the first year of transplantation, when the primary clinical goal is to prevent acute cellular rejection and graft failure. During the subsequent years, however, the major goal of maintenance immunosuppression therapy should be to prevent the insidious onset of progressive allograft dysfunction. During the past several years it became apparent that long-term exposure of the allograft to CNI can result in cumulative damage to the interstitium and tubules. Biopsy studies of the natural history of CAN demonstrate that by five years posttransplantation, 93.5% of patients have evidence of CsA nephrotoxicity and nearly 70% have evidence of CAN Banff grade II or III[6,33].

A meta-analysis has shown that withdrawal of CsA from conventional triple therapy of CsA, azathioprine, and prednisone is associated with an increased risk of acute rejection without affecting the rate of graft failure[34]. Treatment strategies that can replace the long-term use of CNI must prevent the development of acute rejection, including subclinical rejection, and yet at the same time prevent the progression of graft dysfunction due to IF/TA NOS. These strategies could include either replacement of CNI with MMF in patients who are not on such therapy. Or replacement of CNI with Mamalian Target of rapamycin inhibitors (mTOR-I).

Replacement of calcineurin inhibitors or azathioprine with mycophenolate mofetil in patients on triple therapy with cyclosporine, azathioprine and/or steroids

Dudley, et al. randomized 144 patients with negative slopes of 1/serum creatinine for at least three months while on the combination of CsA and azathioprine. These patients were randomized to either weaning of CsA over six weeks and replacement with MMF 2-3 g/day (n = 74), or continuation of the baseline CsA and azathioprine dose (n = 70). Both groups were similar in their demographics, and baseline creatinine clearance was < 38 ml/min. At the end of one year of follow-up, the CNI withdrawal group had positive slopes of 1/serum creatinine in 49% of patients compared to 26% of those who remained on CsA-based therapy. Allograft function assessed by serum creatinine or calculated creatinine clearance improved significantly in the CsA withdrawal group, whereas progressive deterioration of allograft function occurred in the group who continued on CsA. There were no acute rejection episodes. However, six patients developed graft loss, and four of these graft losses developed in the CsA continuation group[35].

Weir, et al. studied 118 kidney transplant recipients with CAN and deteriorating graft func-

tion; MMF therapy was initiated to either eliminate CNI (n = 18) or a 50% reduction in CNI dose (n = 100). During a mean follow-up period of less than two years, more than 50% of patients in the CNI minimization group showed a positive slope (improvement) graft function[36].

Ojo, et al. analyzed the renal transplant scientific registry data with 66,000 patient observations and showed statistically significant improvement in graft survival in those patients whose immunosuppressive regimens contained MMF compared to those who did not receive MMF. In addition, the use of MMF resulted in 27% reduction in the relative risk of allograft failure[37].

Data analysis of 49,666 primary renal allograft recipients reported between October 31, 1988 and June 30, 1998 from the UNOS database demonstrated that continuous use of MMF compared to azathioprine was associated with improved allograft function after one year of continuous therapy, and this protective effect persisted after two years of treatment[38]. These prospective studies and registry data analysis support the concept that the use of MMF is associated with preservation of kidney allograft function.

Withdrawal of calcineurin inhibitors and their replacement with mammalian target of rapamycin inhibitors

Sirolimus and everolimus are mTOR inhibitors. Sirolimus is a microcyclic lactone antibiotic produced from *Streptomyces hygroscopicus*. Everolimus is a derivative of sirolimus. Both bind to the intracellular immunophilin (FK506-binding protein 12), but without inhibiting calcineurin. The mTOR inhibitors inhibit the progression from G0 to G1 cell phase, and interleukins IL-2 and IL-4 dependent proliferation of T as well as B lymphocytes by suppressing the ribosomal protein synthesis. They also block the maturation of the G1-S phase of the cell cycle[39] and inhibit growth factors such as fibroblast growth factor, platelet derived factor, vascular endothelial growth factor and, more importantly, TGF-β1. These pleiotropic effects of mTOR inhibitors have the potential of preventing the proliferation of both hematopoietic and non-hematopoietic cells. These antiproliferative activities also mediate the potential side effects that can be encountered in patients using sirolimus or everolimus[40]. Furthermore, sirolimus inhibits metastatic tumor growth and tumor angiogenesis in *in vivo* mouse models, and therapeutic doses also inhibit the growth of established tumors by decreasing the production of vascular endothelial growth factor. These effects also limit endothelial cell proliferation. The use of sirolimus compared to CNI may reduce the chance of recurrent as well as *de novo* cancers in high cancer-risk transplant patients[41].

Both preclinical and phase II clinical studies demonstrated that addition of sirolimus to the regimen of CsA and azathioprine, with or without prior induction therapy, results in a significant reduction in the rate of acute rejection. However, contrary to these studies, a paradox was noted that combined use of sirolimus and CsA resulted in graft dysfunction. It became apparent that the use of sirolimus with CsA was associated with increased nephrotoxicity. Subsequent studies of CsA withdrawal in such patients resulted in improvement of renal function, and decreased the burden of other CNI-associated side effects.

Data analysis of the Rapamune Maintenance Regimen Study Group[42-45] established that withdrawal of CsA in *de novo* renal transplant recipients who had been on the combination of sirolimus and CsA therapy, resulted in significant improvement in graft function at one year and at four years, along with an improvement in CAN histology scores, albeit at the cost of an increased incidence of acute rejection. Despite an increased rate of acute rejection, graft function at one to four years was significantly superior in the CsA withdrawal group versus the group that remained on a combination of CsA and sirolimus therapy. These observations have been confirmed by others as well, such as the Sirolimus Renal Function Study Group[46] and the UK sirolimus study[47].

The results of these studies showing that CNI withdrawal in kidney transplant recipients on CsA- and sirolimus-based therapies was associated with increased graft and patient survival despite an increase in the rate of acute rejection, led to a wave of single-center studies to explore the impact of CNI elimination in patients with rising creatinine (creatinine creep) in the absence of other reversible factors.

Cohort studies of conversion from calcineurin inhibitors to sirolimus in patients with interstitial fibrosis and tubular atrophy, not otherwise specified (CAN)

Early experience with *de novo* substitution of sirolimus for CNI in kidney recipients with worsening graft function was largely based on small cohort studies from different centers. Each cohort was less than 15 patients. The results were inconsistent on graft function and graft survival due to the heterogeneity of the patient population in these different cohorts[48-50]. However, these results did not deter other investigators from exploring the benefit of converting from CNI- to sirolimus-based therapy in patients with different degrees of graft dysfunction. Invariably, all of these studies were performed in kidney recipients who were on combination of CNI, MMF and low-dose corticosteroid therapy.

Citterlo, et al. used sirolimus with immediate withdrawal of CsA in 19 patients with CAN and worsening graft function. Renal function improved in 36%, remained stable in 21%, and continued to deteriorate in another 42% of patients. Patients with advanced graft dysfunction with mean serum creatinine of more than 2.9±0.9 mg/dl continued to have progressive deterioration of graft function despite withdrawal of CNI therapy[51].

Diekmann, et al.[52] used a strategy of slow withdrawal (1-2 months) of CNI with sirolimus substitution in 59 renal transplant patients with CAN. During the first year of follow-up, improvement in graft function was noted in 54% of the cohort, and graft function continued to deteriorate in another 46%. After controlling for baseline creatinine, the histologic grade of CAN, prior episodes of acute rejection, and baseline proteinuria were the major determinants for progression in CAN despite discontinuation of CNI. As in other studies, all patients were on MMF and steroid therapy.

In continued follow-up for five years following sirolimus conversion in this cohort, the patient and graft survivals were 88 and 38%, respectively. These patients had stable creatinine clearance (33.7±14 ml/min), and with progression in proteinuria (826 ± 860 mg/day) after 3.2 to 6.8 years of follow-up. Baseline proteinuria < 800 mg/day was associated with better graft survival, with a positive predictive value of 90% to predict positive response with sirolimus-based therapy[53].

In other small sample studies of late conversion, Kruger, et al. (n = 12)[54], Renders, et al. (n = 13)[55], and Wu, et al. (n = 16)[56] reported somewhat similar results as described by Diekmann, et al.[53].

Another late conversion study included 60 renal transplant recipients with median serum creatinine of 1.9 mg/dl and median GFR 51 ml/min. During a follow-up of 12 months, less than 4% developed acute rejection. The patient and graft survival rates were 96.7 and 95%, respectively. These patients had several different types of adverse events, including hypercholesterolemia, diarrhea, peripheral edema, rash, and anemia[57].

In a large single center study, recipients of living and deceased kidney transplants (n = 159; two thirds of these patients were high-risk African American) with progressive deterioration in graft function due to biopsy proven CAN were converted from tacrolimus- to sirolimus -based therapy in combination with MMF. An intent-to-treat analysis was performed after excluding the patients (n = 23) in whom sirolimus was discontinued due to different side effects before completing the first three months of therapy while using the loading dose of sirolimus. We demonstrated that 74% of patients had an improvement of graft function. Those who continued to deteriorate following conversion therapy had a mean baseline serum creatinine of 3.8 mg/dl and estimated GFR of < 19 ml/min/1.7 m^2. In addition, time to conversion therapy was more than 34 months is those who continued to deteriorate, compared to 17 months in those with marked improvement in allograft function. The changes in biopsy findings were interesting. A comparison of the allograft biopsies before and at 12 months after conversion demonstrated significant improvements in tubular degeneration, tubular atrophy, and arteriolar hyalinosis, with some progression in interstitial fibrosis[58].

These cohort studies[51,53,55,57-59] demonstrated that: (i) late conversion is not an effective strategy for the prevention of further deterioration in graft function, (ii) advanced allograft dysfunction at the time of conversion therapy is a predictor of continuing deterioration in graft function, and (iii) proteinuria > 800 mg/24 hours is a bad prognostic factor and these patients do not benefit from such conversion therapy.

Randomized controlled studies of conversion from calcieurin inhibitors to sirolimus in patients with interstitial fibrosis and tubular atrophy, not otherwise specified (CAN)

The first randomized study that explored withdrawal of CsA in stable renal transplant patients was reported by Abramowicz, et al.[60]. The recipients of kidney transplants (n = 187) with stable graft function were randomized to continue a baseline triple-drug immunosuppressive regimen of MMF, CsA (Neoral®), and steroids (n = 85), or CsA was converted to sirolimus (n = 85). During nine months of follow-up, biopsy proven acute rejection developed in 11% on sirolimus therapy and < 3% who remained on CsA-based therapy. Conversion was associated with a significant improvement in graft function only after excluding patients who developed acute rejection.

In 2005, Watson, et al.[61] reported the first randomized study (n = 40) in patients with biopsy proven CAN. Discontinuation of CsA with sirolimus substitution resulted in a mean improvement in GFR of 8.5 ml/min from the baseline. This increase was apparent within the first three months after conversion therapy. The patients who remained on CsA continued to lose graft function with a mean loss of GFR of 4.3 ml/min from baseline. Baseline GFR was an important predictor of improvement in graft function following discontinuation of CsA therapy. Early conversion was important to preserve the graft function. More importantly, however, no significant change in proteinuria or rate of acute rejection was noted following conversion to sirolimus therapy in this small group of patients.

Convert Study: This multicenter study enrolled patients with established diagnosis of CAN within 6-60 months posttransplant (n = 830). These patients were randomized either to remain on the CNI-based therapy (n = 275), or CNI was withdrawn and replaced with sirolimus-based therapy (n = 555). At the end of two years, data analysis was stratified according to GFR* groups. All patients remained on center-specific choice of MMF or azathioprine and steroid doses.

For patients with baseline estimated GFR > 40 ml/min at the time of enrollment, on-therapy analysis revealed an overall improvement in GFR in patients randomized to conversion to sirolimus versus maintenance CsA therapy (62.6 ml/min, n = 370, versus 59.9 ml/min, n = 201; p = 0.009) at the end of two years of follow-up. However, nearly 20% of patients randomized to sirolimus had to discontinue the assigned therapy because of adverse events; the most common adverse events included hyperlipidemia, diarrhea, anemia, and edema. The incidence of biopsy proven acute rejection was nearly similar in both groups[62].

At the end of the first two years of the enrollment process, it was apparent that those with baseline GFR < 40 ml/min developed worsening of GFR after conversion and had more adverse events. Subsequent enrollment of patients with GFR < 40 ml/min was terminated at the recommendation of the Data Safety Monitoring Board of the study[63].

An important finding of the CONVERT trial was that improvement in GFR after conversion to sirolimus was inversely related to the degree of urine protein excretion at baseline. The tendency for worsening proteinuria was related to baseline histopathology. A higher Banff total sum score for CAN with a higher percentage of sclerotic glomeruli at baseline was significantly correlated with worsening proteinuria after conversion to sirolimus therapy[63].

Spare the Nephron (STN) study: This was an open-label, prospective, multicenter study that randomized kidney transplant recipients within 30-180 days posttransplant (n = 305) either to continue the current center-specific CNI therapy (CsA or tacrolimus), or CNI was converted to sirolimus-based therapy. All patients received MMF and center-specific doses of steroids. The data analysis of the first 249 patients who completed 12 months of follow-up (CNI/MMF, n = 126 and SRL/MMF, n = 123) was presented during the 2007 American Transplant Congress meeting. Conversion to sirolimus-based therapy was associated with a gain of nearly 6 ml/min iothalamate-based GFR at 12 months (primary endpoint of the study). Biopsy proven acute rejection, graft loss, and patient death were similar in both groups. However, during the first year after randomization nearly 19% of patients in the sirolimus/MMF group had to discontinue the assigned therapy due to adverse events[64].

*GFR was calculated by Nankivell formula.

A recent meta-analysis by Mulay, et al. of conversion studies (CNI to sirolimus-based therapy) with MMF and steroids included five randomized studies (n = 1,040) and 25 cohort studies with different inclusion/exclusion criteria (n = 977) with different degrees of allograft dysfunction and different time intervals after transplantation. Withdrawal of CNI was associated with > 6 ml/min improvement in creatinine clearance[65].

These randomized controlled studies and smaller cohort studies illustrate the risk factors associated with lack of benefit of conversion therapy: (i) advanced graft dysfunction defined either by baseline serum creatinine > 3.0 mg/dl or estimated GFR < 30 ml/min/1.73 m^2, and (ii) proteinuria > 800 mg/24 hours are associated with poor graft outcome following sirolimus-based therapy[51,57,59,66].

Everolimus use in interstitial fibrosis and tubular atrophy, not otherwise specified, (CAN)

To date, the role of everolimus in patients with established diagnosis of CAN has been reported in small cohort studies. A single-center study of 20 kidney transplant patients reported an acute rejection rate of 15% following conversion from CsA to everolimus at seven weeks after transplantation. During a short follow-up period of six months, a significant increase in calculated GFR was described[67].

In a small Chilean study, everolimus was substituted for CsA in patients with Banff grade I and II CAN. Forty-two percent of the cohort had improvement in graft function and none of these patients developed proteinuria[68].

Controversies regarding the conversion from calcineurin inhibitor- to sirolimus-based therapy in patients with interstitial fibrosis and tubular atrophy, not otherwise specified (CAN)

Time to conversion therapy

The optimal timing of conversion in recipients of kidney transplants is unknown, but likely should occur in low-risk patients before significant graft damage occurs. Since it is difficult to routinely perform measured GFR in transplant recipients, often conversion occurs late, which may limit the benefits. Hopefully, newer creatinine or non-creatinine based formulae that will allow precise evaluation of graft function and clinical availability of transcriptome analysis will help to identify at-risk patients for graft damage at an early stage. However, protocol biopsies at different time intervals in the posttransplant period will help to identify recipients with subclinical rejection and the onset of IF/TA NOS at an early stage, before the onset of graft dysfunction. A change in estimated GFR of > 10% is also considered to be a better predictor of graft loss[69]. While most centers perform biopsies based on the changes in the graft function, this deprives us from detecting the early changes as seen in protocol biopsies at one month, three months, and one year post-transplant[70]. Therefore, it is important to consider intervention before changes in surrogate markers of graft function (serum creatinine or estimated GFR) become apparent. This is also conceptually supported by the fact that EMT can be reversed before the onset of advanced fibrogenesis[71].

De novo onset or worsening of existing proteinuria

The development and progression of proteinuria in patients with different degrees of allograft dysfunction is multifactorial and rather complex due to the existence of different degrees of glomerular, endothelial, epithelial, and tubular damage in patients with IF/TA NOS[72]. Several studies have evaluated the clinical implications of proteinuria on the success of CNI conversion to sirolimus-based therapy. Ruiz, et al.[73,74] evaluated the impact of CNI to sirolimus conversion on progression of proteinuria. They assessed proteinuria at baseline and again at six months in 149 patients. These patients were categorized in three groups, based on mean proteinuria at baseline (before conversion): Group 1: ≤ 300 mg/day, n = 64; Group II: > 300-3,500 mg/day, n = 79; Group III: > 3.5 g/day, n = 6 (Fig. 5). Three important findings were established by this study: (i) patients with CAN have varying degrees of proteinuria at baseline, and increasing degree of proteinuria at baseline was associated with increased levels of creatinine; (ii) during a follow-up period of six months after conversion, proteinuria increased in all

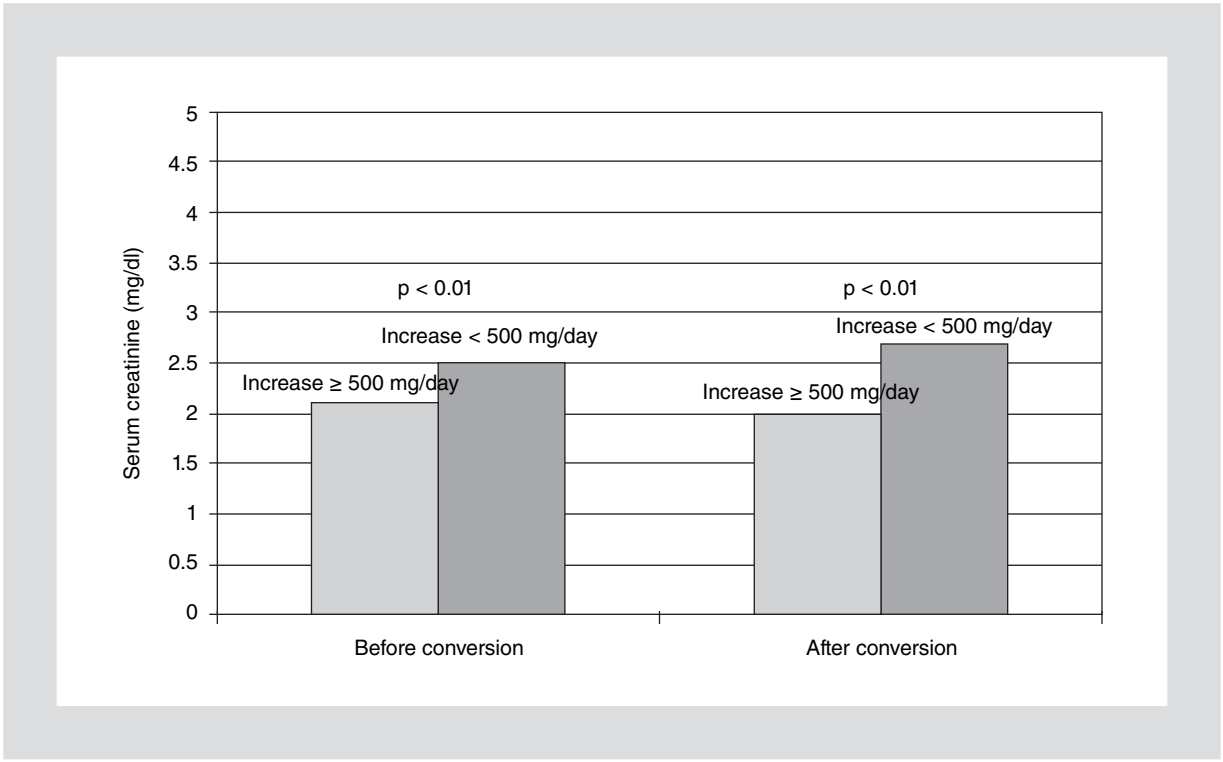

Figure 5. *Serum creatinine values based on the level of proteinuria before and after conversion to sirolimus-based therapy. Mean serum creatinine values of patients with an increase of proteinuria 500 mg/day after conversion are shown with white bars, patients with an increase of > 500 mg/day with black bars. Modified and reproduced with permission from Ruiz, et al.*[74]

three groups, but more significantly in group I and II, and, (iii) graft function as assessed by serum creatinine improved in group I patients, remained unchanged in group II, and worsened in group III patients. Hence, baseline proteinuria should be considered before conversion. Diekman, et al.[52,59,75] suggested that patients with proteinuria > 800 mg/24 hours do not benefit from sirolimus-based therapy. Recent evidence would suggest that worsening of proteinuria after initiation of sirolimus-based therapy could be due to reduced tubular reabsorption of protein[76]. Given these challenges, more work needs to be done to understand the pathophysiology of proteinuria in patients with IF/TA NOS as well as with sirolimus-based therapy.

Minimizing the side effects associated with combination of sirolimus therapy with either mycophenolate mofetil or azathioprine

Most of the cohort and randomized studies have demonstrated that nearly 15-20% of patients may need to discontinue sirolimus therapy due to different types of side effects. These side effects can develop due to bone marrow suppression leading to neutropenia, thrombocytopenia, and anemia, and suppression of cell proliferation in the colon leading to diarrhea. Both hematologic and gastrointestinal side effects are amenable to modification of the MMF dose, or tapering the sirolimus dose to reduce the sirolimus trough levels too[58]. We usually achieve a target sirolimus trough level of 8-10 ng/ml during the first year, 6-8 ng/ml during the second year, and 4-6 ng/ml during the subsequent years after transplantation.

Dyslipidemia manifests itself mostly in the form of increased LDL-cholesterol and triglyceride levels. Most of these lipid abnormalities plateau at three to six months after conversion therapy. In our center, we start patients on statins on the day of sirolimus therapy and monitor serum lipid profile every three months during the first year and subsequently every six months afterwards. Increases in serum lipids may require increasing the dose of statins to the maximum recommended dose[58,77]. Other side effects

associated with sirolimus therapy include acne, mouth ulcers, lymphedema, or peripheral edema that can at times be asymmetric. Therefore, it is important that patients should be counseled regarding the nature and severity of these side effects.

On the contrary, sirolimus use has other long-term benefits such as antifibrotic effects[28,29] that can prevent the progression in interstitial and vascular fibrosis, and antineoplastic properties[78] that may benefit transplant recipients who are at risk for malignancy.

Conversion procedure

The safe procedure of stopping the CNI with initiation of sirolimus therapy is not well defined. In our center, we usually stop the CNI after achieving the therapeutic trough level of sirolimus. Since we started this technique of conversion and without using the loading dose of sirolimus, the rate of acute side effects of sirolimus as well as early acute rejection following conversion therapy were minimized[58].

Conclusion

Treatment of progressive graft dysfunction is a challenge for the future, but it is a daunting task. Due to the lack of robust randomized studies and the lack of generalizability of the currently existing randomized as well as cohort studies, it is difficult to develop a uniform line of action. However, the use of revised Banff criteria that will allow early identification of histologic changes and graft dysfunction may help to optimize the treatment strategies to prevent progressive graft dysfunction. Late CNI withdrawal has achieved variable results, possibly because withdrawal was attempted after the kidney damage was irreversible. Early CNI withdrawal, prior to significant graft damage, has generally improved the graft function, including biomarkers of ongoing fibrosis, and decreased CNI-associated tubular toxicity. Successful withdrawal of CNI appears to be more effective than CNI minimization. However, CNI withdrawal and conversion to sirolimus-based therapy in combination with MMF and with or without corticosteroids improves graft function in patients with different degrees of allograft dysfunction and effectively protects from acute cellular rejection. However, many questions remain, and the tolerability of therapy remains an important concern.

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