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ORIGINAL ARTICLE

Steroid withdrawal at 3 months after kidney transplantation: a comparison of two tacrolimus-based regimens

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Summary

The 6 month prospective, randomized study compared the steroid-sparing potential of two tacrolimus-based regimens after renal transplantation. A total of 489 patients were randomized (1:1) to receive tacrolimus/mycophenolate mofetil (MMF)/steroids ($n = 243$; group Tac/MMF/S) or tacrolimus/azathioprine/steroids ($n = 246$; group Tac/Aza/S). At 3 months, steroids were tapered off in 267 (54.6%) patients free from steroid-resistant acute rejection and with serum creatinine concentrations <160 μmol/l. The incidence of biopsy-confirmed acute rejection at month 3 was lower in group Tac/MMF/S compared with group Tac/Aza/S (18.1% vs. 26.0%,$P = 0.035$). Moreover, more patients in the Tac/MMF/S group met the criteria for steroid withdrawal than in the Tac/Aza/S group (60.5% vs. 48.8%; $P < 0.01$). The incidence of acute rejection during months 4–6 was low in all groups, both for patients on steroid-free dual therapy (Tac/MMF: 2.7%, Tac/Aza: 0.8%) and for patients who continued steroid maintenance therapy (Tac/MMF/S: 3.5%, Tac/Aza/S: 7.1%). Moreover, kidney function was well preserved in steroid-free patients with month 6 median serum creatinine levels of 119.5 μmol/l (Tac/MMF), and 115.1 μmol/l (Tac/Aza). For patients who continued to receive steroids, month 6 median creatinine levels were 130.5 μmol/l (Tac/MMF/S) and 132.8 μmol/l (Tac/Aza/S). The criteria for the selection of patients to discontinue steroids were adequate. Both tacrolimus-based regimens allowed the safe discontinuation of steroids in low-risk patients at month 3. The Tac/MMF combination was superior in the prevention of acute rejections and more patients met the chosen criteria for steroid withdrawal.

Introduction

Acute graft rejection, which is most likely to occur within the first months following transplantation, is a major risk factor for subsequent chronic rejection [1–3]. Given that chronic rejection and death with a functioning graft are the major reasons for late graft loss [1,2], prevention of acute graft rejection is, therefore, of prime clinical importance. Tacrolimus is a cornerstone immunosuppressant in renal transplantation, and has been shown to provide excellent protection from acute rejection and chronic rejection [4–7]. Indeed, large, multicentre, randomized trials have shown that tacrolimus has superior efficacy in preventing acute rejection, corticosteroid-resistant acute rejection and chronic rejection compared with ciclosporin (original formulation) or ciclosporin microemulsion-based regimens [4–7], while maintaining a similarly good safety profile. The pivotal European and US multicentre studies used tacrolimus in combination with azathioprine. More recently, tacrolimus in combination with mycophenolate mofetil (MMF) at a dose of 1 g/day and corticosteroids has been shown previously to be a very effective and well-tolerated regimen in kidney transplantation [8].

As prolonged steroid treatment is associated with a number of deleterious effects, including the development of diabetes mellitus, worsening of hypertension, bone demineralization and disturbed lipid metabolism [1,9], recent study designs in renal transplantation explore the feasibility of withdrawing adjunctive immunosuppressive drugs, in particular the steroids from dual or triple regimens [10]. A large 6-month study was conducted designed specifically to measure the effect of withdrawing MMF or steroids in renal transplantation patients [11]. The incidence of acute rejection between months 3 and 6 appeared to be higher among patients who stopped steroids (5.9%) or who stopped MMF (1.8%) than in patients who continued triple therapy (0.9%). However, the overall 6-month incidence of biopsy-proven acute rejection was comparable in all three groups: 17.0% (triple therapy), 15.1% (steroid stop) and 14.8% (MMF stop). Such findings indicate that adjunctive immunosuppressive therapy can be discontinued early after transplantation, offering the opportunity to minimize the side-effects of long-term immunosuppressive therapy.

As steroid withdrawal may increase the risk of acute rejection in some patients [12], the main challenge in withdrawing steroid treatment is the selection of patients in whom steroid withdrawal is likely to be successful. The present study compared two triple regimens tacrolimus/MMF/steroid (group Tac/MMF/S patients) and tacrolimus/azathioprine/steroid (group Tac/Aza/S patients) with regard to their potential to withdraw steroids in low risk patients after 3 months.

Patients and methods

Patients

The study was conducted in accordance with the principles of the Declaration of Helsinki (South Africa, October 1996) and in compliance with the International Conference on Harmonisation Good Clinical Practice regulations and guidelines. The study protocol was approved by the independent ethics committee for each study centre and written informed consent was obtained before any study-related procedures were conducted.

Patients with end-stage kidney disease were eligible for enrolment if they were aged ≥18 years, were suitable candidates for primary renal transplantation or retransplantation, and were receiving a kidney transplant from a cadaveric or living donor aged 5–65 years. Patients were excluded from the study if they had a panel reactive antibody grade ≥50% within the previous 6 months and/or had a previous graft survival <1 year because of immunological reasons. Patients who were HIV positive, had significant liver disease, severe diarrhoea, vomiting, or an active peptic ulcer were also excluded.

Study design

This was a phase 3, open-label, randomized, multicentre study. Eligible patients hospitalized for a kidney transplant were randomized (1:1) within 12 h of reperfusion to receive tacrolimus with MMF or azathioprine, plus corticosteroids. After the initial screening on day 0 (day of reperfusion), patients were monitored on days 1, 7 and 14 and after 1, 3, 4 and 6 months. Efficacy was assessed by comparing the incidence of, and time to first biopsy-proven acute rejection during months 1–3 and during months 4–6. Additional efficacy criteria included the incidence of and time to first corticosteroid-resistant acute rejection at 3 and 6 months; the proportion of patients eligible for steroid discontinuation at 3 months; the proportion of steroid-free patients at 6 months; graft survival rate; and renal function, as measured by serum creatinine at 3 and 6 months after transplantation. Safety was assessed by monitoring patient survival, adverse events, vital signs and changes in laboratory parameters. Laboratory parameters were assessed on day 0, at 3 and 6 months.

Immunosuppressive treatments

The first dose of tacrolimus was administered within 12 h prior to reperfusion and, where possible, within 3 h prior to anaesthesia. The recommended initial dose for tacrolimus was 0.1 mg/kg orally, twice daily, adjusted as required to maintain the following tacrolimus whole

blood trough levels (measured by the Abbott IMx tacrolimus II assay before the morning dose): 15 ng/ml (range 10–20 ng/ml) from days 0 to 21; 10–15 ng/ml from days 22 to 41; and 5–10 ng/ml from days 42 to 183. Due to the long half-life of tacrolimus, dose adjustments were made no more than twice weekly. Dose modifications were conducted in steps of 25% of the current dose. If the initial tacrolimus dose could not be administered orally or by nasogastric tube, an intravenous (i.v.) dose of one-fifth of the oral daily dose, i.e. 0.04 mg/kg, was administered as a continuous 24-h infusion. Tacrolimus study medication could be withheld at any point during the study if an intolerable adverse event occurred.

Patients in group Tac/MMF/S received 0.5 g MMF orally, twice daily throughout the study. The initial dose was administered within 72 h following reperfusion. Dose reduction was allowed if an MMF-related adverse event occurred, and if the adverse event persisted, MMF treatment could be withdrawn.

Patients in group Tac/Aza/S received the initial dose of azathioprine as soon as possible following transplantation. Patients received an initial loading dose of 2–5 mg/kg/day orally or i.v. and were maintained subsequently on 1–2 mg/kg/day orally or i.v. for at least 6 months. Reduction or suspension of the dose was allowed if a patient's white blood cell count was $<4 \times 10^9$ cells/l.

During the first 3 months of the study, all patients received steroid treatment in addition to MMF or azathioprine adjunctive therapy – daily dose of methylprednisolone (or equivalent) was 500 mg or less at day 0 and 125 mg at day 1, administered as an i.v. bolus. Thereafter, prednisone (or equivalent) was given orally once daily as follows: 20 mg from days 2 to 14; 15 mg from days 15 to 28; 10 mg from days 29 to 42; 5 mg from days 43 to 91.

At 3 months, patients were assigned to a steroid taper if they met the following criteria: the patient was rejection-free and had experienced not more than one steroid-sensitive rejection episode in the first 2 months post-transplant; the patient had serum creatinine <160 μmol/l between days 84 and 91 post-transplant; and the patient was receiving at least 0.5 g/day MMF (if in group Tac/MMF/S) or 1 mg/kg/day azathioprine (if in group Tac/Aza/S). Patients who met the above criteria (120 patients in Tac/Aza/S group and 147 in Tac/MMF/S) had steroids tapered from 5–0 mg between months 3 and 4 according to protocol-specified criteria, local practice, or the needs of the patient.

Diagnosis and grading of rejection episodes

If clinical and/or laboratory signs indicated a rejection episode, a renal biopsy had to be performed prior to the initiation of any anti-rejection therapy. The histological evaluation of the biopsy was performed by the local histopathologist following the BANFF criteria, who was blinded towards the patient's treatment. Acute rejection episodes were categorized according to the treatment administered. A spontaneously resolving acute rejection was defined as a rejection episode which was not treated with new or increasing corticosteroid medication, antibodies or any other medication and resolved irrespective of any tacrolimus dose changes; a corticosteroid sensitive acute rejection was defined as a rejection episode which was treated with new or increasing corticosteroid medication only and resolved; corticosteroid resistant acute rejection was defined as not resolving following treatment with corticosteroids. In case that a rejection episode was not treated with corticosteroids first but only with antibodies, it was nevertheless included in this category. These were further classified into episodes which resolved with additional treatment and those which did not respond to further treatment or were ongoing at the time of study end or patient withdrawal.

Treatment of acute rejections

First line therapy for an acute rejection episode were corticosteroids with the exception of those with a BANFF grade III biopsy where antibodies could be given as first line therapy if it was local practise. The total cumulative dose of steroids used for treatment of an acute rejection episode exceed 2000 mg prednisone or equivalent. If a rejection episode occurred during the steroid taper it was treated as usual and the patient maintained on −10 mg/day prednisone or equivalent after resolution of the episode. An increase in tacrolimus dose in addition to steroid treatment was a therapeutic option if the trough level was below 10 ng/ml. If the rejection episode did not resolve with one course of corticosteroids, as defined above, additional agents such as monoclonal or polyclonal antibodies were used according to local practise.

Statistical analysis

The analyses were based all patients who were randomized to treatment and received at least one dose of study drug (intent-to-treat principle). The number of episodes and incidence of acute rejection were compared between treatments using the chi-square test, or Fisher's exact test (if the expected sample size for any variable was <5).

Times to first acute rejection; first corticosteroid-resistant acute rejection; graft loss, treatment failure and death were analysed using Kaplan–Meier methods; comparisons were made between treatments using the Wilcoxon test. The Greenwood method was used to calculate 3-month survival within each treatment group [13]. The difference in 3-month survival rates between groups was calculated

using Peto's method [14]. Differences in serum creatinine between treatment groups were compared at month 3 and month 6 using a Wilcoxon rank sum test. The incidences of each adverse event were compared between treatment groups using Fisher's exact test.

Results

Patients demographics and disposition

Fifteen centres in four European countries participated in this study. A total of 489 patients were enrolled in the study between November 1999 (first patient enrolled) and 28 August 2001 (last patient visit). All patients received at least one dose of study medication (243 received tacrolimus/MMF/steroids, group Tac/MMF/S; 246 received tacrolimus/azathioprine/steroids, group Tac/Aza/S). The majority of patients (452 patients; 92%) completed the study. Baseline demographic characteristics, viral status and transplant history were similar between both treatment groups (Table 1).

Efficacy

The incidence of biopsy-proven acute rejection was significantly lower in group Tac/MMF/S than in group Tac/Aza/S, with a total of 44 MMF-treated patients (18.1%) compared with 64 azathioprine-treated patients (26.0%) experiencing at least one biopsy-confirmed acute rejection episode during the 3 months after transplantation ($P = 0.035$; Table 2). The 3-month rate of patients free from biopsy-confirmed acute rejection was 81.6% in group Tac/MMF/S, compared with 73.7% in Group Tac/Aza/S ($P = 0.046$). Most acute rejections were corticosteroid sensitive; the incidence of biopsy-confirmed corticosteroid-resistant acute rejection was low in both groups (2.1% vs. 4.5%; $P = 0.134$). Most acute rejections in both groups were histologically assessed as mild (BANFF 1). Graft survival after 3 months was high in both groups (Tac/MMF/S: 96.3% versus Tac/Aza/S: 5.5%; $P = 0.472$).

Median serum creatinine levels were significantly lower in group Tac/MMF/S, compared with group Tac/Aza/S at month 3 (137.0 vs. 150.5 μmol/l; $P = 0.005$).

As a result of the lower average serum creatinine levels and the lower acute rejection rate, more patients in group Tac/MMF/S than in group Tac/Aza/S could be assigned to steroid taper at 3 months [147 patients (60.5%) versus 120 patients (48.8%), respectively $P = 0.009$; Table 2].

After the tapering of steroids in eligible patients, serum creatinine did not change in patients without maintenance steroid therapy. As a result of the assignment, average serum creatinine levels were higher for patient who continued maintenance steroid therapy, but the levels also remained stable over the second half of the study (see Fig. 1).

The incidence of biopsy-confirmed acute rejection was low in both groups during months 4–6 post-transplantation, and was not higher in patients with steroid taper (Tac/MMF: 2.7% and Tac/Aza: 0.8%) than in patients who continued maintenance steroid therapy (Tac/MMF/S: 3.5% and Tac/Aza/S: 7.1%).

Of the patients who completed the study, more the patients in group Tac/MMF/S were steroid-free compared with group Tac/Aza/S (140/226, 61.9% versus 115/226, 50.9%) at month 6.

Table 1. Baseline patient demographic and laboratory characteristics.

	Tac/MMF/S ($N = 243$)	Tac/Aza/S ($N = 246$)
Age (years)		
Mean ± SE (range)	43.8 ± 12.2 (18–70)	42.1 ± 12.3 (19–69)
Sex [*n* (%)]		
Male	156 (64.2)	157 (63.8)
Female	87 (35.8)	89 (36.2)
Viral status at baseline [*n* (%)]		
CMV-positive*	142 (72.1)	148 (75.9)
EBV-positive*	75 (65.2)	79 (64.8)
HBV-positive*	4 (1.6)	11 (4.5)
HCV-positive*	29 (12.0)	20 (8.2)
CMV mismatch: recipient negative, donor positive [*n* (%)]	30 (12.3)	40 (16.3)
Previous transplants [*n* (%)]		
0	229 (94.2)	234 (95.1)
1	14 (5.8)	12 (4.9)

*Variable not recorded for all patients.

N, ITT patients (who received at least one dose of study drug); CMV, cytomegalovirus; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus.

Safety results

Six-month patient survival was similar in both treatment groups (MMF-treated patients: 98.3% versus azathioprine-treated patients: 98.4%; $P = 0.972$). Three patients in group Tac/MMF/S and four patients in group Tac/Aza/S died during the study, and one patient in group Tac/MMF/S died after withdrawal from the study. The causes of death in the TAc/MMF/S group were shock, lung oedema, myocardial infarction and brain swelling; the causes of death in the Tac/Aza/S group were cerebrovascular accident, lung oedema, accidental injury and pneumonia.

Graft survival was also similar at 6 months with 95.0% and 93.5% in the MMF-treated patients and in azathioprine-treated patients ($P = 0.472$). Twelve grafts were lost in MMF-treated patients and 16 grafts were lost in azathioprine-treated patients.

Table 2. Biopsy-confirmed acute rejection.

		Tac/MMF/S ($N = 243$)		Tac/Aza/S ($N = 246$)
Months 1–3 following transplantation				
Acute rejection [*n* (%)]		44 (18.1)		64 (26.0)*
Steroid-resistant acute rejection		5 (2.1)		11 (4.5)
	Tac/MMF ($N = 147$)	Tac/MMF/S ($N = 85$)	Tac/Aza ($N = 120$)	Tac/Aza/S ($N = 113$)
Months 4–6 following transplantation				
Acute rejection [*n* (%)]	4 (2.7%)	3 (3.5)	1 (0.8)	8 (7.1)
Steroid-resistant acute rejection	0	0	0	2 (1.8%)

N, ITT patients (who received at least one dose of study drug).
*$P = 0.035$, chi-square test.

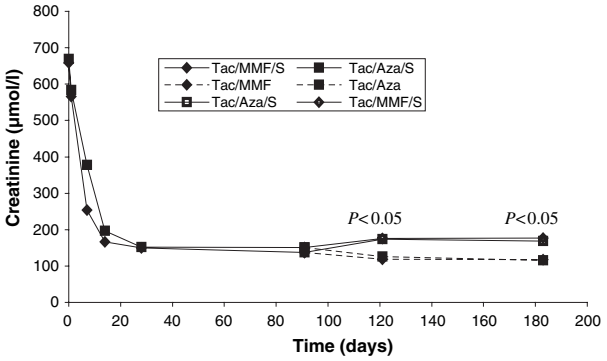

Figure 1 Median values of serum creatinine levels measured during the study.

Table 3. Adverse events experienced by ≥5% of patients in either treatment group Tac/MMF/S descending order of frequency.

Preferred term	Tac/MMF/S ($N = 243$) [*n* (%)]	Tac/Aza/S ($N = 246$) [*n* (%)]	*P*-value*
Kidney function abnormal	45 (18.5)	67 (27.2)	0.019
Kidney tubular necrosis	23 (9.5)	39 (15.9)	0.033
Urinary tract infection	28 (11.5)	34 (13.8)	
Primary graft dysfunction	19 (7.8)	28 (11.4)	
Diabetes mellitus	27 (11.1)	27 (11.0)	
Leukopenia	7 (2.9)	21 (8.5)	0.001
CMV infection	12 (4.9)	14 (5.7)	
Diarrhoea	13 (5.3)	7 (2.8)	
Tremor	13 (5.3)	7 (2.8)	

N, ITT patients (who received at least one dose of study drug). CMV, cytomegalovirus.
*$P < 0.05$, Fisher's exact test.

The percentage of patients reporting adverse events during the study was similar in the two treatment groups, with 77.8% in group Tac/MMF/S patients and 81.3% in group Tac/Aza/S patients. The most frequently reported adverse events are shown in Table 3. The incidences of abnormal kidney function, kidney tubular necrosis and leukopenia were significantly higher in group Tac/Aza/S compared with group Tac/MMF/S. Some less frequently reported adverse events occurred in the MMF-receiving patients than in the azathioprine-treated patients (bronchitis: 4.9% vs. 1.2%, herpes zoster 2.9% vs. 0.0%, hypercholesterolaemia: 2.5% vs. 0.0% and gastritis: 2.1% vs. 0.0%). In patients without pre-existing glucose metabolism disorders, more patients in the Tas/MMF/S group received long-term (>30 days) insulin treatment compared with the Tac/Aza/S group (9.1% vs. 4.4%).

Discussion

This was the first multicentre study in a European setting and in the absence of antibody induction to compare the efficacy and safety of tacrolimus/MMF and tacrolimus/azathioprine, with and without steroids taper, in kidney transplant recipients. The MMF-containing regimen was significantly more effective than the azathioprine-containing regimen in preventing acute rejection. However, the 3-month incidence of corticosteroid-resistant biopsy-confirmed acute rejection was low in both groups.

As acute rejection is a significant risk factor for chronic rejection and subsequent long-term graft loss [1–3,15], the lower rate of acute rejection within the first months achieved with the tacrolimus/MMF combination compared with the tacrolimus/azathioprine combination may also have long-term implications. The difference in the incidence of acute rejection between the two groups cannot be explained by a disparity in the tacrolimus and/or steroid therapy. Initial doses and target whole blood trough levels of tacrolimus were those recommended in a previous large-scale European study [4] and the trough levels were maintained within the target range throughout the study in both groups. Moreover, the doses of azathioprine and MMF were

within the protocol-defined ranges throughout the study, and the mean daily dose of corticosteroids was similar in both study groups at all time points (data not shown).

In this study, low-risk patients with a good graft function were selected for steroid withdrawal while patients with a more difficult clinical course remained on steroids. The percentage of patients eligible for steroid discontinuation at 3 months was significantly higher in group Tac/MMF/S (60.5%) than in group Tac/Aza/S (48.8%). Steroid withdrawal was performed successfully in both treatment groups, and the selected patients could be managed on a dual tacrolimus/MMF regimen as well as on a tacrolimus/azathioprine regimen. At study end, the steroid-free patients had good kidney function, as assessed by median serum creatinine concentrations. The criteria chosen for the selection of patients to withdraw steroids were, therefore, successful, as the rate of rejection did not increase after discontinuation of steroids. Given the well-known side-effects associated with steroid use [1,9], such findings seem to represent an advance in the management of transplant patients.

Both treatments were generally well tolerated, with the majority of reported adverse events typical for patients undergoing renal transplantation. Abnormal kidney function, kidney tubular necrosis, urinary tract infection and diabetes mellitus were the most commonly reported adverse events. The overall safety profile was similar with both treatment regimens, although there was a tendency for more kidney function disorders in the Tac/Aza/S group and more infectious complications in the Tac/MMF/S group.

In conclusion, both tacrolimus triple regimens were effective and safe, and resulted in low incidences of acute rejection. The criteria chosen for assigning patients to steroids taper were adequate. Steroids could safely be withdrawn in both groups without a rebound in acute rejection rate or a deterioration of renal function. The combination of tacrolimus and MMF being more effective in the prevention of acute rejection and achieving a better mean renal function at month 3, allowed more patients to meet the criteria for steroid withdrawal. The successful withdrawal of steroids suggests some long-term benefits for those patients who continued on a dual immunosuppressive regimen.

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References

1. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi A. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 2002; **346**: 580.
2. Kreis H, Ponticelli C. Causes of late renal allograft loss: chronic allograft dysfunction, death and other risk factors. *Transplantation* 2001; **71**: S5.
3. Basadonna GP, Matas AJ, Gillingham KJ, *et al.* Early versus late acute renal allograft rejection: impact on chronic rejection. *Transplantation* 1993; **55**: 993.
4. Mayer AD, Dmitrewski J, Squifflet JP, *et al.* Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation* 1997; **64**: 436.
5. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS, FK506 Kidney Transplant Study Group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. *Transplantation* 1997; **63**: 977.
6. Margreiter R. Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. *Lancet* 2002; **359**: 741.
7. Mayer AD. Chronic rejection and graft half-life: five-year follow-up of the European Tacrolimus Multicenter Renal Study. *Transplant Proc* 2002; **34**: 1491.
8. Squifflet JP, Bäckman L, Claesson K, *et al.* Dose optimization of mycophenolate mofetil when administered with a low dose of tacrolimus in cadaveric renal transplant recipients. *Transplantation* 2001; **72**: 63.
9. Prasad GV, Nash MM, McFarlane PA, Zaltzman JS. Renal transplant recipient attitudes toward steroid use and steroid withdrawal. *Clin Transplant* 2003; **17**: 135.
10. Kasiske BL, Chakkera HA, Louis TA, Ma JZ. A meta-analysis of immunosupression withdrawal trials in renal transplantation. *J Am Soc Nephrol* 2000; **11**: 1910.
11. Squifflet JP, Vanrenterghem Y, van Hooff JP, Salmela K, Rigotti P, The European Tacrolimus/MMF Transplantation Study Group. Safe withdrawal of corticosteroids or mycophenolate mofetil: results of a large, prospective, multicenter, randomized study. *Transplant Proc* 2002; **34**: 1584.
12. Hricik DE, O'Toole MA, Schulak JA, Herson J. Steroid-free immunosuppression in cyclosporine-treated renal transplant recipients: a meta-analysis. *J Am Soc Nephrol* 1993; **4**: 1300.
13. Lee ET. Statistical Methods for Survival Data Analysis. New York: John Wiley and Sons, 1992.
14. Collett D. Modelling Survival Data in Medical Research. London: Chapman and Hall, 1994.
15. Jindal RM, Hariharan S. Chronic rejection in kidney transplants. An in-depth review. *Nephron* 1999; **83**: 13.