J Am Soc Nephrol 13: 1365–1373, 2002

Withdrawal of Cyclosporine or Prednisone Six Months after Kidney Transplantation in Patients on Triple Drug Therapy: A Randomized, Prospective, Multicenter Study

PETER J. H. SMAK GREGOOR,* RUUD G. L. DE SÉVAUX,† GERRY LIGTENBERG,‡ ANDRIES J. HOITSMA,† RONALD J. HENÉ,‡ WILLEM WEIMAR,* LUUK B. HILBRANDS,† and TEUN VAN GELDER*

**Department of Internal Medicine, University Hospital Rotterdam, Rotterdam, the Netherlands; †Department of Nephrology, University Medical Centre St. Radboud, Nijmegen, the Netherlands; and ‡Department of Nephrology, University Medical Centre Utrecht, Utrecht, the Netherlands.*

Abstract. Uncertainty exists regarding the necessity of continuing triple therapy consisting of mycophenolate mofetil (MMF), cyclosporine (CsA), and prednisone (Pred) after kidney transplantation (RTx). At 6 mo after RTx, 212 patients were randomized to stop CsA ($n = 63$), stop Pred ($n = 76$), or continue triple drug therapy ($n = 73$). The MMF dose was 1000 mg twice daily, target CsA trough levels were 150 ng/ml, and Pred dose was 0.10 mg/kg per d. Follow-up was until 24 mo after RTx. Biopsy-proven acute rejection occurred in 14 (22%) of 63 patients after CsA withdrawal compared with 3 (4%) of 76 in the Pred withdrawal group ($P = 0.001$) and 1 (1.4%) of 73 in the control group ($P = 0.0001$). Biopsy-proven chronic rejection was present in one patient in the control group, in nine patients after CsA withdrawal ($P = 0.006$ *versus* control group); and in four patients after discontinuation of Pred (NS). Graft loss occurred in two *versus* one patient after CsA or Pred withdrawal, respectively, and in two patients in the control group (NS). Patients who successfully withdrew CsA had a significantly lower serum creatinine during follow-up. Pred withdrawal resulted in a reduction in mean arterial pressure, and the total cholesterol/HDL ratio increased. In conclusion, rapid CsA withdrawal at 6 mo after RTx results in a significantly increased incidence of biopsy-proven acute and chronic rejection. Pred withdrawal was safe and resulted in a reduction in mean arterial pressure. However, patient and graft survival and renal function 2 yr after RTx were not different among groups.

The addition of mycophenolate mofetil (MMF) to cyclosporine (CsA) and prednisone (Pred) results in a decrease in the incidence of acute rejections during the first 6 mo after kidney transplantation (1–4). Whether or not MMF has an inhibitory effect on the development of chronic rejection, either resulting from fewer early acute rejection episodes or as a specific MMF-related effect, is still a matter of debate (4,5,6). Meanwhile, the possibility of over-immunosuppression associated with long-term continuation of triple drug therapy is a matter of concern. Furthermore, both CsA and Pred have specific drug-related adverse effects on cardiovascular risk factors that may negatively influence long-term outcome. Therefore, we performed a randomized, prospective multicenter study to compare the effect of withdrawing CsA or Pred from a triple drug regimen consisting of MMF, CsA, and Pred at 6 mo after renal transplantation in stable renal transplant patients, with patients continuing triple drug therapy as controls.

Materials and Methods

Between January 1997 and January 1999, 313 patients undergoing kidney transplantation in the university hospitals of Rotterdam, Utrecht, and Nijmegen in the Netherlands entered a study that evaluated the cyclosporine-sparing effect of MMF in the first 6 mo after transplantation, the results of which have been published in this journal (7). After completion of 6 mo of follow-up, 212 (68%) of 313 of these patients were enrolled and randomized to participate in this subsequent multicenter, open-label trial of CsA or Pred withdrawal.

During the first 6 mo after transplantation, 22 patients had lost their graft and 8 patients died with a functioning graft. Excluded from randomization were patients with two or more acute rejections during the first 6 mo after transplantation ($n = 15$), patients with biopsy-proven chronic vascular rejection ($n = 3$), patients with proteinuria of more than 3 g/d ($n = 2$), patients with an unstable graft function ($n = 9$), and patients not treated with triple drug therapy (MMF, CsA, Pred) at the time of randomization ($n = 29$). At the time of randomization, seven patients refused to participate in the study. In five cases, the treating physician decided not to ask the patient for participation because of multiple HLA-mismatches, previous severe acute rejection, or liver function disturbances. One patient was lost to follow-up before randomization. The study design was approved by the institutional review boards of the three participating hospitals, and written informed consent was obtained from all participants.

Received November 2, 2001. Accepted December 17, 2001.
Correspondence to: Dr. Peter Smak Gregoor, Department of Internal Medicine, Room D-412, University Hospital Rotterdam, P.O. Box 2040, 3000 CA Rotterdam, the Netherlands. Phone: 31-10-4639222; Fax: 31-10-4366372; E-mail: smakgregoor@inw1.azr.nl

1046-6673/1305-1365
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000013298.11876.BF

Randomization Procedure

Patients were randomly assigned to one of the three treatment groups in a 1:1:1 ratio, with stratification for cadaveric/living related transplant, for center, and for the number of acute rejections during the first 6 mo after transplantation. Randomization was carried out by opening a sealed envelope with the lowest available study number.

Immunosuppression

Patients were treated with 1000 mg of MMF twice daily, 0.1 mg/kg Pred per day, and CsA targeted at trough levels between 125 and 175 ng/ml (from 3 mo after transplantation). The microemulsion formulation of CsA (Neoral; Novartis, East Hanover, NJ) was used in all patients. No induction antibody therapy was used. Dose reduction or interruption of MMF treatment was allowed in cases of leukocytopenia or anemia, primary cytomegalovirus (CMV) infection, or severe gastrointestinal side effects. In the patients randomized for discontinuation of CsA, the CsA dose was reduced by 50% for 2 wk before complete cessation while increasing the prednisone dose to 0.15 mg/kg per d and continuing 2 g of MMF daily. In nine patients, the CsA dose was reduced by 25% every 3 wk before complete discontinuation. The patients randomized for discontinuation of Pred were tapered off the Pred to 0 mg in 10 wk (according to protocol) while continuing CsA and MMF in unchanged dosages.

Acute rejections were treated primarily with 1000 mg of intravenous methylprednisolone during three consecutive days. Steroid-resistant rejections were treated with anti–T cell therapy, either rabbit polyclonal antithymocyte globulin (ATG) or a mouse anti-CD3 monoclonal antibody (WT32) (8). If patients in one of the withdrawal groups needed anti–T cell rejection treatment, therapy with CsA or Pred was reinstituted. CMV prophylaxis with ganciclovir or CMV hyperimmune globulin was prescribed during anti–T cell therapy in patients at risk for CMV disease (donor and/or recipient seropositive).

Assessments

At baseline, the medical history, physical examination, routine laboratory tests, lipid profile, and histocompatibility data were obtained. Vital signs, body weight, and the results of routine laboratory measurements were recorded every month. Data on rejection episodes, CsA nephrotoxicity, concomitant medication, adverse events, and infections were gathered throughout the entire study period. CsA whole blood levels were measured with a monoclonal antibody against the CsA parent molecule, using the fluorescence-polarization immunoassay on an Abbott TDx analyser (Abbott Laboratories, North Chicago, IL) or with an enzyme-multiplied immunoassay on a COBAS-MIRA analyser (Dade-Behring, San José, CA). A biopsy was performed in cases of deteriorating graft function without an obvious prerenal or postrenal cause, suspected CsA nephrotoxicity, or in cases of increasing proteinuria. No protocol biopsies were performed.

Biopsies were examined by the local pathologist and were classified according to the Banff 1993 biopsy scoring system (grade 1, mild rejection; grade 2, moderate rejection; grade 3, severe rejection) (9). Patients were presumed to have acute rejection if antirejection treatment without prior biopsy resulted in a decrease in serum creatinine without an obvious prerenal or postrenal cause. The creatinine clearance was estimated according to the Cockcroft and Gault method (10). Infections were classified by using the Centers for Disease Control definitions for nosocomial infections (11).

Statistical Analyses

Primary end points for analysis were first biopsy-proven acute or chronic rejection between 6 mo (*i.e.,* time of randomization) and 24 mo after transplantation. Secondary end points were patient and graft survival, renal function at 1 and 2 yr after transplantation, the incidence of infections and malignancies, and changes in BP and lipid metabolism.

Data were analyzed on an intention-to-treat basis. For comparisons of numerical data within groups, a paired nonparametric test was performed (Wilcoxon signed rank test). When appropriate, a *t* test was performed. For comparisons of numerical data among different groups, a nonparametric ANOVA was performed (Kruskal-Wallis test). Comparison of time to first biopsy-proven acute rejection was performed using the Kaplan-Meier procedure with log rank testing. Multiple logistic regression analysis was performed to determine risk-factors associated with the occurrence of acute rejection after randomization. The following variables were entered: gender, age, panel reactive antibodies (PRA), number of transplantation, postmortal or living donor, number of HLA mismatches, serum creatinine at randomization, biopsy-proven rejection during the first 6 mo, and randomization group.

A second analysis, on treatment, was performed for renal function at 1 and 2 yr after transplantation and changes in lipid metabolism at 2 yr after transplantation. For renal function, patients with or without calcineurin inhibitors were compared; comparison among groups was performed with a Mann-Whitney test, and a repeated measurements analysis (general linear model) was performed to exclude a putative influence of a baseline difference between the two groups. Comparison of numerical data for changes in lipid metabolism was performed with nonparametric ANOVA (Kruskal-Wallis test).

Results are given as median with range or as mean with SD, unless stated otherwise. $P < 0.05$ was considered statistically significant; all tests were two-sided. Calculations were performed using the software programs, SPSS 8.0 for Windows (SPSS Inc., Chicago, IL) and Graphpad Instat, version 3.00 for Windows (Graphpad Software Inc., San Diego, CA).

Results

Six months after transplantation, 63 patients were randomized for discontinuation of CsA, 76 patients for discontinuation of prednisone, and 73 patients for continuation of triple therapy. Baseline characteristics are summarized in Table 1; no statistically significant differences existed among the groups. There were no black patients in this study cohort.

Biopsies

During the study period, a total of 47 renal biopsies were performed: 33 first, 13 second, and 1 third biopsy. In the CsA withdrawal group, there were 19 first, 9 second, and 1 third biopsies. In the Pred withdrawal group, there were 9 first and 3 second biopsies. In the control group, there were 5 first and 1 second biopsies.

Acute Rejections

In Table 2, all first biopsy-proven acute rejections after randomization are shown. The incidence of acute rejection was significantly higher after withdrawal of CsA than after withdrawal of steroids or during continuation of triple therapy. As depicted in Figure 1, most rejections occurred within 3 mo after conversion from triple to double therapy. The median time from randomization to first biopsy-proven acute rejection was 74 d (26 to 244 d) and 72 d (22 to 211 d) for the CsA and Pred withdrawal groups, respectively.

Table 1. Baseline characteristics at time of randomization, 6 mo after kidney transplantation. There are no statistically significant differences among the three groups

Parameter	MMF/Pred	MMF/CsA	MMF/CsA/Pred
n patients	63	76	73
Age (yr)	52 (20 to 72)	52 (19 to 68)	51 (19 to 70)
Female/male	21/42	24/52	27/46
First/second graft	57/6	68/8	64/9
Postmortal/living donor	48/15	58/18	54/19
HLA mismatches[b]			
HLA-A	0.78 ± 0.66	0.87 ± 0.52	0.68 ± 0.53
HLA-B	0.90 ± 0.58	1.0 ± 0.65	0.66 ± 0.58
HLA-DR	0.71 ± 0.66	0.95 ± 0.60	0.67 ± 0.53
PRA >10%			
historical (%)	43	39	48
recent (%)	17	13	23
Acute rejection ≤6 mo (biopsy-proven)	10	11	8
CsA			
dose (mg/d)[b]	283 ± 102	288 ± 85	288 ± 84
level (ng/ml)[b]	160 ± 39	158 ± 40	158 ± 42

[a] MMF, mycophenolate mofetil; CsA, cyclosporine; Pred, prednisone.
[b] Mean ± SD.

Table 2. First biopsy-proven acute rejections, according to the BANFF 1993 classification, and anti-rejection therapy needed per patient per group

Parameter	MMF/Pred	MMF/CsA	MMF/CsA/Pred
n patients	63	76	73
Acute rejection	14[ab]	3[b]	1[a]
BANFF I	5	2	1
BANFF ≥II	9	1	0
Anti-rejection therapy			
methylprednisolone	13	4	1
anti–T cell antibodies	6	2	0

[a] Pairwise comparison among groups, $P = 0.0001$.
[b] Pairwise comparison among groups, $P = 0.0014$.

In a multiple logistic regression model, the only variable that was significantly related to the occurrence of biopsy-proven acute rejection after randomization was the group for which patients were randomized ($P = 0.0003$). Neither number of HLA mismatches nor rejection during the first 6 mo were predictive of acute rejection after CsA or Pred withdrawal at 6 mo.

In the CsA withdrawal group, there were two patients with a presumed acute rejection. No biopsy was performed in these two patients, but a course of methylprednisolone resulted in recovery of the serum creatinine to initial values. Of the nine patients who tapered their CsA dosage during a 9-wk period, only one patient developed a biopsy-proven acute rejection. However, this was not significantly different from the rejection incidence (13 of 54) in patients who discontinued CsA in 2 wk. A second biopsy-proven acute rejection occurred in five patients in the CsA withdrawal group; in three of five cases, this second acute rejection was steroid-resistant. One patient in the CsA withdrawal group was treated with methylprednisolone for a presumed second rejection (not biopsy-proven). In the Pred withdrawal group, one patient developed a second biopsy-proven acute rejection that responded to treatment with methylprednisolone. There were no presumed rejections in this group.

The only patient randomized for triple drug therapy who developed an acute rejection had stopped MMF 9 d earlier because of side effects.

Chronic Rejections and CsA Nephrotoxicity

In the CsA withdrawal group, nine patients had a biopsy showing histologic changes compatible with chronic rejection. In five of these patients, there were no concomitant signs of acute rejection. The incidence of biopsy-proven chronic rejection in the CsA withdrawal group (9 of 63) was significantly higher than in the control group (1 of 73; $P = 0.006$). Four patients in the Pred withdrawal group had histologic evidence

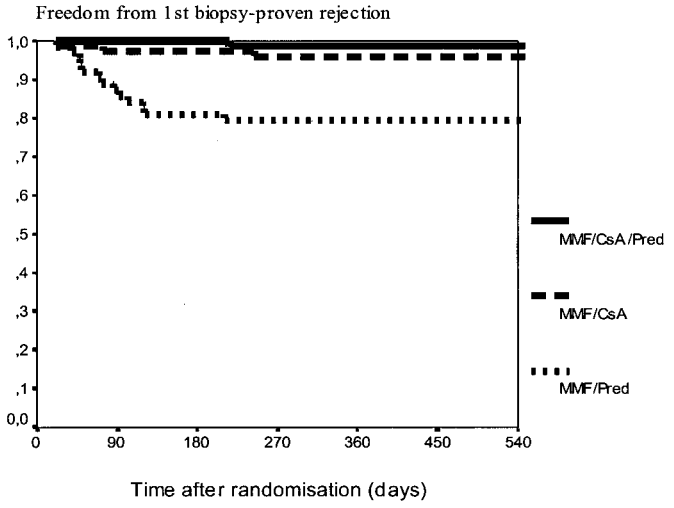

Figure 1. Kaplan-Meier survival curve that demonstrates the time to first biopsy-proven acute rejection for all groups from 6 mo after transplantation during a follow-up period of 18 mo. MMF, mycophenolate mofetil; CsA, cyclosporine; Pred, prednisone.

of chronic rejection, and two of these patients had concomitant acute rejection. When comparing the incidence of chronic rejection in biopsies only, there was no statistically significant difference among all groups; 9 of 33 *versus* 4 of 19 *versus* 1 of 9 for the CsA and Pred withdrawal and control groups, respectively.

Histologic changes compatible with CsA nephrotoxicity were present in one patient in the CsA withdrawal group, in three patients in the Pred withdrawal group, and in four patients in the control group.

Graft Failure, Patient Death, and Treatment Failure

Graft failure without patient death occurred in 2 of 63 patients in the CsA withdrawal group. The cause of graft failure was chronic rejection in both patients. Graft failure, without patient death, occurred once in the Pred withdrawal group because of chronic rejection. In 2 patients in the control group, immunosuppressive medication was stopped completely, followed by graft failure. The reasons for cessation of immunosuppressive medication were severe myositis and posttransplant lymphoma (the latter patient died 2 mo later). Patient death with functioning graft only occurred in the groups continuing CsA (3 of 149). In one case, the cause of death was documented myocardial infarction; two patients were found dead at home, presumably as a result of fatal myocardial infarction (Table 3). Treatment failure, defined as changes in immunosuppressive therapy or reinstitution of the discontinued drug, resulted from various causes (Table 4). In the CsA withdrawal group, calcineurin inhibitors were mostly restarted after acute rejection episodes requiring anti–T cell therapy (protocol driven). Pred withdrawal was uneventful for almost all patients.

Graft Function, Proteinuria, and BP

When comparing renal function among the three groups (intention-to-treat analysis), there were no statistically significant differences in serum creatinine or creatinine clearance among the three groups at 6, 12, and 24 mo after transplantation (Table 5). In addition, there were no significant changes in serum creatinine or creatinine clearance within the three groups during follow-up (intention-to-treat analysis).

However, 24% of patients in the CsA withdrawal group used calcineurin inhibitors at 12 and 24 mo after transplantation, which might negate a positive effect of CsA withdrawal on renal function. When comparing renal function in patients with or without calcineurin inhibitors (on-treatment analysis), a statistically significant difference in serum creatinine at 12 and 24 mo after transplantation was present. The median serum creatinine at 6, 12, and 24 mo for patients with or without calcineurin inhibitors were: 135 μmol/L (61 to 411) *versus* 125 μmol/L (65 to 267), NS; 135 μmol/L (59 to 368) *versus* 119 μmol/L (68 to 226), $P = 0.01$; and 144 μmol/L (64 to 295) *versus* 124 μmol/L (72 to 332), $P = 0.001$, respectively. The repeated measurements analysis on renal function in time demonstrated only a statistically significant difference between patients with or without calcineurin inhibitors ($P = 0.001$).

At randomization, proteinuria (>0.5 g/d) was present in 18% of patients in the CsA withdrawal group, 16% in the Pred withdrawal group, and 15% in the control group. After 18 mo of follow-up, these percentages were 18% in the CsA withdrawal group compared with 20% in the Pred withdrawal group and 12% in the control group (no significant differences or changes).

In Table 6, the results regarding BP are shown. Only in the Pred withdrawal group was a statistically significant reduction

Table 3. Patient and graft survival after conversion per group after 1.5-yr follow-up. Incidence of malignancies per group[a]

Parameter	MMF/Pred	MMF/CsA	MMF/CsA/Pred
n patients	63	76	73
Graft failure	2	1	2
Patient death	0	2	2
Malignancies	0	1	2
PTLD	0	0	1
skin (non-melanoma)	0	1	1

[a] PTLD, posttransplantation lymphoproliferative disorder.

Table 4. Treatment failure per group during 18 mo of follow-up after randomization[a]

Parameter	MMF/Pred	MMF/CsA	MMF/CsA/Pred
n patients	63	76	73
Medication never stopped	1	1	NA
Medication restarted	14[c]	4[c]	NA
Medication stopped			
MMF	3	0	5
CsA	NA	0	1
Pred	0	NA	0
all	0	0	1
Total *n*	18[b]	5[b]	7

[a] NA, not applicable.
[b] Pairwise comparison among groups, $P = 0.03$.
[c] Pairwise comparison among groups, $P = 0.004$.

Table 5. Serum creatinine and creatinine clearance (Cockcroft-Gault) for all groups (median and range) from time of randomization (month 6) and 12 and 24 mo after transplantation

Group	Month 6	Month 12	Month 24
MMF/Pred			
creatinine (μmol/L)	118 (65 to 267)	117 (68 to 228)	123 (72 to 332)
creatinine clearance (ml/min)	62 (30 to 100)	66 (31 to 100)	64 (18 to 104)
MMF/CsA			
creatinine (μmol/L)	130 (61 to 242)	137 (70 to 274)	137 (65 to 293)
creatinine clearance (ml/min)	61 (31 to 115)	58 (30 to 117)	58 (28 to 103)
MMF/CsA/Pred			
reatinine (μmol/L)	123 (65 to 411)	124 (59 to 368)	125 (64 to 276)
reatinine clearance (ml/min)	65 (20 to 156)	63 (22 to 121)	65 (34 to 116)

Table 6. BP and number of antihypertensive drugs needed at time of randomization and after 1.5-yr follow-up after conversion (mean ± SD.)

Parameter	Month 6	Month 24	*P*
Mean arterial pressure (mmHg)			
MMF/Pred	105 ± 1.4	101 ± 1.5	0.037
MMF/CsA	104 ± 1.4	101 ± 1.4	0.017
MMF/CsA/Pred	104 ± 1.3	107 ± 2.9	NS
Antihypertensive drugs (*n*)			
MMF/Pred	1.2 ± 0.12	1.5 ± 0.14	0.027
MMF/CsA	1.3 ± 0.13	1.4 ± 0.13	NS
MMF/CsA/Pred	1.3 ± 0.11	1.7 ± 0.13	<0.001

in mean arterial pressure observed without a concomitant increase in the number of antihypertensive drugs.

Infections and Malignancies

The incidence of infections did not differ among groups: 1.4 infections/patient in the CsA withdrawal group, 1.2 infections/patient in the Pred withdrawal group, and 1.3 infections/patient in the control group. There also was no difference in the distribution or type of infections among the three groups (Table 7).

Only three malignancies occurred during the 18-mo follow-up (Table 3). One posttransplant lymphoma was present in the control group. This patient was treated with r-ATG in the first 6 mo after transplantation because of an acute rejection episode. The other two malignancies were skin carcinomas (non-melanoma). One of these patients had had a previous transplantation.

Lipid Metabolism

To appreciate the immediate as well as the chronic effects of withdrawal of CsA and Pred on lipid metabolism, values at the

Table 7. Number of infections for all groups during follow-up (18 mo), after randomisation

Infection	MMF/Pred	MMF/CsA	MMF/CsA/Pred	*P*
n patients	63	76	73	
Viral infections				NS
herpes simplex	5	5	8	
herpes zoster	2	3	0	
cytomegalovirus	4	1	2	
Opportunistic infections				NS
candida stomatitis	3	3	1	
oesofagitis	0	1	1	
Bacterial infections				NS
urinary tract	36	31	33	
pneumonia/bronchitis	3/2	3/8	5/10	
upper respiratory tract	13	16	16	
skin	7	6	3	
gastrointestinal tract	4	3	3	
other	1	1	4	
Sepsis	3	0	2	NS
Culture-negative infection	3	12	10	NS
Total	85	93	98	NS

time of randomization are compared with those obtained at 3 mo and at 18 mo after randomization (Table 8). The withdrawal of either CsA or Pred was followed by a rapid fall in total cholesterol levels. However, discontinuation of Pred also resulted in a transient significant decrease in HDL-cholesterol. Consequently, there was an unbeneficial rise in the total/HDL cholesterol ratio in this group 3 mo after randomization. Among the three groups, there was an equally distributed percentage of patients treated with cholesterol-lowering drugs (mostly statins).

The on-treatment analysis for all three groups revealed a statistically significant difference in the total cholesterol at 24 mo for the Pred withdrawal group compared with all three groups at the time of randomization: 5.65 mmol/L compared with 6.5, 6.6, and 6.6 mmol/L, respectively. There were no statistical differences in the ratio of total/HDL cholesterol at 6 mo or 24 mo after transplantation among the three groups.

Discussion

Treatment with the combination of MMF, CsA, and Pred has decreased the incidence of acute rejection to 20% in the first half-year after kidney transplantation (1–4). Long-term graft

Table 8. Effect of withdrawal of CsA or Pred, compared with continuation of MMF/CsA/Pred therapy, on lipid metabolism at 6, 9, and 24 mo (mean ± SD)

Group	Month 6	Month 9	Month 24
MMF/Pred			
total cholesterol (mmol/L)	6.63 ± 1.81	6.04 ± 1.64	6.16 ± 1.37
ratio total/HDL	5.8 ± 2.6	5.3 ± 2.7	4.9 ± 1.7
triglycerides (mmol/L)	2.56 ± 1.98	2.30 ± 2.66	2.18 ± 1.90[a]
cholesterol-lowering medication (%)	0	2	16
MMF/CsA			
total cholesterol (mmol/L)	6.69 ± 1.42	6.1 ± 1.24	5.69 ± 1.22
Ratio total/HDL	6.0 ± 2.4	6.6 ± 2.8	5.7 ± 2.5
triglycerides (mmol/L)	2.59 ± 1.31	2.62 ± 1.39	2.8 ± 1.29[a]
cholesterol-lowering medication (%)	0	5	18
MMF/CsA/Pred			
total cholesterol (mmol/L)	6.66 ± 1.62	6.61 ± 1.54	6.19 ± 1.28
ratio total/HDL	6.0 ± 2.4	5.7 ± 2.2	5.1 ± 1.9
triglycerides (mmol/L)	2.62 ± 2.12	2.33 ± 1.13	2.23 ± 1.22
cholesterol-lowering medication (%)	6	4	15

[a] ANOVA (Kruskal-Wallis test) for comparison between groups $P < 0.05$

survival, excluding patient death as a cause of graft failure, is primarily influenced by the occurrence of chronic rejection with subsequent development of renal failure (12,13). The reduced survival of kidney transplantation patients is largely a result of the high risk of cardiovascular disease and malignancies in this population (14). Unwanted side effects of both CsA and Pred, such as hyperlipidemia and hypertension, contribute to the increased risk for developing cardiovascular disease (15,16). MMF has no adverse effect on cardiovascular risk factors. Therefore, discontinuation of either CsA or Pred might positively influence the cardiovascular risk profile, albeit with a small risk of acute rejection or even graft loss (17–19). The 3-yr follow-up from the European MMF study indicated a modest but not statistically significant effect of the addition of MMF on graft survival, which was attributed solely to the lower incidence of acute rejection (5). Data from the US renal transplant scientific registry demonstrated a reduction in the occurrence of chronic rejection for patients treated with MMF, independent of acute rejection, compared with patients treated with azathioprine in combination with calcineurin-inhibitors with or without Pred (20). As long as data demonstrating the need for continuation of full-dose triple drug therapy to achieve superior long-term patient and graft survival are not available, the risks of over-immunosuppression favor discontinuation of one or more drugs.

In our study, the patients discontinuing CsA had the highest incidence of acute rejection (22%), and these acute rejections were severe (≥grade II) in 9 of 14 cases. Severe acute vascular rejections are known to have a poor prognosis for long-term graft survival (21). Our data are in variance with the results of a recent meta-analysis by Kasiske *et al.* (22), which reported a risk for acute rejection after CsA withdrawal of 11% without increased graft loss during long-term follow-up (>5 yr). A possible contributing factor to the increased incidence of biopsy-proven rejections in our study might be the rapid CsA withdrawal. Although no significant difference was observed in our study between patients with two different withdrawal-schedules, lower rejection rates have been described with a longer period of stepwise CsA withdrawal (18,23). Despite the higher incidence of acute rejections after discontinuation of CsA, no detrimental effect on creatinine clearance was noted. This might be caused by a simultaneously occurring disappearance of CsA-associated nephrotoxicity during the first 3 mo after withdrawing CsA. The on-treatment analysis of patients with or without calcineurin inhibitors confirmed the positive effect of CsA withdrawal on renal function.

Pred withdrawal did not increase the incidence of acute rejection episodes compared with the control group. Similar results were previously reported by Grinyó *et al.* (24), although the risk for acute rejection after Pred withdrawal was 14% in a meta-analysis (22). However, the continuation of CsA and MMF was not used as baseline immunosuppression after Pred withdrawal in most studies included in this meta-analysis.

Two multicenter trials of Pred withdrawal from 3 mo after kidney transplantation in patients maintained on CsA and MMF demonstrated an incidence of acute rejection at 1 yr after transplantation of 30.8% (US trial) and 25% (European trial), respectively (17,25). The US trial had the highest incidence of acute rejection in African Americans (39.6%) compared with 16% in non–African Americans (25). Possible explanations for the lower incidence of acute rejections between our study and the US and the European trials might be the time of Pred withdrawal after transplantation (6 mo *versus* 3 mo) and how rapidly Pred was withdrawn (10 wk *versus* 8 wk). No African Americans were present in our study. No induction therapy was given in our study, in contrast to 27% of patients in the US and in the European trial. However, no protective effect of induction therapy could be demonstrated in the US trial (underpowered test). The European trial demonstrated an incidence of biopsy-proven acute rejection of 16% compared with 27% in the withdrawal patients with or without induction therapy, respectively, a difference that was not statistically significant (17).

There appears to be an increased incidence of chronic rejection in the CsA withdrawal group compared with the other groups. It must be stressed however that there was a substantially higher number of biopsies performed in the CsA withdrawal group and the indication for the biopsies was the suspicion of an acute rather than a chronic rejection in most cases.

Although no significant differences exist in patient survival among groups, the causes of death were cardiovascular in patients continuing CsA. In the Pred withdrawal group, there was a significant decrease in mean arterial pressure without the need for more medication; patients continuing triple therapy required more antihypertensive medication to prevent a rise in mean arterial pressure. This positive effect of Pred withdrawal on BP has previously been reported for patients withdrawing prednisone at 3 mo or 1 yr after kidney transplantation (26,27). Long-term follow-up data (>5 yr) of patients withdrawing CsA from 3 mo after transplantation showed less cardiovascular deaths, less hypertension, and better renal function compared with patients continuing CsA (28). In our study, withdrawal of CsA was also followed by a decrease in mean arterial pressure, but this was achieved by an increase in antihypertensive medication. As a consequence of the intention-to-treat analysis, the effects of discontinuation of CsA are obscured by the proportion of patients in whom this drug was reintroduced (29%). In conversion studies with patients switching from CsA to azathioprine or MMF, beneficial effects on BP and lipid profile were also noted (18,26,29).

Three months after withdrawing either CsA or Pred, a substantial decrease in total cholesterol was observed. However, a more favorable cardiovascular risk-profile, reflected by a lower total/HDL cholesterol ratio, was only present in the CsA withdrawal group at 3-mo follow-up. There were no statistically significant differences in lipid parameters among the three groups at the end of follow-up. This may be due to the fact that >15% of the patients in all groups used cholesterol-lowering drugs. It should furthermore be noted that 77% of patients used calcineurin inhibitors at that time. On-treatment analysis of changes in the total cholesterol from randomization until the end of follow-up only demonstrated a beneficial effect for patients withdrawing Pred.

There was no evidence of over-immunosuppression or an increased cardiovascular risk in these patients, although the follow-up period of 18 mo does not allow firm conclusions regarding these issues.

Do the results of our study help decide which immunosuppressive regimen is preferred as maintenance therapy? The relatively short follow-up of 18 mo potentially underestimates the benefits of improving the cardiovascular risk-profile in the CsA and Pred withdrawal groups. Likewise, CsA-induced interstitial fibrosis with subsequent loss of renal function may only become apparent after prolonged use of this drug. Nevertheless, the increased number of acute rejections in the CsA withdrawal group could deter clinicians from following this strategy. There is a clear need for screening tests that identify those patients at increased risk for acute rejection after tapering of immunosuppressive medication. We recently showed that measurement of the frequency of precursor cytotoxic T lymphocytes in peripheral blood allows the identification of a subgroup of patients in whom tapering of immunosuppression was safe (30,31). In the future, such tests may aid physicians not only in the selection of patients in whom drug treatment can be tapered but also to know which degree the level of immunosuppression can be reduced in the individual patient.

Acknowledgments

We thank Ms. Mario van Helden for expert assistance in collecting the data and Roche Pharmaceuticals, Mijdrecht, the Netherlands, for financial support.

References

1. A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. The Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. *Transplantation* 61: 1029–1037, 1996
2. Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. European Mycophenolate Mofetil Cooperative Study Group. *Lancet* 345: 1321–1325, 1995
3. Sollinger HW: Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U.S. Renal Transplant Mycophenolate Mofetil Study Group. *Transplantation* 60: 225–232, 1995
4. Halloran P, Mathew T, Tomlanovich S, Groth C, Hooftman L, Barker C: Mycophenolate mofetil in renal allograft recipients: A pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. The International Mycophenolate Mofetil Renal Transplant Study Groups. *Transplantation* 63: 39–47, 1997
5. Mycophenolate mofetil in renal transplantation: 3-year results from the placebo-controlled trial. European Mycophenolate Mofetil Cooperative Study Group. *Transplantation* 68: 391–396, 1999
6. Mycophenolate mofetil in cadaveric renal transplantation. US Renal Transplant Mycophenolate Mofetil Study Group. *Am J Kidney Dis* 34: 296–303, 1999
7. de Sévaux RGL, Smak Gregoor PJH, Hené R, Hoitsma AJ, Vos P, Weimar W, van Gelder T, and Hilbrands L B: A controlled trial comparing two doses of cyclosporine in conjunction with mycophenolate mofetil and corticosteroids. *J Am Soc Nephrol* 12: 1750–1757, 2001
8. Tax WJ, van de Heijden HM, Willems HW, Hoitsma AJ, Berden JH, Capel PJ, Koene RA: Immunosuppression with monoclonal anti-T3 antibody (WT32) in renal transplantation. *Transplant Proc* 19: 1905–1907, 1987
9. Solez K, Axelsen RA, Benediktsson H, Burdick JF, Cohen AH, Colvin RB, Croker BP, Droz D, Dunnill MS, Halloran PF: International standardization of criteria for the histologic diagnosis of renal allograft rejection: The Banff working classification of kidney transplant pathology. *Kidney Int* 44: 411–422, 1993
10. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
11. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM: CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 16: 128–140, 1988
12. Myers BD, Newton L, Oyer P: The case against the indefinite use of cyclosporine. *Transplant Proc* 23: 41–42, 1991
13. Arend SM, Mallat MJ, Westendorp RJ, van der Woude FJ, van Es LA: Patient survival after renal transplantation: More than 25 years follow-up. *Nephrol Dial Transplant* 12: 1672–1679, 1997
14. Kasiske BL: Risk factors for accelerated atherosclerosis in renal transplant recipients. *Am J Med* 84: 985–992, 1988
15. Kobashigawa JA, Kasiske BL: Hyperlipidemia in solid organ transplantation. *Transplantation* 63: 331–338, 1997
16. Porter GA, Bennett WM, Sheps SG: Cyclosporine-associated hypertension. National High Blood Pressure Education Program. *Arch Intern Med* 150: 280–283, 1990
17. Vanrenterghem Y, Lebranchu Y, Hené RJ, Oppenheimer F, Ekberg H: Double-blind comparison of two corticosteroid regimens plus mycophenolate mofetil and cyclosporine for prevention of acute renal allograft rejection. *Transplantation* 70: 1352–1359, 2000
18. Schrama YC, Joles JA, van Tol A, Boer P, Koomans HA, Hené RJ: Conversion to mycophenolate mofetil in conjunction with stepwise withdrawal of cyclosporine in stable renal transplant recipients. *Transplantation* 69: 376–383, 2000
19. Kasiske BL, Guijarro C, Massy ZA, Wiederkehr MR, Ma JZ: Cardiovascular disease after renal transplantation. *J Am Soc Nephrol* 7: 158–165, 1996
20. Ojo AO, Meier-Kriesche HU, Hanson JA, Leichtman AB, Cibrik D, Magee JC, Wolfe RA, Agodoa LY, Kaplan B: Mycophenolate mofetil reduces late renal allograft loss independent of acute rejection. *Transplantation* 69: 2405–2409, 2000
21. van Saase JL, van der Woude FJ, Thorogood J, Hollander AA, van Es LA, Weening JJ, van Bockel JH, Bruijn JA: The relation between acute vascular and interstitial renal allograft rejection and subsequent chronic rejection. *Transplantation* 59: 1280–1285, 1995
22. Kasiske BL, Chakkera HA, Louis TA, Ma JZ: A meta-analysis of immunosuppression withdrawal trials in renal transplantation. *J Am Soc Nephrol* 11: 1910–1917, 2000
23. Heim-Duthoy KL, Chitwood KK, Tortorice KL, Massy ZA, Kasiske BL: Elective cyclosporine withdrawal 1 year after renal transplantation. *Am J Kidney Dis* 24: 846–853, 1994
24. Grinyo JM, Gil-Vernet S, Seron D, Cruzado JM, Moreso F, Fulladosa X, Castelao AM, Torras J, Hooftman L, Alsina J: Steroid withdrawal in mycophenolate mofetil-treated renal allograft recipients. *Transplantation* 63: 1688–1690, 1997
25. Ahsan N, Hricik D, Matas A, Rose S, Tomlanovich S, Wilkinson A, Ewell M, McIntosh M, Stablein D, Hodge E: Prednisone

withdrawal in kidney transplant recipients on cyclosporine and mycophenolate mofetil–a prospective randomized study. Steroid Withdrawal Study Group. *Transplantation* 68: 1865–1874, 1999
26. Hilbrands LB, Hoitsma AJ, Koene KA: Randomized, prospective trial of cyclosporine monotherapy *versus* azathioprine-prednisone from three months after renal transplantation. *Transplantation* 61: 1038–1046, 1996
27. Hollander AA, Hene RJ, Hermans J, van Es LA, van der Woude FJ: Late prednisone withdrawal in cyclosporine-treated kidney transplant patients: A randomized study. *J Am Soc Nephrol* 8: 294–301, 1997
28. Hollander AA, van Saase JL, Kootte AM, van Dorp WT, van Bockel HJ, van Es LA, van der Woude FJ: Beneficial effects of conversion from cyclosporin to azathioprine after kidney transplantation. *Lancet* 345: 610–614, 1995
29. Hilbrands LB, Demacker PN, Hoitsma AJ, Stalenhoef AF, Koene RA: The effects of cyclosporine and prednisone on serum lipid and (apo)lipoprotein levels in renal transplant recipients. *J Am Soc Nephrol* 5: 2073–2081, 1995
30. van Besouw NM, van der Mast BJ, de Kuiper P, Smak Gregoor PJH, Vaessen LM, IJzermans JN, van Gelder T, Weimar W: Donor-specific T-cell reactivity identifies kidney transplant patients in whom immunosuppressive therapy can be safely reduced. *Transplantation* 70: 136–143, 2000
31. Smak Gregoor PJH, van Gelder T, van Besouw NM, van der Mast BJ, IJzermans JN, Weimar W: Randomized study on the conversion of treatment with cyclosporine to azathioprine or mycophenolate mofetil followed by dose reduction. *Transplantation* 70: 143–148, 2000