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Original Article

Sirolimus-based triple immunosupression with antithymocyte globulin induction in expanded criteria donor kidney transplantation

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SUMMARY:

Background: Target of rapamycin inhibitors have presented similar graft and patient outcomes with no evidence of drug-induced nephrotoxicity when compared with calcineurin inhibitors. The principal aim of this study is to demonstrate the efficacy of sirolimus-based triple immunosuppression with antithymocyte globulin induction in expanded donor kidney transplantation.

Methods: Twenty-seven primary expanded criteria donor kidney transplant recipients were recruited. The severity of kidney damage was qualified by zero-hour biopsies. Protocol biopsies were performed at 1 year to assess the chronic allograft damage. Death, graft function, proteinuria and adverse events were systematically analysed during the study period.

Results: The mean follow up was 20.2 months. Patient and graft survival was 100% with a mean glomerular filtration rate (GFR) of 53.1 ± 4.9 mL/min at last follow up. The cumulative incidence of acute rejection was 11% at the last follow up. At 1 year, mean creatinine, GFR and proteinuria were 1.84 mg/dL, 52.3 mL/min, 651.5 mg/day, respectively. Four patients required surgical intervention due to urinary complications and recovered successfully. Two patients developed acute graft dysfunction due to acute tubular necrosis which was presumably drug related. Ten patients developed relapsing urinary tract infections and three patients had pneumonia. No infectious death occurred throughout the study period. Baseline renal structure was preserved in 13 biopsies at 1 year post transplant. Five patients demonstrated progressive but mild tubular atrophy or interstitial fibrosis in their protocol biopsies. The mean chronic allograft damage index scores at baseline and at 1 year from biopsy were 2.57 ± 0.23 and 2.83 ± 0.23, respectively ($P = 0.046$).

Conclusions: Low-dose sirolimus-based triple immunosuppresion with antibody induction offered a safe clinical outcome in expanded criteria donor kidneys with the achievement of stable renal function and favourable recipient outcomes throughout the short term. However, mild progression of histological damage and increased risk of bacterial infection are a major concern. Additionally, the benefit (if any) of the low acute rejection rate on long-term graft outcome is still undetermined.

KEY WORDS: **expanded criteria donor, chronic allograft nephropathy, sirolimus.**

Calcineurin inhibitors (CNI), which were introduced during the early 1980s, achieved favourable short-term graft outcomes with noticeable reductions in acute rejection rates. Indeed, while it is true that the rate of long-term function has not changed dramatically with the use of CNI, it is also clear that CNI have significantly prolonged the half-life of grafted kidneys. In spite of the well-known drawbacks of CNI drugs, they are currently the gold standard of immunosuppression in kidney transplantation. Paradoxically, this early graft survival benefit with the modest improvement in longer-term graft survival is overshadowed by progressive and inferior long-term histopathological changes in renal paranchyme.[1,2] Nankivell *et al.* described the phases of injury during the evolution of chronic allograft nephropathy and emphasized the progressive nature of microvascular and ischaemic glomerular injury beyond the first year of the transplant in every patient maintained on a

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CNI regime. In addition, this study demonstrated significant histopathological changes in 96.8% of the protocol biopsies at 10 years.[3]

The introduction of target of rapamycin (TOR) inhibitors, with their unique mechanism of action, promised to overcome CNI-related nephrotoxicity. TOR inhibitors prevented smooth muscle cell proliferation both *in vitro* and *in vivo* rat aortic transplantation models, and data from human studies identified the potential of sirolimus (SRL) to control IL-2- and IL-15-driven proliferation of human T cells by arresting the cell cycle at the late G1 stage.[4] The purpose of this prospective non-randomized study was to investigate the impact of SRL-based triple immunosuppression on the composite efficacy failure rate (biopsy-proven acute rejection (BPAR), graft loss and death) in a group of recipients undergoing primary renal transplantation with expanded criteria donor kidneys (ECDK). The secondary objective was to demonstrate by protocol biopsies the efficacy of SRL in the prevention of alloantigen-dependent vascular changes leading to chronic allograft nephropathy. The principal rationale was the avoidance of CNI nephrotoxicity in those patients who had received kidneys with pre-existing damage.

MATERIALS AND METHODS

Twenty-seven (13 female, 14 male) renal recipients between 20 and 60 years of age undergoing primary cadaveric and ECDK transplantation were included in this study. Patients with severe hypercholesterolaemia (>350 mg/dL) or hypertriglyceridaemia (>500 mg/dL) at dialysis or with a history of solid organ malignancy within the last 4 years were excluded. Also, patients demonstrating current panel reactive antibody titre >10% were exempted from allocation.

Baseline biopsies (zero-hour biopsies) were collected and evaluated within 12 h, and patients were recruited to the study protocol as soon as the definitive reading by the local pathologist was attained. Sections were stained with haematoxylin and eosin, PAS, Masson's trichrome and methenamine silver. The severity of kidney damage on zero-hour biopsies was established by the protocol as described by Karpinski *et al.* in 1999.[5] The principle of this protocol is the quantitative evaluation of glomerular global sclerosis, tubular atrophy, interstitial fibrosis and arteriolar narrowing, expressed as a percentage. Final grades from 0 to 3, 4 to 6, and 7 to 12 indicate mild, moderate and severe renal tissue damage, respectively. Only kidneys with biopsy scores from 1 to 6, which indicate mild to moderate damage, were considered eligible.

Kidney biopsies were performed at 12 months per protocol in all patients. Furthermore, all suspected rejection episodes and graft dysfunctions were evaluated by a graft core biopsy following the exclusion of urinary tract obstruction by ultrasound, DTPA scan and MRI pyelography. The revised Banff 1997 system was used for grading of acute allograft rejection. A retrospective control population (including 12 patients with ECDK transplantation) who had at least one post-baseline kidney biopsy was constituted. All patients were assigned to cyclosporin-A (CyA)-based triple immunosuppression and similar antithymocyte globulin (ATG) induction protocol.

The objective was to determine whether the SRL group was superior to the CyA group with respect to mean acute rejection, complication and glomerular filtration rate (GFR) at the last follow up. Renal function was assessed by measuring serum creatinine (SCr) and GFR by using the Nankivell formula[6], whereas proteinuria was determined by quantitative analysis of 24 h urine specimen. The complete blood count, biochemistry, lipid profile and renal function parameters were collected at the first, third, sixth and twelfth month post transplant and during the last follow up for statistical analysis. The mean values obtained during the four terms of the first year of transplantation were compared with the current values using the Mann–Whitney *U*-test.

All patients received induction therapy using ATG (Fresenius, Bad Homburg, Germany) at day zero through the third postoperative day, with the intention of keeping CD3 levels (<50 cell) at least two consecutive days before the initiation of SRL. SRL levels were measured by microparticle enzyme immunoassay technology with the use of available kit (Abbott Laboratories, Abbott Park, IL, USA) in IMx analyser. The SRL dose was adjusted to keep the trough level between 5 and 10 ng/mL during the first 6 months and between 3 and 8 ng/mL afterwards.

All patients received mycophenolic acid (MPA, Myfortic tablet (Novartis, Basel, Switzerland)) and prednisolone (P) in addition to SRL, as per protocol. MPA dosage was adjusted according to the recipient's body weight and targeted at 1080, 900, 720 mg/day, for patients with more than 70 kg, between 55 and 70 kg, and less than 55 kg body weight respectively. Each patient received the same steroid protocol consisting of 0.3 mg/kg per day oral prednisone for the first month post transplant, tapered to 0.2 mg/kg per day for the subsequent 2 months and reduced to a maintenance dose of 0.1 mg/kg per day. The ferritin level was determined by chemiluminescent method with the commercially available kit (Immulite 2000, BioDPC, Los Angeles, CA, USA). Urine protein quantification was carried out by the pyrogallol method (Chromatest, Linear Chemicals, Barcelona, Spain) in an auto-analyser (Olympus AU5200, Tokyo, Japan). Low-density lipoprotein levels were determined by the Friedewald formula. Cytomegalovirus (CMV) prophylaxis was carried out by ganciclovir infusions during ATG induction and extended until CMV-DNA titres in whole blood specimens became undetectable in every patient. This was followed by maintenance oral acyclovir therapy (800 mg/day) together with fungal and *Pneumocystis prophylaxis* for a period of 6 months. Data are expressed as mean ± SEM. For non-parametric data, the Mann–Whitney *U*-test was used, and $P < 0.05$ was considered significant.

RESULTS

Twenty-seven primary expanded criteria donor kidney transplant recipients were recruited into the study. All patients received the allograft from a deceased donor.

Table 1 The demographic data of rapamycine and CNI groups

	Rapamycine group		Retrospective control group	
	n	mean ± SEM	*n*	mean ± SEM
Follow up (months)	27	20.2 ± 1.8	12	44.6 ± 5.3
Recipient age (years)	27	43.0 ± 1.5	12	38.8 ± 3.9
Donor age-cadaveric (years)	27	67.7 ± 1.3	12	63.0 ± 0.9
Weight (kg)	27	65.5 ± 2.8	12	71.1 ± 4.9
BMI (kg/m^2)	27	24.2 ± 0.8	12	22.6 ± 1.0
Cold ischaemia time (h)	27	16.8 ± 1.7	12	12.4 ± 1.9
MPN (mg/kg per day)†	27	0.09 ± 0.01	12	5.41 ± 0.7
MPA (mg/day)†	27	646.7 ± 53.9	12	466.3 ± 75.6
Rapamycin/CyA or FK dose (mg/day)†	27	1.85 ± 0.16	12	135.8 ± 21.2
Rapamycin/CyA or FK level (ng/mL)†	27	5.69 ± 0.41	12	247.5 ± 105.6
ATG dose (mg/day)	27	259.3 ± 14.7	12	217.1 ± 24.9
CD3 (mm^3)	26	50.9 ± 8.5	10	42.3 ± 4.7
Systolic BP (mmHg)†	27	128.5 ± 2.5	12	120.0 ± 3.5
Diastolic BP (mmHg)†	27	81.1 ± 1.5	12	78.3 ± 1.7

†At last follow up. ATG, antithymocyte globulin; BMI, body mass index; CNI, calcineurin inhibitor; CyA, cyclosporin-A; MPA, mycophenolic acid; MPN, methylprednisolone.

Cadaveric donors were favourably matched because two HLA-DR matches and a maximum of one mismatched antigen at the HLA-A or HLA-B locus were mandatory for allocation.

The demographic data of the study and the retrospective control groups are shown in Table 1. The donor histology score ranged between 2 and 5, with a mean of 2.57. The mean age of the recipients and donors were 43.0 ± 1.5 and 67.7 ± 1.3 years, respectively. The mean cold ischaemia time was 16.8 ± 1.7 h. None of the patients were lost to follow up. The cause of renal failure was unknown in five patients. Among the remainder, end-stage renal disease was related to glomerulonephritis in eight, interstitial nephritis in four, membranoproliferative glomerulonephritis in two, nephrosclerosis in two, diabetes mellitus in two, focal segmental glomerulosclerosis in two, Alport syndrome in one and polycystic kidney disease in one patient. The mean post-transplant follow up was 20.2 ± 1.8 months. The incidence of BPAR was 11% (three patients) at 1 year. Two patients developed Banff IB acute rejection on day 51 and day 120 and both recovered uneventfully with steroid pulse treatment. One patient had experienced an episode of Banff IIB acute rejection on day 15 and required antithymocyte antibody in association with alternate-day parenteral immunoglobulin therapy.

The protocol biopsy of these patients revealed complete clearance of inflammation with no residual or progressive tissue destruction (Figs 1–3). Acute rejection after 1 year of transplantation has not been observed in any of the patients in this series. SRL trough levels ranged between 2.8 and 13.0 ng/mL, with a mean of 5.69 ± 0.41 ng/mL. MPA trough levels were not monitored. Mean daily doses of SRL and MPA were 1.85 ± 0.16 and 646.7 ± 53.9 mg, respectively. The mean daily dose of prednisolone per kilogram body weight was 0.09 ± 0.01 mg. Increased susceptibility to infection was evident despite the administration of suboptimal doses of immunosuppressive drugs with this regimen. In fact, 10 patients developed relapsing infection of the urinary tract. The only predisposing factors identified were early distal ureteral obstruction in four patients and the administration of pulse steroid or ATG therapy in three patients during acute rejection episodes. Moreover, three patients had pneumonia, and they underwent diagnostic bronchoscopy with bronchoalveolar lavage. The offending organisms were *Escherichia coli* and *Klebsiella pneumonia* for the urinary tract infections and *Pseudomonas aeroginosa* for lower respiratory tract infections.

There were no systemic fungal infections or CMV disease in this study. Four patients (33%) in the control group developed serious infections that required hospitalization. Two had bacterial pneumonia; one had systemic herpes infection with neural involvement; and the last patient had CMV infection. All were treated properly and recovered without replase. Four patients (15%) required surgical intervention due to major complications of the urinary tract occurring at post-transplant days 16, 18, 96 and 98. The former two patients experienced fibrosis and necrosis of the distal portion of the ureter and recovered after the revision of vesicoureteral anostomosis. The latter two patients had segmental ureteral fibrosis below the ureteropelvic junction, which was surgically corrected with an anostomosis between the bladder and the renal pelvis.

Results of complete blood count, differential counts and blood chemistry at the last follow up are shown in Table 2. Mean values for leucocyte, neutrophil, platelet and erythrocytes were within normal ranges, and there were no patients with clinically notable high or low values during the study period. Comparison of the mean values of biochemistry and haematologic parameters at 1 year and at the last follow up was not statistically significant ($P > 0.05$). Eighteen recipients (66%) required antihypertensive drugs, and 15 of them had pre-existing hypertension at the time of transplantation. Fifteen (55%) patients were treated with statins because of the new onset of lipid elevations after

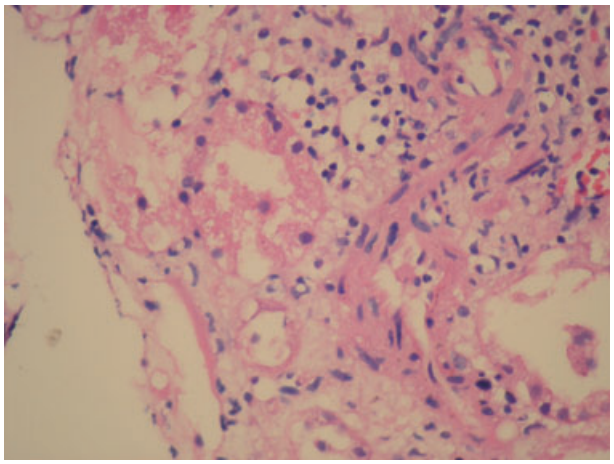

Fig. 1 Endarteritis in grade IIB acute rejection (haematoxylin and eosin ×400).

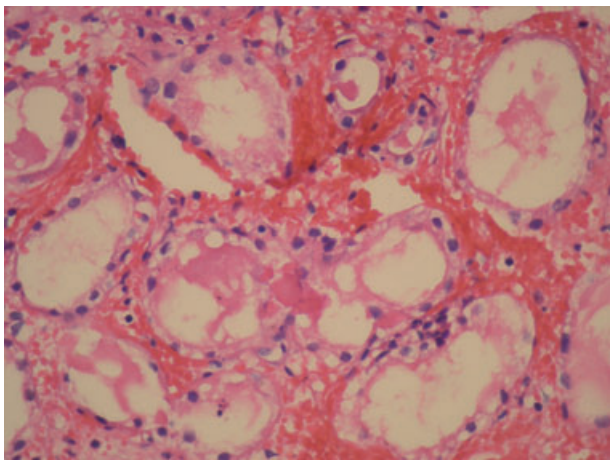

Fig. 2 Interstitial haemorrhage in grade IIB acute rejection (haematoxylin and eosin, ×400).

transplantation. The incidence of delayed graft function was 7% (two cases), and the average duration of post-transplant maintenance dialysis was 21 and 40 days in these patients. Patient and graft survival was 100%, with a mean GFR of 53.1 ± 4.9 mL/min at last follow up.

Early graft dysfunction, which indicates a creatinine value of more than 2 mg/dL by 6 months post transplant, was observed in six patients (22%). Current SCr values and the corresponding quantitative protein excretions are: 2.4, 2.8, 3.0, 3.4, 3.26, 3.2 mg/dL and 418, 500, 1159, 750, 1062, 2464 mg/24 h, respectively, in this group. Among the patients with early graft dysfunction, two required weekly erythropoetin for the treatment of post-transplant anaemia for a period of 6 months, and the corresponding GFR were 24.0 and 31.2 mL/min. Another two patients had radiologic evidence of aseptic necrosis of the femur head during the fifth and ninth month post transplant. Daily prednisone dosage was reduced to 5 mg for both patients, and they were

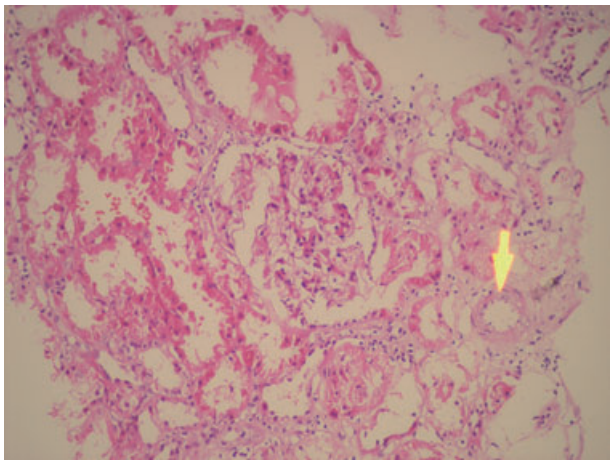

Fig. 3 Normal appearance of glomerulus, tubulus and artery (arrow) after therapy (haematoxylin and eosin, ×20).

simply treated with monthly parenteral biphosphonates and daily calcium carbonate supplementation. None of them required surgical intervention. Furthermore, one patient in this group experienced an adverse cardiac event 6 months after transplantation and underwent triple coronary bypass procedure with an uneventful recovery. Surprisingly, two patients developed mild acute graft dysfunction 1 and 7 months after attaining baseline creatinine levels. Renal biopsy specimens showed characteristic features of acute tubular necrosis (ATN) without myoglobin casts in Masson's trichrome stains (Fig. 4). They were not volume depleted, had no history of statin or ACE-inhibitor use and had had normal serum creatine kinase levels during graft dysfunction.

During February 2007, 24 of the 27 patients completed at least 1 year of follow up. Nineteen recipients (70%) were biopsied at the first year as per protocol, and the transplant tissue was qualified by allograft damage scoring system as proposed by Karpinski *et al.*[5] Five patients did not approve the protocol biopsy at 1 year because of excellent graft function. The difference between chronic allograft damage scores of the two terms (zero hour and 1 year post transplantation) was analysed by Mann–Whitney *U*-test, and the mean tissue damage scores were 2.57 and 2.83, respectively ($P < 0.046$). No further chronic damage was observed in 13 of the biopsies when compared with baseline histological findings (Figs 5,6). The comparative histology in the remaining six biopsies were representative of progressive histological damage merely composed of mild tubular atrophy and interstitial fibrosis (Figs 7,8).

At the last follow up, the mean creatinine, GFR and quantitative protein excretion values were 1.97 ± 0.18 mg/dL, 53.1 ± 4.9 mL/min and 692.5 ± 146.2 mg/day, respectively. All of the patients in the SRL group continued on study medication, and SRL was not interrupted for longer than 15 consecutive days for any reason. On the contrary, two patients in the control group required discontinuation of CyA due to biopsy-proven drug toxicity and switched to

Table 2 The complete blood count and chemistry of rapamycine and CNI groups at last follow up

	Rapamycine group			
	n	mean ± SEM	*n*	mean ± SEM
Htc (%)	27	34.7 ± 1.0	12	38.5 ± 1.7
Hb (g/dL)	27	11.6 ± 0.3	12	11.6 ± 1.1
MCV (fL)	27	81.2 ± 1.0	12	87.1 ± 1.7
WBC ($\times 10^3/\mu L$)	27	7.79 ± 0.5	12	8.67 ± 0.5
PLT ($\times 10^3/\mu L$)	27	262.8 ± 21.1	12	233.0 ± 22.4
RBC ($\times 10^6/\mu L$)	25	4.29 ± 0.16	12	4.44 ± 0.21
Ferritin (ng/mL)	25	813.4 ± 102.4	7	785.1 ± 196.1
Urea (mg/dL)	27	62.8 ± 6.9	12	58.4 ± 12.7
Creatinine (mg/dL)	27	1.97 ± 0.18	12	1.75 ± 0.3
K (mg/dL)	27	4.1 ± 0.1	12	4.3 ± 0.1
P (mg/dL)	26	3.8 ± 0.2	12	3.6 ± 0.2
Albumin (g/dL)	27	4.2 ± 0.1	12	4.2 ± 0.1
Glucose (mg/dL)	27	92.3 ± 3.7	12	80.6 ± 2.0
Cholesterol (mg/dL)	27	219.2 ± 10.0	12	189.2 ± 10.0
Triglycerides (mg/dL)	27	190.3 ± 18.7	12	191.0 ± 28.3
HDL (mg/dL)	27	54.0 ± 2.5	12	45.5 ± 2.3
LDL (mg/dL)	27	127.2 ± 8.2	12	104.8 ± 7.8
Urine protein (mg/day)	20	692.5 ± 146.2		–
GFR (mL/min)	27	49.6 ± 4.2	12	58.4 ± 5.9

CNI, calcineurin inhibitor; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GFR, glomerular filtration rate; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

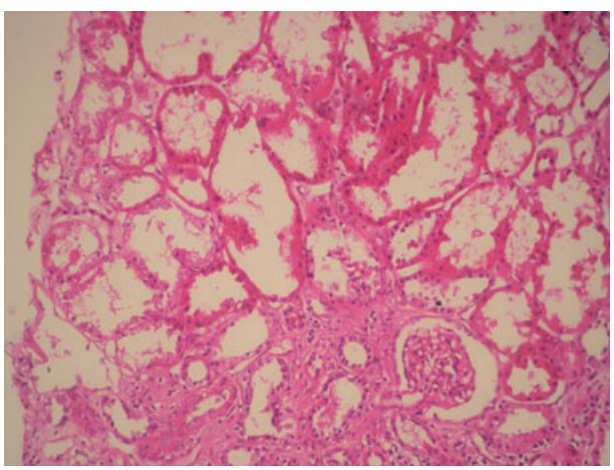

Fig. 4 Acute tubular necrosis (haematoxylin and eosin, ×20).

SRL with a consequent improvement in renal function. The third patient in the control group discontinued CyA because of systemic herpes infection and was assigned to double-drug regimen (MPA + steroids). Mortality rate and graft loss was nil, both in the study and in the control groups.

The mean GFR and serum creatinin values at the last follow up were 53.1 ± 4.9 mL/min *vs* 58.3 ± 5.9 mL/min ($P = 0.258$) and 1.97 ± 0.18 mg/dL *vs* 1.75 ± 0.31 mg/dL ($P = 0.271$), respectively, in the SRL and the CyA groups. The difference between renal function parameters were not significant. The only significant difference was detected between lipid profiles of the groups. The mean total cholesterol, low-density lipoprotein and high-density lipoprotein values were 189.2 ± 9.9 mg/dL *vs* 218.1 ± 9.9 mg/dL ($P = 0.031$), 104.8 ± 7.7 mg/dL *vs* 127.2 ± 8.2 mg/dL ($P = 0.049$) and 45.5 ± 2.34 mg/dL *vs* 53.9 ± 2.49 mg/dL ($P = 0.031$) in the CyA and the SRL groups, respectively.

DISCUSSION

Worldwide shortage of cadaveric donors has encouraged transplant teams to use organs from marginal deceased donors. Marginal kidneys are more susceptible to CNI-induced iatrogenic damage, and graft failure rate in those kidneys was reported to be at least 70% higher than that of ideal donors. Nevertheless, kidney transplantation, even with the use of marginal organs, has provided survival benefit and cost-effectivity when compared with chronic dialysis.[7] We designed a single-centre prospective clinical trial to test the efficacy of a CNI sparing regimen, consisting of SRL + MPA + P, in association with ATG induction, in 27 cadaveric ECDK transplant recipients. UNOS policy 3.5 recently extended its criteria to include all donors over 60 years of age or donors between 50 and 59 years of age with at least two of the three variables (terminal SCr >1.5 mg/dL, history of hypertension and death by cerebrovascular accident), as expanded criteria donors (ECD).[7] However, histological assessment of the donor tissue, which is the quantitative analysis of the nephron mass and intact tubular structure, is lacking in this definition. For this reason, the consistency of UNOS policy 3.5 with the histological scoring system of Karpinski *et al.* was mandated for every possible ECD in our series (Figs 6,7).

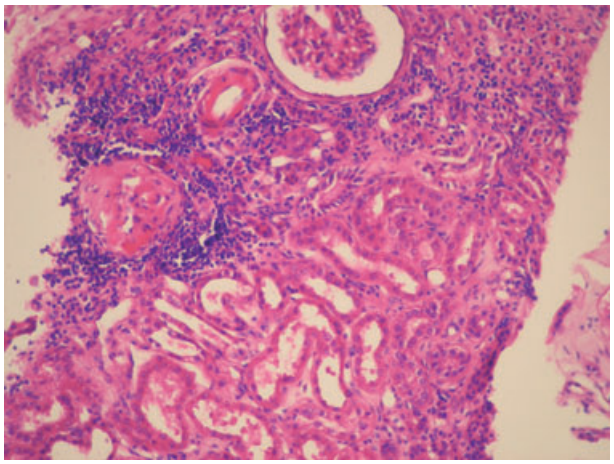

Fig. 5 Global sclerosis, interstitial infiltration and arteriolar hyalinosis (zero-hour biopsy) (haematoxylin and eosin, ×200).

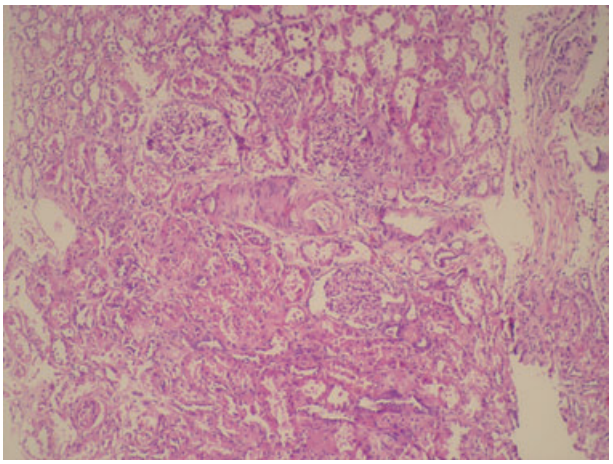

Fig. 7 Baseline biopsy of glomeruli, interstitium, tubuli and artery (zero-hour biopsy) (haematoxylin and eosin, ×10).

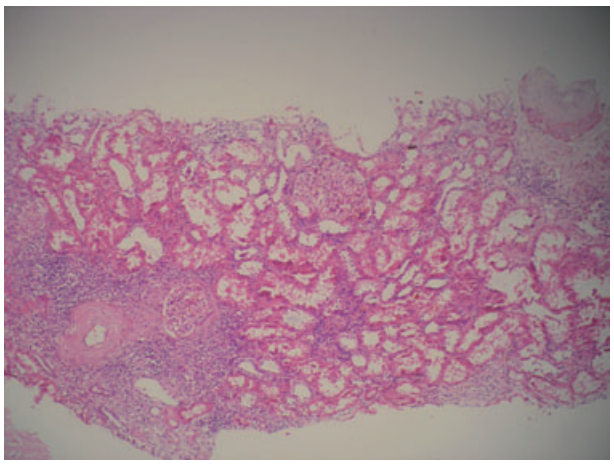

Fig. 6 Interstitial infiltration, periglomerular fibrosis, arteriolar hyalinosis on the left, intimal fibrosis on the right (protocol biopsy) (haematoxylin and eosin, ×10).

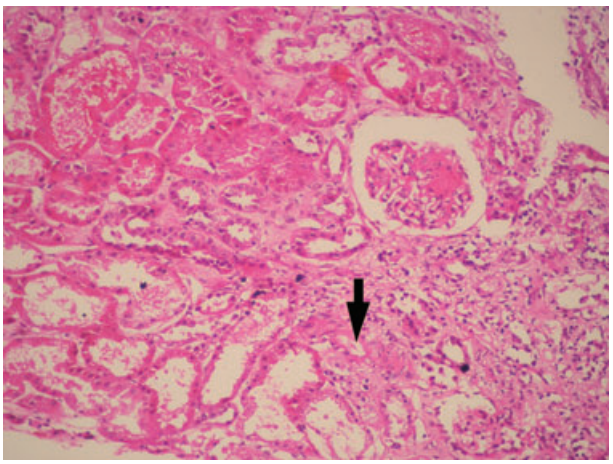

Fig. 8 Segmantal sclerosis of glomerulus, tubular atrophy and arteriolar hyalinosis (arrow) (protocol biopsy) (haematoxylin and eosin, ×20).

In a former prospective randomized trial including primary cadaveric renal recipients, SRL + azathioprine + P combination (41 patients) was compared with CsA + azathioprine + P (42 patients) regimen. Induction immunosuppression was not administered in both groups. Although patient and graft survival figures were similar (>90%) at 12 months, BPAR rates were 41% in the SRL group and 38% in the CyA group, which are unacceptably higher than the protocols used in the current clinical practice. In addition, dose-dependent lipid elevations and thrombocytopenia were more common in the SRL group.[8]

In another pivotal study in which mycophenolate mofetil (MMF, 2 g/day) replaced azathioprine, the composite end-points (graft and patient survival) were similar (>90% at 12 months in both groups), but the incidence of BPAR was reduced to 27.5% and 18.4% for the SRL and the CyA groups, respectively.[9] The GFR was consistently higher in SRL-treated patients. These two studies showed non-inferiority of SRL-based triple immunosuppression from the CyA-based triple drug regimens. These figures were improved further by basiliximab induction to CyA or SRL plus MMF and prednisone in a randomized trial including 61 primary kidney-only recipients. The 2 year actual patient and graft survival rates were 93.5% and 93.5% versus 100% and 93.3%, respectively, for the SRL and the CyA groups. Although the difference was not statistically significant, SRL patients experienced fewer BPAR when compared with CyA-treated patients (6.5% *vs* 16.6%). Interestingly, daily urinary protein excretion was similar and less than 1.0 g/24 h in both groups. SRL-treated patients showed significantly better GFR and SCr values and had more Banff (Grade 0) protocol biopsy readings at 2 years, suggesting superior renal function with preserved histology.[10]

In a contemporary prospective randomized trial comparing SRL–MMF–prednisone with FK506–MMF–prednisone in de novo kidney transplants essentially composed of living-related donors, Larson *et al.* reported similar patient (98% *vs* 96%) and graft (94% *vs* 92%) survival rates, respectively. With the contribution of ATG induction, the incidence of acute rejection at 1 year was 10% in FK506 and 13% in SRL groups. No difference was observed in mean GFR and chronic allograft damage indices in protocol biopsies at 1 year of transplantation in both groups.[11]

Similar to the previous prospective clinical studies,[10,11] there is no doubt that antibody induction contributed to improved kidney function, as shown by the low delayed graft function and BPAR rates (7% and 11%, respectively) in our clinical trial. The increased incidence or prolonged recovery of delayed graft function has been reported in deceased donor kidney recipients receiving rapamycin. These patients were twice as likely to remain on dialysis due to prolonged ATN when compared with patients without rapamycin.[12] In a recent retrospective study, the incidence of histological ATN was 10.5% in 543 renal recipients using rapamycin, and the one and only identifiable cause was myoglobinuria, which was present in 25% of the ATN patients.[13] However, there was no evidence of any predisposing factor such as severe volume depletion, Gram-negative endotoxemia or rhabdomyolysis-induced myoglobinuria in the two patients with ATN in our series. These patients successfully recovered from ATN shortly after the daily rapamycin dosage was halved.

Nineteen (70%) patients were biopsied at the first year post transplant, as per protocol. Chronic allograft damage grade was virtually identical to baseline histology and no progressive damage was observed in 13 of the biopsies. However, six patients had evidence of focal tubular atrophy with or without fibrous connective tissue replacement. The preservation of renal structure by SRL-based triple immunosuppression in our study was consistent with the previous prospective randomized trial of a similar regimen.[10] Nevertheless, inconsistent with the previous randomized trials of TOR inhibitors which demonstrated high incidence of bone marrow suppression,[9] white blood cell and platelet counts in our study were within normal range and stabilized after the third month of transplantation in approximately all patients.

Unfortunately, the development of relapsing infection even with low immunosuppression was frequent in our series. Thirty-seven per cent of the patients had serious infections, which in turn required readmission and prolonged hospitalization. However, all of the patients recovered uneventfully with the exception of patients with relapsing urinary tract infection who showed mild histological evidence of progressive tubulointerstitial damage on their follow-up biopsy specimens.

The small number of patients included in this pilot study and the absence of a randomized control group with CNI-based immunosuppression might be accepted as a drawback. However, this study has the ability to use the patients as their own controls to report on the changes between 0 day and 1 year. A minimum of a 5 year follow up is necessary to predict the long-term surrogate end-points for graft and patient outcomes and the hazards of hyperlipidaemia-induced cardiac problems.

Although not a clean control group was constituted, the comparison of the prospective study group with the retrospective control group yielded non-inferior renal function during a follow up of approximately 2 years. Our results are encouraging within a short time-frame and indicate that low-dose SRL-based triple immunosuppression with antibody induction offers a safe clinical outcome in ECD kidneys without exposing the recipient to an enhanced risk of acute rejection and graft failure. However, a mild progression of the pre-existing tubulointerstitial histological damage and frequent bacterial infection are a major concern.

REFERENCES

1. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N. Engl. J. Med.* 2000; **342**: 605–12.
2. Kaplan B, Meier-Kriesche HU. Renal transplantation: A half century of success and the long road ahead. *J. Am. Soc. Nephrol.* 2004; **12**: 3270–71.
3. Nankivell BJ, Borrows RJ, Fung CLS, O'Connell PJ, Allen RDM, Chapman JR. The natural history of chronic allograft nephropathy. *N. Engl. J. Med.* 2003; **349**: 2326–33.
4. Sehgal SN. Rapamune (RAPA, rapamycin, sirolimus): Mechanism of action. Immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin. Biochem.* 1998; **31**: 335–40.
5. Karpinski J, Lajoie G, Cattran D *et al.* Outcome of kidney transplantation from high-risk donors is determined by both structure and function. *Transplantation* 1999; **67**: 1162–7.
6. Nankivell BJ, Gruenewald SM, Allen RD, Chapman JR. Predicting glomerular filtration rate after kidney transplantation. *Transplantation* 1999; **59**: 1683–9.
7. Ojo OA. Expanded criteria donors: Process and outcomes. *Semin. Dial.* 2005; **18**: 463–8.
8. Groth CG, Backman L, Morales JM *et al.* Sirolimus (rapamycin)-based therapy in human renal transplantation: Similar efficacy and different toxicity compared with cyclosporine. *Transplantation* 1999; **67**: 1036–42.
9. Kreis H, Cisterne JM, Land W *et al.* Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation* 2000; **69**: 1252–60.
10. Flechner SM, Kurian SM, Solez K *et al.* De novo kidney transplantation without use of calcineurin inhibitors preserves renal structure and function at two years. *Am. J. Transplant.* 2004; **4**: 1776–85.
11. Larson TS, Dean PG, Stegall MD *et al.* Complete avoidance of calcineurin inhibitors in renal transplantation: A randomized trial comparing sirolimus and tacrolimus. *Am. J. Transplant.* 2006; **6**: 514–22.
12. Mc Taggart RA, Gottlieb D, Brooks J *et al.* Comparison of outcomes after delayed graft dysfunction: Sirolimus-based versus other calcineurin-inhibitor sparing induction immunosuppression regimens. *Transplantation* 2004; **78**: 475.
13. Pelletier R, Nadasdy T, Nadasdy G *et al.* Acute renal failure following kidney transplantation associated with myoglobinuria in patients treated with rapamycin. *Transplantation* 2006; **82**: 645–50.