Transpl Int (2001) 14: 370–383

ORIGINAL ARTICLE

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Single-center experience with tacrolimus versus cyclosporine-Neoral in renal transplant recipients

Received: 17 July 2000
Revised: 31 May 2001
Accepted: 21 June 2001

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Abstract Tacrolimus has proven to be superior to cyclosporine-Sandimmune with regard to the prevention of acute rejections, but data comparing tacrolimus with Neoral are scarce. A total of 128 consecutive renal transplant recipients was studied. The patients were treated with Neoral-based ($n = 74$) or tacrolimus-based ($n = 54$) immunosuppressive regimens. Survival analyses (Cox regression analysis) were performed on an intention-to-treat basis. Renal function and cardiovascular risk profile were analyzed by means of a repeated-measures analysis of variance (ANOVA) up to 12 months after transplantation. Immunological features were less favorable in the tacrolimus group. Two-year patient and graft survival were comparable. Acute-rejection-free survival was 82% in the tacrolimus group versus 40% in the Neoral group ($P < 0.0001$). The severity of the rejections (1997 Banff classification) was comparable ($P = 0.43$). Immunological graft loss (3.7% vs 12.2%, $P = 0.02$) and conversion because of rejection (0% vs 28.4%, $P < 0.001$) were less in the tacrolimus group. A higher proportion (68.5% vs 14.9%, $P < 0.001$) was successfully put on monotherapy. Creatinine clearance, proteinuria, and fractional uric acid clearance were similar. In the tacrolimus group mean blood pressure was comparable, but patients needed less antihypertensive drugs ($P < 0.001$) and, even with fewer patients on lipid-lowering drugs, total cholesterol was lower (5.2 vs 6.0 mmol/l, $P = 0.003$). Treatment for post-transplant diabetes mellitus was 18.5% versus 10.8% ($P = 0.22$). In both groups, antidiabetic medication could be withdrawn for most patients. This study indicates that tacrolimus is superior to cyclosporine-Neoral in preventing acute rejection with comparable patient and graft survival rates. Because of a lower need for treatment of hypertension and hypercholesterolemia, the cardiovascular risk profile is more favorable. A considerable proportion of patients can be successfully weaned off co-medication and treated with tacrolimus monotherapy.

Keywords Tacrolimus · Cyclosporine · Neoral · Survival · Rejection · Clinical outcome

Abbreviations *ANOVA* Analysis of variance · *HLA* Human leukocyte antigen · *HUS* Hemolytic uremic syndrome · *MMF* Mycophenolate mofetil · *PRA* Panel-reactive antibodies · *r-ATG* Rabbit anti-thymocyte globulin

Introduction

Cyclosporine A (Sandimmune, Sandoz/Novartis, Switzerland) was introduced in renal transplantation in the 1970s [4]. The major advantage was a reduction in the number of acute rejections and improvement in 1-year graft survival [8, 12, 48]. Its impact on medium- and long-term graft survival has not been firmly established [11]. Chronic allograft rejection is a major cause of graft loss. Prior acute rejections, especially steroid-resistant and recurrent rejections, appear to be an important contributory factor [1, 2, 16, 22, 23, 24, 25, 45, 50]. In 1995, in the Netherlands, cyclosporine-Sandimmune was replaced by cyclosporine-Neoral (Novartis, Switzerland), a new microemulsion formulation. This formulation has a more rapid and consistent absorption, leading to lower intra-patient variability [9]. In controlled trials, Neoral has proven to be superior to Sandimmune in the prevention of acute rejection [20, 31, 34].

Randomized multicenter trials have shown the superiority of tacrolimus (Prograf, Fujisawa, Japan)-based immunosuppressive regimens to cyclosporine (Sandimmune) with regard to the prevention of acute and steroid-resistant rejection [17, 26, 33, 40, 52]. Recently, a meta-analysis confirmed the results of the separate trials [21]. Data comparing tacrolimus with Neoral in renal transplantation are scarce. One retrospective study showed a lower incidence of acute rejections with tacrolimus versus Neoral [10]. Another group published two interim reports of a prospective trial [19, 29]. In the first report, with relatively low initial tacrolimus target levels, a similar incidence of acute rejection was reported for tacrolimus and Neoral [29]. In the second report, tacrolimus was superior to Neoral [19].

With regard to side effects, tacrolimus administration resulted in advantages in cardiovascular risk profile. The incidence of hyperlipidemia was lower [5, 14, 17, 19, 33]. Although the incidence of hypertension in the main prospective trials was comparable [26, 33], there are now also indications that less anti-hypertensive drugs are needed with tacrolimus [13, 17, 19, 36, 42]. Another advantage was a steroid-sparing effect [17, 42]. Furthermore, the incidence of gingival hyperplasia and hirsutism, seen with cyclosporine, was remarkably lower in tacrolimus-treated patients [26, 33]. An important disadvantage of tacrolimus was a higher incidence of post-transplant diabetes mellitus [26, 33, 52]. Also, tremor, pruritus, and alopecia were more frequently observed [26, 33].

Because data comparing tacrolimus with Neoral are limited and prospective data have only been published by one group, we analyzed all of our patients treated with standard immunosuppressive therapy based on cyclosporine-Neoral and compared them with patients treated with tacrolimus-based immunosuppressive regimens in the same time period.

Materials and methods

All patients receiving consecutive renal transplants between July 1995 and October 1997 were included in the study. Combined kidney and pancreas transplant recipients were excluded. Follow-up data were collected until May 1, 1999, death, or graft failure.

Immunosuppression

Neoral

Standard initial immunosuppression from July 1995 until mid-September 1997 consisted of Neoral and steroids (prednisolone, 10–20 mg/day). Neoral was initially administered intravenously before surgery in a starting dose of 4 mg/kg per 24 h and switched to oral intake (4 mg/kg b.i.d.) within 48 h after surgery. In the case of an uneventful course, prednisolone was tapered to 7.5 mg at month 1 and to 5 mg at month 3. In recipients without rejection, prednisolone was further tapered to 0 mg in the months that followed.

Recipients with panel-reactive antibodies (PRA) greater than 85% in either peak or current serum and recipients with immunological failure of a previous graft also received azathioprine at 1 mg/kg ($n = 4$). Tapering prednisolone in these patients was restricted to 5 mg. All recipients with living-related grafts except one also received azathioprine ($n = 11$).

Whole blood trough cyclosporine target levels (radioimmunoassay; Syva, Dade-Behring, USA) were initially 0.15–0.20 mg/l in recipients receiving Neoral and steroids and 0.10–0.15 mg/l in recipients receiving Neoral, steroids, and azathioprine. The dose was gradually adjusted after 3 months to target levels of 0.10–0.15 mg/l in recipients receiving Neoral and steroids, and 0.05–0.10 mg/l in recipients receiving triple therapy with the addition of azathioprine.

Forty consecutive Neoral-treated patients (54.1%) participated in a placebo-controlled Dutch multicenter trial that investigated whether the addition of isradipine (Lomir, Novartis, Switzerland) to standard immunosuppressive therapy would result in an improvement in transplant outcome.

Tacrolimus

During the same period, indications for tacrolimus were: participation in a multicenter trial (FG-220) ($n = 25$), compassionate use ($n = 23$), or standard therapy ($n = 6$), after the introduction of tacrolimus in the Netherlands in mid-September 1997. Tacrolimus was only administered orally, with starting doses of 0.10–0.15 mg/kg twice daily, within 12 h after transplantation. Whole blood tacrolimus trough target levels (micro-particle immunoassay, IMx, Abbot Laboratories, Abbott Park, Ill., USA) were 10–20 ng/ml within the first 3 months after transplantation. The dose of tacrolimus was gradually adjusted to target levels of 5–7 ng/ml after 6 months.

All patients received steroids (10–20 mg prednisolone). In the case of an uneventful course, prednisolone was tapered to 7.5 mg at month 1 and further to 5 mg at month 3. In recipients without rejection, prednisolone was gradually withdrawn in the months that followed.

The FG-220 multicenter trial compared the value of adding mycophenolate mofetil (MMF, Cellcept, Roche, Switzerland) in doses of 1 and 2 g/day. All adults receiving a cadaveric transplant were eligible for this trial, with the exception of those with present or previous malignancies, liver disease, or recent infection. Twenty-five consecutive recipients participated in this trial. Seven recipients received 2 g MMF/day, eight were administered 1 g, and ten received only tacrolimus and steroids.

Twenty-three recipients received tacrolimus on a compassionate-use basis for the following indications:

1. Highly immunized patients with contra-indications to azathioprine or previous use of recombinant anti-thymocyte globulin (r-ATG) ($n = 7$)
2. Early graft loss of a previous transplant because of acute rejection or primary nonfunction with cyclosporine ($n = 2$)
3. Intolerance to, or severe side effects from, cyclosporine (hemolytic uremic syndrome (HUS)) ($n = 3$)
4. High-risk transplants due to living-unrelated donors ($n = 7$), high urgency, or poor human leukocyte antigen (HLA)-match ($n = 2$)
5. Prior use of tacrolimus due to participation in a previous trial ($n = 2$)

Two patients of subgroup 4 also received MMF at 1 g/day: one living-unrelated graft and one high-urgency patient with a poor HLA-match. Among the six patients who received tacrolimus as standard therapy, one patient with a living-related donor received MMF at 0.5 g/day. Azathioprine at 1 mg/kg was added for nine patients: five with a living-unrelated graft, one with a living-related graft, two re-transplants (HUS), and one highly immunized recipient.

Conversion

In the case of severe side effects and persistent or steroid-resistant rejections, patients could be switched to the opposite drug.

Clinical outcome parameters

Primary endpoints were patient survival, graft survival, acute-rejection-free survival, and the severity of rejection episodes. Graft loss was defined as return to dialysis, independent of its cause, or death with a functioning graft. Acute rejection was defined on clinical criteria within the first 6 months after transplantation and confirmed by ultrasound-guided needle core biopsy. Episodes during which treatment against acute rejection was given and for which no biopsy could be performed due to contra-indications were considered as acute rejection. There was clinical suspicion of rejection in the case of an unexplained rise or insufficient decrease in serum creatinine, with or without other signs, such as fever, tenderness of the graft, decreased renal perfusion on nuclear scan, or hypertension. Contra-indications for biopsy were bleeding disorders, uncontrolled severe hypertension, or neonatal kidneys. In the case of delayed graft function (need for dialysis during the first week after transplantation), a protocol biopsy was performed at week 1 to rule out rejection as the cause of nonfunctioning of the graft. Rejection treatment consisted of a course of three doses of 500–1000 mg methylprednisolone. In the case of steroid-resistant or vascular rejection, a 10-day course of r-ATG (RIVM, Bilthoven, The Netherlands) was used. A pathologist classified the biopsies in a blinded fashion according to the 1997-revision of the Banff classification [35].

Secondary endpoints were the course of renal function and the cardiovascular risk profile, as they may be drug-related. Renal function parameters were creatinine clearance, calculated by means of the Cockcroft-Gault formula and expressed per 1.73 m^2 body surface area [6]; proteinuria, expressed as g protein/mol creatinine excretion [39]; and fractional uric acid clearance [= (urinary uric acid × serum creatinine / urinary creatinine × serum uric acid) × 100 %].

The cardiovascular risk profile was assessed by measuring systolic and diastolic blood pressure, total cholesterol, and incidence of diabetes. Blood pressure was measured in the sitting position during outpatient clinic visits, according to the method of Riva-Rocci. The mean blood pressure [defined as diastolic blood pressure + (systolic – diastolic blood pressure) / 3] was calculated. The use of drugs for hypertension or hypercholesterolemia was established from the medical record. Diuretics were not included, because they could have been used for reasons other than hypertension (e.g., edema or congestive heart failure). We classified patients for post-transplant diabetes if oral antidiabetic drugs or insulin were used at any time after transplantation and if no diagnosis of diabetes had been established before transplantation.

Statistical analysis

For comparison of baseline characteristics, we used the Student's *t*-test or, in the case of nonparametrical distribution, the Mann-Whitney test. For comparison of categorical variables, Pearson's χ^2-test was used. To estimate patient survival, graft survival, and acute-rejection-free survival we used the Cox proportional-hazards regression analysis. For the estimation of patient survival and graft survival, we included the following variables: use of tacrolimus or Neoral, use of azathioprine or MMF, HLA-A-, HLA-B-, and HLA-DR-mismatch (0 vs ≥ 1), transplant type (postmortem vs living), transplant number (1 vs > 1), preservation solution (University of Wisconsin solution vs histidine-tryptophan-ketoglutarate vs machine preservation), both warm (minutes) and cold (hours) ischemia times, peak PRA level (%), donor age (years), donor gender (male vs female), recipient age (years), recipient gender (male vs female), original renal disease (glomerulonephritis vs pyelonephritis vs nephrosclerosis vs diabetes mellitus type I vs diabetes mellitus type II vs other), and the presence of rejection within the first 6 months (yes vs no). For the estimation of acute-rejection-free survival, donor gender, both warm and cold ischemia times, transplant type, preservation solution, and the original renal disease were not suspected as being risk factors and were thus not analyzed. A basic model was tested for, which had to include only direct odds ratio effects that were statistically significant. When two factors were highly associated, the one that was more strongly related to the dependent variable was chosen. The possibility of interactions between factors in the basic model (different relative effects within different subgroups) was not taken into account since too many additional terms would have had to have been included to test for such effects. These analyses were made on an intention-to-treat basis.

Changes in clinical outcome variables were assessed by a repeated-measures analysis of variance (ANOVA). In the case of nonparametrical distribution, a log transformation was performed. Recipients were included until graft loss, conversion to the opposite drug, or the moment they were lost to follow-up. Measurements were made at 3, 6, 9, and 12 months after transplantation. Orthogonal polynomial contrasts were used to calculate linear, quadratic, etc. effects separately therein. The other factor was between patients: tacrolimus versus Neoral. The time-by-group interaction indicates differences in polynomial time trends. Overall effects are calculated in *F* ratios with degrees of freedom (*df*) and *P* values for nonsignificance. When Mauchly's *W*-test showed *P* values lower than 0.05, sphericity of the variance-covariance matrix of repeated measures was assumed to be violated. Univariate tests of *F* ratios were then conservatively corrected using an epsilon value suggested by Greenhouse and Geisser to correct degrees of freedom. Specific contrasts were only interrupted when overall effects compromising them showed statistical significance. A *P* value below 0.05 was considered to be statistically significant.

Table 1 Baseline characteristics. Values are expressed as mean (range) or as numbers (%) (*CyA* cyclosporine A, *PRA* panel reactive HLA-antibodies, *HB* heart-beating)

	CyA-Neoral ($n = 74$)	Tacrolimus ($n = 54$)	P value
Age (years)	48.5 (17.5–73.4)	51,2 (20.6–67.5)	0.25[a]
Gender male / female (n)	47 (63.5) / 27 (36.5)	36 (66.7) / 18 (33.3)	0.71[b]
Mean arterial pressure (mmHg)	103.5 (70–140)	104.7 (73–137)	0.62[a]
Hypertension (n)	66 (89.2)	48 (88.9)	0.96[b]
Anti-hypertensive drugs	1.46 (0–4)	1.48 (0–5)	0.32[b]
Cholesterol (mmol/l)	5.5 (3.1–8.9)	5.3 (2.5–7.1)	0.54[a]
On lipid-lowering drugs (n)	9 (12.2)	6 (11.1)	0.86[b]
Diabetes mellitus (n)	5 (6.8)	2 (3.7)	0.58[b]
HLA-A (≥ 1 mismatch) (n)	49 (66.2)	40 (74.1)	0.34[b]
HLA-B (≥ 1 mismatch) (n)	49 (66.2)	43 (79.6)	0.10[b]
HLA-DR (≥ 1 mismatch) (n)	41 (55.4)	36 (66.7)	0.20[b]
Transplant number (n)			0.004[b]
= 1	66 (89.2)	37 (68.5)	
> 1	8 (10.8)	17 (31.5)	
PRA peak serum (n)			0.03[b]
≤ 5 %	60 (81.6)	33 (61.1)	
= 5–85 %	9 (12.2)	15 (27.8)	
≥ 85 %	3 (4.1)	5 (9.3)	
missing	2 (2.7)	1 (1.9)	
Donor age (years)	44.3 (4.4–72.5)	46.0 (4.8–69.6)	0.58[a]
Donor gender male / female (n)	42 (56.8) / 32 (43.2)	27 (50.0) / 27 (50.0)	0.45[b]
Donor type (n)			0.004[b]
post-mortal HB	46 (62.2)	30 (55.6)	
post-mortal non-HB	16 (21.6)	14 (25.9)	
living-related	12 (16.2)	3 (5.6)	
living-unrelated	0 (0.0)	7 (13.0)	
Cold ischemia time (h)[d]	27.6 (16–43)	25.3 (8–50)	0.12[a]
Warm ischemia time 1 (min)	14.8 (0–192)	17.3 (0–148)	0.44[c]
Warm ischemia time 2 (min)	34.3 (18–65)	35.6 (23–80)	0.47[c]

[a] Student's t-test
[b] Pearson's χ^2-test
[c] Mann-Whitney test
[d] Living donor excluded

Results

In the study period, 128 consecutive renal transplantations were performed. Seventy-four recipients primarily received Neoral and 54 received tacrolimus. Immunological features were less favorable in the tacrolimus group (Table 1). More patients underwent a re-transplantation (31.5 % vs 10.8 %, $P = 0.004$, χ^2-test). More patients were immunized or highly immunized (PRA 5–85 %: 27.8 % vs 12.2 %, and PRA ≥ 85 %: 9.3 % vs 4.1 %, $P = 0.03$, χ^2-test). In addition, more patients received a kidney from living-unrelated donors (13 % vs 0 %). Other donor and transplant characteristics were comparable between the two groups (Table 1). Causes of death of the donors and type of preservation did not differ significantly either between the two groups. Differences did exist in the diagnosis of renal insufficiency ($P = 0.006$, χ^2-test): more patients with chronic glomerulonephritis (35.2 % vs 14.9 %) and nephrosclerosis (18.5 % vs 8.1 %) were included in the tacrolimus group; more patients with polycystic kidney disease (21.6 % vs 9.3 %) and miscellaneous causes (36.5 % vs 16.7 %) were included in the Neoral group. Diabetic nephropathy was the diagnosis in 3.7 % of the tacrolimus group versus 6.8 % of the Neoral group.

MMF was included in the primary immunosuppression for 18 patients: 15 participated in a tacrolimus-based trial, three received MMF on a compassionate-use basis. The use of azathioprine was not significantly different in the two groups (16.7 % in the tacrolimus group vs 20.3 % in the Neoral group, $P = 0.61$, χ^2-test).

Primary endpoints

Patient survival

In the tacrolimus group, four patients died with a functioning graft (7.4 %) versus three (4.1 %) in the Neoral group ($P = 0.39$, χ^2-test). In both groups two patients died of infectious complications and one patient of se-

Table 2 Cox proportional-hazards regression analysis (*OR* odds ratio, *CI* confidence interval, *df* degrees of freedom, *PRA* panel reactive HLA-antibodies, *WIT-2* second warm ischemia time, *CIT* cold ischemia time)

Risk factor	OR	95% CI	*df*	*P* value
Patient survival				
age recipient (/year)	1.14	1.01–1.29	1	0.006
donor age (/year)	1.06	0.99–1.13	1	0.05
peak PRA (/%)	1.03	1.01–1.06	1	0.006
Neoral vs tacrolimus	0.85	0.16–4.5	1	0.85
Graft survival				
HLA-DR (≥ 1 mismatch vs 0)	2.9	1.1–7.4	1	0.01
donor age (/year)	1.03	1.00–1.06	1	0.01
WIT-2 (/min)	1.04	1.01–1.08	1	0.03
CIT (/h)	1.05	1.01–1.10	1	0.01
Neoral vs tacrolimus	0.8	0.4–1.8	1	0.61
Rejection-free survival				
HLA-DR (≥ 1 mismatch vs 0)	2.5	1.4–4.5	1	0.002
Neoral vs tacrolimus	4.6	2.3–9.2	1	< 0.0001

vere hemorrhage. The other patient in the tacrolimus group died of a myocardial infarction.

In the Cox regression analysis, recipient age, donor age, and peak PRA level were significant risk factors contributing to patient survival (Table 2). The type of immunosuppression (Neoral-based vs tacrolimus-based) did not contribute significantly to patient survival. The odds ratio for Neoral versus tacrolimus was 0.85 (95% confidence interval: 0.2–4.5; $P = 0.85$). The predicted 2-year survival was 98% in the tacrolimus group and 99% in the Neoral group.

Graft survival

Graft loss and graft loss, censored for death, were comparable between both groups (tacrolimus vs Neoral: 24.1% vs 20.2%, $P = 0.61$, and 16.7% vs 16.2%, $P = 0.95$, respectively, χ^2-test). When focusing on graft failure due to immunological causes, significantly fewer failures were observed in the tacrolimus group (3.7% vs 12.2%, $P = 0.02$, χ^2-test).

In the Cox regression analysis, the presence of an HLA-DR-mismatch, donor age, cold ischemia time, and second warm ischemia time were significant risk factors contributing to graft survival (Table 2). The type of immunosuppression (Neoral-based vs tacrolimus-based) did not contribute significantly to graft survival. The odds ratio for Neoral versus tacrolimus was 0.82 (95% confidence interval: 0.4–1.8; $P = 0.61$). The predicted 2-year graft survival was 84% in the tacrolimus group and 87% in the Neoral group (Fig. 1).

In the Cox regression analysis of graft survival, censored for death, only the presence of an HLA-DR-mismatch contributed significantly to graft failure: the odds ratio was 5.1 (95% confidence interval: 1.5–17.4; $P = 0.002$). Again, the type of immunosuppression did not contribute significantly to graft failure. The odds ratio for Neoral versus tacrolimus was 0.95 (0.4–2.2; $P = 0.90$). The predicted 2-year graft survival, censored for death, was 87% in both groups.

Acute rejection

Fifty-four patients were treated for acute rejection. All but four of these episodes were biopsy-confirmed. Three patients had no biopsy performed due to contraindications, and for one patient there was insufficient bi-

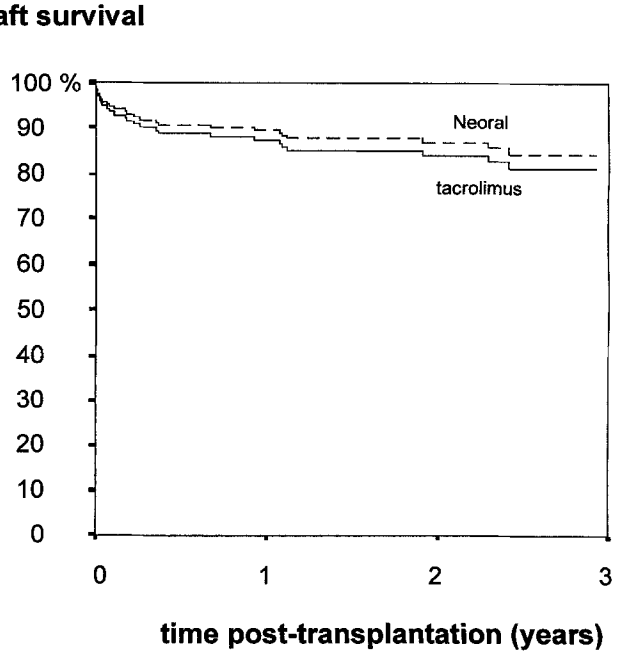

Fig. 1 Predicted graft survival. Significant risk factors using Cox proportional-hazards regression analysis were the presence of a mismatch in HLA-DR, donor age, and cold as well as second warm ischemia time. The odds ratio for Neoral vs tacrolimus was 0.8 (95% confidence interval: 0.4–1.8, $P = 0.61$)

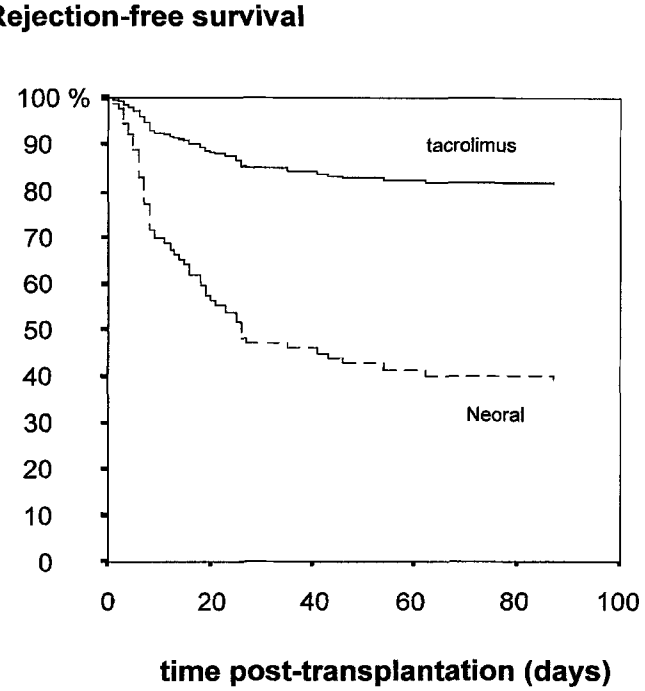

Fig. 2 Predicted acute-rejection-free survival. Significant risk factors using Cox proportional-hazards regression analysis were the presence of a mismatch in HLA-DR and the use of Neoral vs tacrolimus: odds ratio 4.6 (95 % confidence interval: 2.3–9.2, $P < 0.0001$)

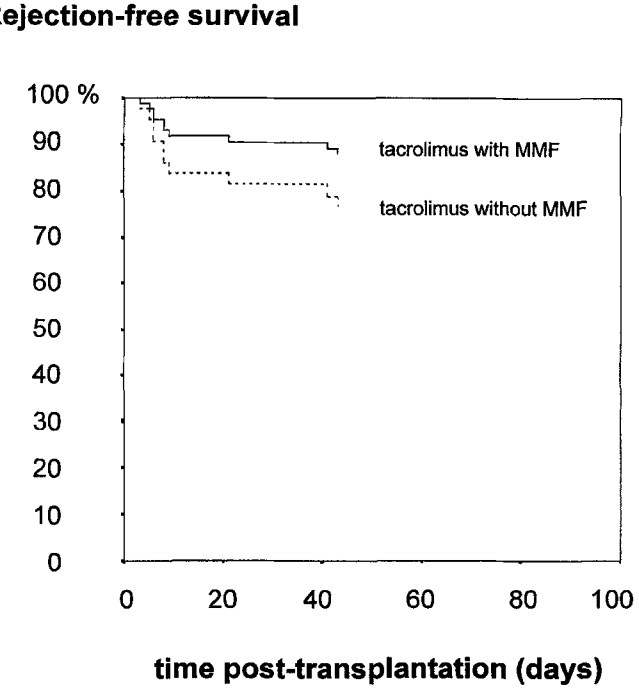

Fig. 3 Predicted acute-rejection-free survival using Cox proportional-hazards regression analysis among patients treated with tacrolimus without mycophenolate mofetil (*MMF*) ($n = 36$) vs those treated with both tacrolimus and MMF ($n = 18$): odds ratio 2.0 (95 % confidence interval: 0.4–9.7, $P = 0.37$)

opsy material. Nevertheless, in connection with a second episode of acute rejection, the diagnosis could be biopsy-confirmed for two of these patients. The remaining two patients showed good clinical response upon antirejection treatment with a return of serum creatinine to baseline.

In the tacrolimus group, significantly fewer acute rejections were observed: 18.5 % versus 59.5 % for primary acute rejection within the first 6 months ($P < 0.001$, χ^2-test) and 7.4 % versus 33.8 % for second acute rejection ($P < 0.001$, χ^2-test). In the tacrolimus group, fewer patients were treated with r-ATG: 5.6 % versus 13.5 %, but this did not reach statistical significance ($P = 0.14$, χ^2-test).

In the Cox regression analysis, the use of Neoral and the presence of an HLA-DR-mismatch were significant risk factors contributing to acute-rejection-free survival (Table 2). The odds ratio for Neoral versus tacrolimus was 4.6 (95 % confidence interval: 2.3–9.2; $P < 0.0001$). The predicted acute-rejection-free survival was 82 % in the tacrolimus group versus 40 % in the Neoral group (Fig. 2).

To test the possibility that the observed difference was due to the use of MMF in a subgroup of tacrolimus-treated patients, we analyzed acute-rejection-free survival in two ways: (1) for patients with tacrolimus without MMF ($n = 36$) versus patients with tacrolimus and MMF ($n = 18$), and (2) for patients with tacrolimus without MMF ($n = 36$) versus the Neoral group ($n = 74$). The first analysis showed that the relative risk of rejection was not significantly higher in the patients without MMF (odds ratio 2.0, 95 % confidence interval: 0.4–9.7; $P = 0.37$). The estimated acute-rejection-free survival was 77 % in the patients treated without MMF versus 88 % for the patients treated with tacrolimus and MMF (Fig. 3). The second analysis showed that the increased risk of rejection in the Neoral group remained highly significant (odds ratio for Neoral 3.8, 95 % confidence interval: 1.8–8.1; $P = 0.0006$). The predicted rejection-free survival was 78 % in the tacrolimus group versus 40 % in the Neoral group. The relative distribution of the severity of the rejection episodes was not significantly different between the patients in the tacrolimus and the Neoral groups ($P = 0.43$ for the Banff score of the first rejection and $P = 0.31$ for the maximum Banff score under the initial drug, χ^2-test; Table 3).

Significantly more patients were converted from Neoral to tacrolimus than from tacrolimus to Neoral (31.1 % vs 3.7 %, $P < 0.001$, χ^2-test). The main reason for conversion from Neoral to tacrolimus was an episode of acute rejection (21 out of 23 patients). No patients were converted from tacrolimus to Neoral be-

Table 3 Severity of rejection according to the 1997 Banff classification (*CyA* cyclosporin A)

	CyA-Neoral ($n = 44$)	Tacrolimus ($n = 10$)	P value (χ^2-test)
Banff score first rejection			0.43
borderline	3 (6.8)	3 (30.0)	
1 A	17 (38.6)	3 (30.0)	
1 B	13 (29.5)	2 (20.0)	
2 A	5 (11.4)	2 (20.0)	
2 B	1 (2.3)	0 (0.0)	
3	0 (0.0)	0 (0.0)	
hyperacute	1 (2.3)	0 (0.0)	
no histology	4 (9.1)	0 (0.0)	
Maximum Banff score under initial drug			0.31
borderline	2 (4.5)	2 (20.0)	
1 A	14 (31.8)	2 (20.0)	
1 B	16 (36.4)	3 (30.0)	
2 A	5 (11.4)	3 (30.0)	
2 B	4 (9.1)	0 (0.0)	
3	0 (0.0)	0 (0.0)	
hyperacute	1 (2.3)	0 (0.0)	
no histology	2 (4.5)	0 (0.0)	

cause of rejection. Three patients were converted after the first episode of acute rejection, 14 after the second, and four after the third. Sixteen patients (76.2%) were spared further acute rejections. In the remaining five patients, renewed acute rejection occurred 12, 29, 34, 180, and 1139 days after conversion. In two of these five patients, the rejection occurred after steroid withdrawal. In three of the five patients, the grafts were lost due to ongoing rejections.

In the tacrolimus group, steroids could be withdrawn from significantly more patients (79.6% vs 41.9%, $P < 0.001$, χ^2-test). At the time of steroid withdrawal, only four patients still used MMF (9.3% of the patients in which the steroids were stopped). After the discontinuation of steroids, three patients had an acute rejection 34, 126, and 482 days after steroid withdrawal. None were in the initial tacrolimus group.

At the end of the follow-up period, 70.4% of the patients in the tacrolimus group versus 27.0% in the Neoral group were actually on monotherapy ($P < 0.001$, χ^2-test). Censored for conversion, 68.5% in the tacrolimus group versus 14.9% in the Neoral group were on monotherapy ($P < 0.001$, χ^2-test).

Secondary outcome parameters

Because a considerable proportion of the patients in the Neoral group were converted to tacrolimus, only those patients who were still on the primary drug at 12 months after transplantation and who had a complete follow-up were considered for the analysis of possibly drug-related clinical outcome parameters. In the tacrolimus group, one patient was converted, four patients had died, five grafts had failed, and two patients had an incomplete follow-up. In the Neoral group, 15 patients were converted, three patients had died, eight grafts had failed, and two patients had an incomplete follow-up. So, 88 patients were eligible, 42 in the tacrolimus group and 46 in the Neoral group.

Renal function

Creatinine clearance increased during the first year in both groups and stabilized between 9 and 12 months (Fig. 4). At none of the time points were the differences between the two groups statistically significant ($P > 0.30$).

Proteinuria was log-transformed because of a non-parametrical distribution. Log-transformed proteinuria showed a decrease in both groups up to 9 months and an increase afterwards (Fig. 5). Differences in proteinuria were not statistically significant between the two groups at any time point ($P > 0.20$).

Fractional uric acid clearance decreased in a similar way in both groups (Greenhouse-Geisser-corrected F ratio = 10.85 by 2 and 206 *df*, $P < 0.001$). There were no statistically significant differences between the two groups at any time point ($P > 0.25$).

Cardiovascular risk profile

Mean blood pressure showed no significant trend with time (F ratio = 1.06 by 3 and 258 *df*, $P = 0.37$; Fig. 6). Differences between the two groups were not significant at any time point ($P > 0.10$).

Although blood pressure was comparable for both groups, patients in the Neoral group used significantly

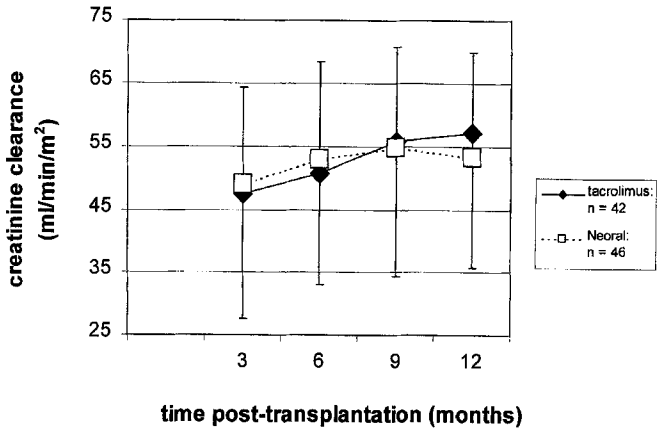

Fig. 4 Creatinine clearance. In both groups creatinine clearance increased during the first year and stabilized between 9 and 12 months after transplantation (ANOVA). Differences at each time point were not significant ($P > 0.30$, Student's t-test)

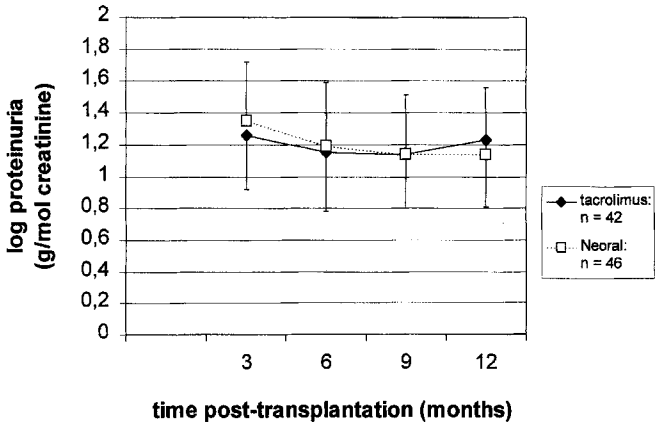

Fig. 5 Proteinuria. Proteinuria was log-transformed due to non-parametrical distribution. In both groups proteinuria decreased up to 9 months after transplantation and increased thereafter (ANOVA). Differences at each time point were not significant ($P > 0.20$, Student's t-test)

more anti-hypertensive drugs at 6, 9, and 12 months ($P = 0.09$, $P = 0.008$, $P = 0.001$, and $P < 0.001$ at 3, 6, 9, and 12 months, respectively, χ^2-test; Fig. 7). At 12 months after transplantation, 21.4% of the patients in the tacrolimus group versus 4.3% in the Neoral group were free of the use of anti-hypertensive drugs. Three drugs or more were used by 7.1% in the tacrolimus group versus 52.2% in the Neoral group.

Total cholesterol was significantly higher at all time points in the Neoral group ($P = 0.04$, $P = 0.03$, $P < 0.001$, and $P = 0.003$ at 3, 6, 9, and 12 months after transplantation, respectively, Student's t-test; Fig. 8). Notwithstanding the higher total cholesterol in the Neoral group, more patients in this group used lipid-lowering drugs, statistically significant up to 6 months

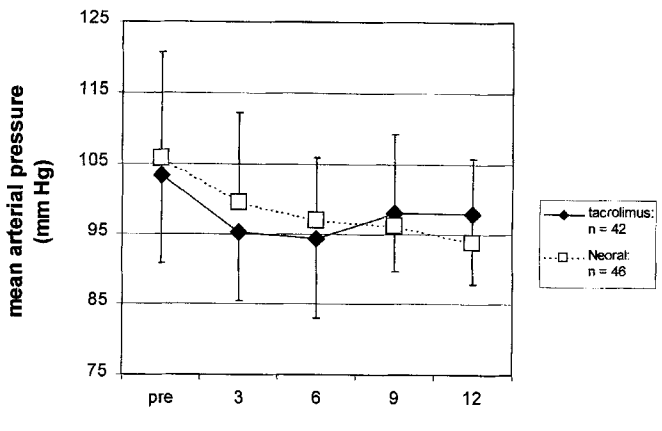

Fig. 6 Mean arterial pressure. The mean arterial pressure showed no significant trend in time (ANOVA). Differences at each time point were not significant ($P > 0.10$, Student's t-test)

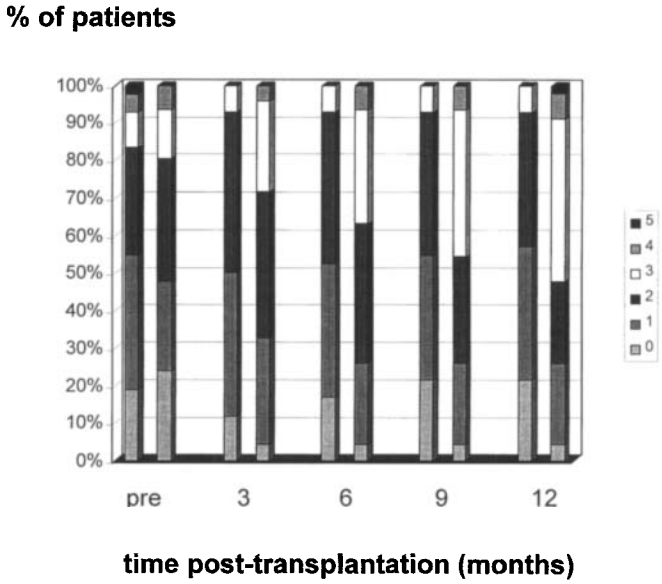

Fig. 7 Anti-hypertensive medication. *Right column* at each time point: Neoral group ($n = 46$); *left column:* tacrolimus group ($n = 42$). The number of anti-hypertensive drugs the patients were using is expressed in the columns. $P = 0.09$, 0.008, 0.001, and < 0.001 at 3, 6, 9, and 12 months after transplantation, respectively (χ^2-test). Diuretics are not included

($P = 0.004$ and $P = 0.004$ at 3 and 6 months after transplantation, respectively, χ^2-test; Fig. 9).

The overall incidence of post-transplant diabetes mellitus on an intention-to-treat analysis was higher in the tacrolimus group, but the difference was not statistically significant (18.5% in the tacrolimus group vs 10.8% in the Neoral group, $P = 0.22$, χ^2-test). The need for treatment with insulin at any time after transplantation was nearly identical (7.4% in the tacrolimus group

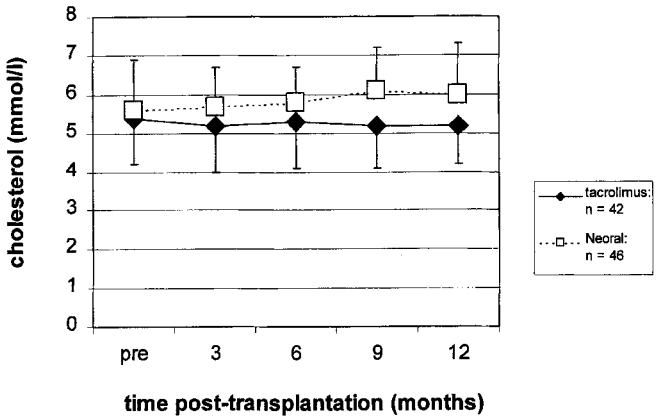

Fig. 8 Total cholesterol. Total cholesterol showed no significant trend in time (ANOVA). Differences at each time point were significant: $P = 0.04$, 0.03, < 0.001, and 0.003 at 3, 6, 9, and 12 months after transplantation, respectively (Student's t-test)

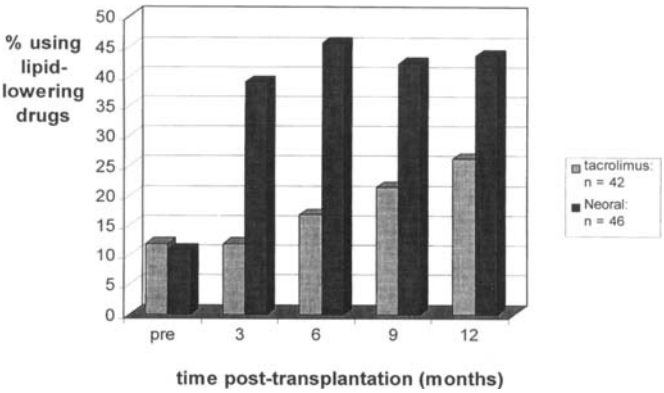

Fig. 9 Use of lipid-lowering drugs. Differences between the tacrolimus and Neoral group were significant at 3 and 6 months after transplantation: $P = 0.004$, 0.004, 0.09, and 0.18 at 3, 6, 9, and 12 months, respectively (χ^2-test)

vs 6.8% in the Neoral group, $P = 0.89$, χ^2-test). In connection with the patients needing treatment for diabetes, all anti-diabetic medication could be withdrawn for 70% in the tacrolimus group versus 50.0% in the Neoral group ($P = 0.51$, χ^2-test).

Conversion for side effects

Besides conversion for acute rejection, two other patients were converted from Neoral to tacrolimus 1076 and 628 days after transplantation. For one patient it was because of gingival hyperplasia, and for the other it was because of osteoporosis and the need for steroid withdrawal despite a prior acute rejection. The further course of these two patients was uneventful.

Two patients were converted from tacrolimus to Neoral 505 and 25 days after transplantation because of side effects possibly related to tacrolimus: Guillain-Barré syndrome and thrombotic thrombocytopenic purpura, respectively. After conversion, no acute rejections were observed, but in the latter patient MMF was added due to prior rejections. This graft failed due to the recurrence of focal segmental glomerulosclerosis.

Discussion

In former trials, tacrolimus-based immunosuppression had been shown to be superior to cyclosporine (Sandimmune) with regard to the prevention of acute and steroid-resistant rejection [17, 26, 33, 40, 52]. Neoral, the new formulation of cyclosporine, has proven to be superior to Sandimmune in the prevention of acute rejection [20, 31, 34]. Criticism arose that it has not yet been proven that tacrolimus is superior to Neoral. One group from Cardiff had published two interim reports of a prospective trial. In the first report, tacrolimus and Neoral yielded similar results [29]. In the second report, tacrolimus was superior to Neoral [19]. In our single-center study, we compared all consecutive renal transplant recipients treated with Neoral- or tacrolimus-based immunosuppression during the same time period. Because tacrolimus was not yet registered in the Netherlands, for much of the period studied, a substantial number of the recipients received this drug on a compassionate-use basis. This is reflected in a less favorable immunological profile in the tacrolimus group. More patients underwent re-transplantation and had positive PRA levels. With regard to living donors, more grafts were received from living-unrelated donors. On the other hand, a proportion of tacrolimus-treated patients received MMF. Patient and graft survival were comparable, but we observed a substantial lower rate of first and relapsing acute rejections and graft loss due to immunological causes in the tacrolimus group.

The predicted 2-year patient survival rate was 98% in the tacrolimus group. This is comparable to the findings of other studies with tacrolimus [10, 17, 26, 33, 42, 43, 52, 55]. In this study, a similar patient survival rate was observed between tacrolimus- and Neoral-based immunosuppression. This is in concordance with similar patient survival in studies comparing tacrolimus with cyclosporine [17, 26, 33, 42, 52] and in a retrospective study comparing tacrolimus with Neoral [10].

The 2-year predicted graft survival rate with tacrolimus was 84%. This is comparable to what has previously been reported with tacrolimus [11, 17, 19, 26, 33, 42, 43, 52, 55]. In previous studies comparing tacrolimus with cyclosporine, graft survival was comparable [17, 26, 33, 43, 52]. In our study comparing tacrolimus with Neoral, graft survival was the same as that reported by

Ghasemian et al. [10]. In contrast, in his interim report, Jurewicz reported a better graft survival with tacrolimus than with Neoral [19].

Acute-rejection-free survival was halved in the tacrolimus group. The rate of acute rejection in the tacrolimus group was 18.5%, comparable to the 19.4% [40] and 25.9% [26] in former European multicenter trials. By participating in those trials, our center acquired a lot of experience with tacrolimus. Such experience is important since, in a European multicenter trial [51], the rate of acute rejection was lower in centers with experience with this drug [46, 51]. In American and Japanese studies, the rate of rejection was higher (30%–55%) [33, 42, 43, 44, 55]. Race (in this case, being black) and age (being young) are risk factors for rejection [15, 23, 30, 38]. Differences in these factors in the patient groups could be an explanation for the higher rate of acute rejection with tacrolimus.

In the tacrolimus group, 25 patients (46.3%) participated in a trial which could have led to bias in selection and management. Within the trial period, nearly all consecutive transplant patients participated in that study. Five well-matched living-related transplants and only one patient who met the exclusion criteria were treated with Neoral. Beside that, four living-unrelated transplants in that period were treated with tacrolimus outside of the trial. If any selection bias had occurred, it would have been in favor of the Neoral group. In addition, an even larger proportion in the Neoral group (54.1%) participated in a trial.

Furthermore, outside of the trial period, the majority of tacrolimus-treated patients belonged to a high-risk category. Thus, for the whole population studied, selection bias was in favor of the Neoral group. This is reflected in a significantly higher proportion of re-transplants and higher PRA levels in the tacrolimus group. Also, HLA-matching was better in the Neoral group.

Throughout the study period, all patients were treated by the same group of surgeons and physicians. Pre- and post-transplant management and diagnosis and treatment of rejection were unchanged and were the same for Neoral-treated and tacrolimus-treated patients.

The addition of MMF to the immunosuppressive regimen of a certain number of patients in the tacrolimus group does not fully explain the high rejection-free survival in this study because rejection-free survival in patients treated with tacrolimus without MMF was 77%, compared to 88% in patients treated with tacrolimus and MMF ($P = 0.37$). After exclusion of the patients who received MMF, the difference with the Neoral group (78% vs 40%) remained highly significant ($P = 0.0006$). Moreover, the duration of the use of MMF was relatively short. Only one patient received MMF throughout the study period. For the remaining patients, MMF was stopped after a mean of 106 days (range: 6–254 days).

The rate of rejection in the Neoral group in this study resembles the rate found earlier in Sandimmune-treated recipients [20, 23, 31, 42]. In recent studies, the rate of rejection with Neoral is around 40% [10, 19, 20, 29, 31, 34]. In some of these studies, the upper target trough level of cyclosporine was higher, with values of up to 0.3–0.4 mg/l [10, 34]. Higher dosing results in a lower rate of acute rejection [23, 41], but poses the risk of dose-dependent nephrotoxicity [27] and may even have a negative impact on graft survival [23]. Also, in most studies, more patients received triple therapy with the addition of azathioprine or MMF [10, 19, 20, 29, 31] and, where mentioned, the steroid dose was higher [19, 29]. In one study, high-risk recipients were excluded [34].

In our study, nearly all rejections were biopsy-proven, and classification as borderline was scarce. Thus, the high incidence of acute rejection cannot be the result of overtreatment of clinical suspicion of acute rejection. To test the possibility of underdosing of Neoral, blood levels at the time of first rejection were analyzed. The mean cyclosporine trough level was 0.22 mg/l (SD = 0.11). Only nine patients with rejection (20.5%) had a level below the lower target level. Thus, only in a minority might underdosing be the explanation for rejection; it cannot account for the relatively high incidence of rejection in Neoral-treated patients.

In the studies comparing the rejection rate among patients treated with tacrolimus versus Neoral, Ghasemian et al. and Jurewicz, in the second interim report of the Cardiff study group, reported a comparable rate of acute rejection with tacrolimus and twice-as-high an incidence in the Neoral group [10, 19]. Yet in the initial interim report of the Cardiff study group, Morris-Stiff et al. reported a substantially higher rate of acute rejection (40%) with tacrolimus [29]. This latter observation can probably be explained by their relatively low initial tacrolimus target levels of 5–15 ng/ml. It is well proven that the risk of acute rejection is enhanced when initial target levels are below 10 ng/ml [49].

An important risk factor for acute rejection is the degree of HLA-matching, especially for HLA-DR [23, 37]. When corrected for this risk factor, the rate of acute rejection for tacrolimus-treated patients, compared to Neoral-treated patients, was still highly significant. From the reported data, differences in HLA-DR-mismatches between this study and other studies could not be obtained [17, 33, 42, 43, 55]. It therefore remains unclear whether poorer matching for HLA-DR is an additional explanation for the higher rate of acute rejection with tacrolimus in those studies.

Acute rejection, and especially steroid-resistant rejection, is a risk factor for the development of chronic allograft rejection, a major cause of graft loss after 1 year [1, 2, 16, 22, 23, 24, 25, 45, 50]. In our study, no improvement in graft survival was found, but the lower in-

cidence of acute rejections might have had an impact on improvement in long-term graft survival [11].

When a rejection occurred, the relative severity was comparable between tacrolimus and Neoral. This means that tacrolimus equally reduces all grades of rejection, not only borderline and grade 1. Our study is, to our knowledge, the first one that demonstrates the lower incidence of all grades of acute rejection with tacrolimus in relation to Neoral. In a previous multicenter study, the same result was found with Sandimmune [26].

A unique property of tacrolimus is that, if irreversible rejection occurs after conversion from cyclosporine to tacrolimus, the graft can be saved [18, 28, 33, 53, 54]. Similar observations after conversion from Neoral to tacrolimus are scarce [10, 29]. In our study, 76.2 % of the patients converted to tacrolimus for acute rejection with Neoral experienced no further rejection. Thus, the results obtained with Sandimmune could be confirmed.

Steroid-related side effects are a major cause of morbidity in renal transplant recipients [3]. In nearly 80 % of the patients in the tacrolimus group could steroids be withdrawn. None had a rejection after withdrawal, and only 9.3 % of these patients received MMF at the time of steroid withdrawal. Other groups have also reported a reasonable number of patients without steroids, ranging from 35 % to 55 % [42, 43, 44]. Only one study reported a comparison with Sandimmune-treated patients, in which all Sandimmune-treated patients received some dose of steroids [42]. We were able to withdraw steroids in 42 % of the Neoral-treated patients, but that was significantly less than in the tacrolimus group. It was recently demonstrated that it is probably safe to withdraw steroids in cyclosporine-treated, non-black patients when MMF is added [47]. In our study, 68.5 % of the patients in the tacrolimus group were successfully put on monotherapy versus only 14.9 % in the Neoral group. In our opinion, the safety of steroid withdrawal in patients without acute rejection is another advantage of tacrolimus.

It has been reported that the need for treatment with r-ATG for steroid-resistant rejection was significantly lower with tacrolimus than with either Sandimmune [26, 33] or Neoral [10]. Although we also observed a lower need for r-ATG, the difference was not statistically significant, as in the study of Jurewicz [19].

Both tacrolimus and cyclosporine are nephrotoxic drugs [27]. In our study, renal function in patients with a functioning graft 12 months after transplantation was comparable with tacrolimus and Neoral. This is in accordance with previous reports in which tacrolimus was compared to Sandimmune [17, 26, 33, 40, 42, 52] or Neoral [10, 29]. In contrast, Jurewicz reported a lower serum creatinine in the tacrolimus group than in the Neoral group [19]. Data on differences in proteinuria between tacrolimus and Sandimmune or Neoral have not been published. We found a similar rate of proteinuria between tacrolimus and Neoral. Also, fractional uric acid clearance was comparable in our study. In another study, serum uric acid increased more over time with Neoral, but the difference with tacrolimus was not significant and could have been influenced by the higher serum creatinine in the Neoral group in that study [19].

Both hypertension and hyperlipidemia have been associated with long-term graft outcome [7, 32]. The targets for blood pressure and cholesterol were the same for both groups, whether or not the patients participated in a trial. Differences in management of these parameters cannot account for differences between the groups. The cardiovascular risk profile in our study was more favorable with tacrolimus than with Neoral. Although mean arterial pressure was comparable, the need for anti-hypertensive drugs was lower in the tacrolimus group and more patients were without anti-hypertensive treatment. This is in accordance with previous reports in which tacrolimus was compared to both Sandimmune [17, 33, 40, 42] and Neoral [19].

Total cholesterol was also lower in the tacrolimus group, even with fewer patients taking lipid-lowering drugs. Previous studies have shown similar results when comparing tacrolimus with Sandimmune [17, 33, 42] or Neoral [19, 29]. After steroid withdrawal, both hypertension and the lipid profile improve [47]. In our study, a considerable proportion of tacrolimus-treated patients were not on steroids. This could have influenced the more favorable cardiovascular risk profile. However, the more favorable lipid profile persists [5, 14, 33], and there are indications that less anti-hypertensive treatment is necessary with tacrolimus in studies with equal doses of steroids [13, 36]. Thus, the results in our study can probably not be totally explained by the lower use of steroids.

A considerable proportion of patients in the Neoral group (25.4 % of patients with 1-year follow-up) in our study were converted to tacrolimus, in all cases because of acute rejection. Since we wanted to evaluate the differences in drug-related side effects between the two drugs and the development of parameters over time after transplantation, analyses were made after censoring for conversion. This could have influenced the composition of the study groups. We re-analyzed the parameters on an intention-to-treat basis. One year after transplantation, the following parameters analyzed by means of the Student's *t*-test were still comparable between the tacrolimus group ($n = 44$) and the Neoral group ($n = 61$): creatinine clearance: 56.0 ml/min vs 50.0 ml/min ($P = 0.12$), log-transformed proteinuria: 1.28 g/mol creatinine vs 1.20 g/mol creatinine ($P = 0.39$), fractional uric acid clearance: 9.5 % vs 9.7 % ($P = 0.87$), and mean blood pressure: 97.7 mmHg vs 95.0 mmHg ($P = 0.25$). In the tacrolimus group, still less patients used anti-hypertensive drugs (mean 1.3 vs 2.3, $P < 0.001$). The differences in cholesterol (5.4 mmol/l vs 5.8 mmol/l, $P = 0.10$)

and the percentage of patients on lipid-lowering drugs (27.3% vs 38.7%, $P = 0.37$) were attenuated, but still less favorable in the Neoral group. We also analyzed the possible influence of MMF on secondary outcome. All clinical outcome parameters were well comparable between patients treated with tacrolimus and MMF and those treated with tacrolimus without MMF. Thus, MMF seems to play no role for these parameters.

In previous studies comparing tacrolimus with Sandimmune, the incidence of post-transplant diabetes was higher in patients taking tacrolimus and ranged from 15% to 20% [17, 26, 33, 43, 52]. We also found a comparable incidence of 18.5% in the tacrolimus group, with a similar need for treatment with insulin as in the Neoral group at any time after transplantation. After tapering the dose of tacrolimus and withdrawing steroids, 70% of the patients with post-transplant diabetes could stop taking all medication. The reversibility of post-transplant diabetes is in accordance with other studies [17, 26, 43, 55]. The percentage of patients who persisted on antidiabetic medication was similar with tacrolimus and Neoral. In the first interim report of the ongoing prospective study, Morris-Stiff et al. reported a similar incidence of diabetes with tacrolimus and Neoral [29], but in that study tacrolimus was initially relatively low-dosed.

In summary, this study compared tacrolimus-based and Neoral-based immunosuppression. A substantially lower rate of first and relapsing acute rejection and fewer failures due to immunological causes were observed with tacrolimus. Furthermore, a higher proportion of patients could safely have steroids withdrawn and be put on monotherapy. We hereby extend the findings that tacrolimus is more effective than either Sandimmune or Neoral. Renal function was comparable, but the cardiovascular risk profile was more favorable, with lower total cholesterol and fewer patients needing drugs for the treatment of hypertension and hypercholesterolemia. In the initial phase after transplantation, the incidence of post-transplant diabetes mellitus was higher, but after lowering the dose of tacrolimus and withdrawing from steroids, the need to treat post-transplant diabetes was identical.

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