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ORIGINAL ARTICLE

Early conversion from cyclosporine to tacrolimus increases renal graft function in chronic allograft nephropathy at BANFF stages I and II

Thorsten Marcard,[1] Katrin Ivens,[1] Bernd Grabensee,[1] Reinhart Willers,[2] Udo Helmchen,[3] Lars Christian Rump[1] and Cornelia Blume[1]

1 Department for Nephrology, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany
2 Rechenzentrum, Heinrich-Heine-University Duesseldorf, Duesseldorf, Germany
3 Nierenregister of the Institute for Pathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany

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Correspondence
PD Dr med. Cornelia Blume, Klinik fuer Nephrologie, Heinrich-Heine-Universitaet Duesseldorf, Moorenstrasse 5, 40225 Duesseldorf, Germany. Tel.: +49 211 8104086; fax: +49 211 8104086; e-mail: hblume@cityweb.de

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Summary

Switching from cyclosporine to tacrolimus without steroid pulse was suggested as a therapeutic option in chronic allograft nephropathy (CAN). Thirty-one renal transplant recipients with CAN were prospectively converted from cyclosporine to tacrolimus (group A), in parallel 31 matched cyclosporin A (CsA) patients (group B) without CAN were followed up for 30 months. In six matching patients of groups A and B inulin and para-aminohippurate (PAH)-clearances and mycophenolate were measured over a span of 3 months. Transplant biopsies of group A were scored according to BANFF. While group A presented with transplant dysfunction compared with group B before switching (2.7 ± 0.16 mg/dl vs. 1.7 ± 0.09 mg/dl; $P < 0.001$), transplant function was equal 30 months later: it ameliorated in group A (2.0 ± 0.18 mg/dl vs. 2.7 ± 0.16 mg/dl; $P < 0.001$) and decreased in group B (1.9 ± 0.13 mg/dl vs. 1.7 ± 0.09 mg/dl, $P < 0.05$). Especially, patients with biopsy scores I and II according to BANFF benefited from tacrolimus. Within 3 months, mycophenolate acid (MPA) levels increased under tacrolimus ($P < 0.05$) whereas inulin and PAH-clearances remained unchanged. At switching, antihypertensive treatment was more intense in group B, but this difference evened out. Adverse side effects were more frequent under tacrolimus. Patients with mild to moderate CAN significantly benefited from switching to tacrolimus. Increased MPA-levels under tacrolimus might have contributed to this effect.

Introduction

In the recent 20 years, the transplant-survival-rate in our centre was approximately 83.4% 1 year after kidney transplantation. In contrast, the 5-year-survival-rate was only 65.9% (Katrin Ivens and Bernd Grabensee, unpublished data), which is concordant with that in other centres. One major cause for this frequent transplant function loss is represented by the chronic allograft nephropathy (CAN) with a prevalence of 60–70% in the allograft biopsy findings [1,2]. Clinically, this disease is apparent with a slowly deteriorating graft function, often accompanied by proteinuria. The specific histological sign of chronic rejection is a doubling of the basal membrane in the glomeruli [3,4]. There are different factors involved in the pathology of CAN: ischaemic damaging, destroyed tissue after reperfusion, ineffectively or nontreated rejection episodes, nephrotoxicity caused by high trough levels of calcineurin-inhibitors and donor quality [5]. Uncontrolled hypertension [6] and increased serum lipids [7] can aggravate the progression of transplant function loss.

The effectiveness of tacrolimus as 'rescue'-therapy in steroid-resistant rejections was often described [8]. In stable Chinese cadaveric renal transplant recipients with

cyclosporine or tacrolimus therapy, paired kidney analysis yielded a significantly better renal function for tacrolimus patients than for cyclosporine patients after 3 years [9]. Therefore, it has been suggested that switching from cyclosporine to tacrolimus can also prolong transplant graft survival in patients with CAN. As minimization of cyclosporine in CAN has shown the same stabilizing effect as switching to tacrolimus, it was not clear whether this was only as a result of decreased toxicity of the used calcineurin inhibitor (CNI) [10]. CAN may be caused by the whole range of possible damaging factors in a renal transplant, but may also include chronic rejection with specific immunological processes underlying. Experimental studies on rats suggest that tacrolimus, but not cyclosporine, can significantly prevent this chronic rejection in a renal graft and therefore may have a greater potency as an immunosuppressant [11]. Cyclosporine withdrawal was also associated with better blood pressure profiles and decreased serum lipids and decreased blood pressure and serum cholesterol has been demonstrated in clinical trials contributing to a significantly decreased cardiovascular risk after kidney transplantation [12]. Two multicentre studies from Europe and USA showed that the incidence of acute rejection is significantly lower under tacrolimus than under cyclosporine [13,14]. The estimated transplant-halftime was longer and acute rejections within 5 years after renal graft transplantation (NTX) was less frequent under tacrolimus [13]. This may suggest that tacrolimus is less nephrotoxic than cyclosporine with a higher immunosuppressive potency. The chronic nephrotoxicity of cyclosporine is equivalent to an irreversible interstitial fibrosis in the renal allograft that develops in some patients over 6–12 years [15]. A study dealing with the effectiveness of switching from cyclosporine to tacrolimus must therefore discuss the possibility that the amelioration of transplant function may only reflect the withdrawal of cyclosporine.

The aim of this study was to evaluate the benefit of a long-lasting switching from cyclosporine to tacrolimus in patients with CAN for transplant survival. Besides, the impact of tacrolimus on blood pressure profiles and serum lipids should be evaluated. On the other hand, possible side effects of tacrolimus such as new onset of diabetes or neurological disorders should be monitored to give a realistic assessment of possible risks under tacrolimus therapy. To analyse the underlying reason for the effectiveness of tacrolimus in contrast to cyclosporine, we performed para-amminohippuurate (PAH) and inulin clearances at switching and 3 months after in subgroups of groups A and B as a measure of nephrotoxicity for both substances. Furthermore, concomitant mycophenolate acid (MPA) concentrations were assessed using a 8-h-kinetic and HPLC-determination. Group B was chosen as a patient group without proven CAN to assess the spontaneous progression of transplant function over time.

In a retrosperspective pilot study, we have already presented data of 17 patients with biopsy proven rejection after switching to tacrolimus. Treatment with tacrolimus significantly reduced the progression of renal graft deterioration. Renal transplant function was stabilized within the observation time of 12.3 months because the decrease in the glomerular filtration rate (GFR) from -7.92 ± 1.13 to -0.04 ± 0.90 ml/min (1/creatinine/year*100, $P < 0.03$). After switching to tacrolimus, serum lipids in these patients were significantly decreased compared to values under cyclosporine as well as blood pressure values and the number of antihypertensive drugs. The pilot study therefore clearly showed a positive impact of tacrolimus on transplant function and possible risk factors for the progression of CAN [16].

Patients and methods

Patients

Between 2000 and 2004, 31 patients [group A; $n = 31$, 16 men and 15 women, age 45.2 ± 11.4 years, body mass index (BMI) 25.4 ± 3.6] with transplant dysfunction after kidney transplantation at our centre diagnosed with transplant dysfunction mainly caused by a biopsy-proven chronic transplant rejection (see Table 2) were switched from cyclosporin A (CsA) to tacrolimus and followed up in a prospective clinical study for 30 months. In parallel to group A, we observed 31 patients (group B, $n = 31$, 14 men, 17 women, aged 46.7 ± 12.1 years, BMI 24.5 ± 5.9) comparable in age and gender and matched according to transplant age (group A: 21.4 ± 24.6 months vs. group B: 18.4 ± 21.8 months, NS) to group A patients. Group B patients did not present a transplant dysfunction and rested on CsA therapy. This was performed to evaluate the spontaneous progression of transplant function after kidney transplantation. Among the study patients, there were 51 deceased kidney grafts and 11 living donor grafts. There were 29 females and 33 male recipients. Group A patients included 10 patients with more than one renal allograft and nine patients who were inducted with either anti-thymocyte globulin (ATG) or Muromonab Orthoclone OKT^{R}3 (OKT) when they received their actual kidney allograft. In group A, 11 patients were lost to follow up; in group B, 10 patients were not followed up until the end of observation time. In each group, one patient died during observation time.

Immunosuppression

Group A was switched to tacrolimus without prior steroid pulse therapy. The average CsA-trough level of group A

before switching was 168.1 ± 52.2 ng/ml, whereas group B patients presented with a CsA-through-level of 164.9 ± 48.9 ng/ml. After switching to tacrolimus, group A showed a mean trough level of 10.9 ± 3.63 ng/ml within the first 4 weeks and a trough level of 8.6 ± 2.6 ng/ml thereafter. In both groups, 25/31 patients were co-medicated with MMF and steroids, 6/31 patients only with steroids.

Observation time and monitoring

The mean observation time in group A was 26.9 ± 13.8 and 27.2 ± 11.9 months in group B. During the whole study, transplant function was followed up three monthly according to serum creatinine and Cockroft–Gault [17] estimates of the GFR:

$$\text{Men: estimated GFR} = \frac{(140 - \text{age}) \times (\text{body mass})}{72 \times \text{creatinine (mg/dl)}}$$

$$\text{Women: estimated GFR} = \frac{(140 - \text{age}) \times (\text{body mass})}{85 \times \text{creatinine (mg/dl)}}$$

Furthermore, the mean blood pressure, the serum lipids and drug adverse side effects of the immunosuppressives were monitored three monthly using a data bank.

BANFF classification

Renal biopsies of group A were additionally analysed according to BANFF criteria for CAN [3] by the Institute for Pathology, University Hospital of Hamburg-Eppendorf (Udo Helmchen, Director of the Renal Register). Here, predominantly interstitial fibrosis and tubular atrophy were found and scored (grade I for mild, grade II for moderate). Furthermore, signs of cyclosporine toxicity as arteriolar hyaline thickening were described as well as signs of T-cell-mediated rejection (grading for interstitial infiltration I a and I b) indicating acute inflammatory episodes as well as acute tubular necrosis. Table 2 summarizes the biopsy findings of group A patients.

Subgroups C and D

Six patients from each group A and B (defined as groups C and D) who additionally were on mycophenolate mofetil (MMF) as co-medication received an inulin and dextrane clearance as well as a 8-h-kinetic of MPA and its metabolites MPA-glucuronide (MPAG) and acyl-MPA-glucuronide (acyl-MPAG) using the 'area under the curve (AUC)' method [18]. These tests were performed before switching to tacrolimus and 3 months after.

For the 8-h-kinetic of mycophenolate, blood samples before and 1, 2, 3, 5 and 8 h after taking MMF were taken from each patient in groups C and D and mixed with ethylene-di-amine-tetraacetate (EDTA). These samples were frozen and preserved for analysis of concentrations of the active drug MPA as well as its metabolites MPA-glucuronide and acyl-MPA-glucuronide using the 'high-performance liquid chromatography' (HPLC) by co-workers of the Department of Clinical Chemistry at the Georg-August-University Goettingen with kind permission from Victor W. Armstrong (Director of the department).

Effective renal blood flow (ERBF) and GFR were measured according to the clearance of sodium PAH (Clinalfa, Bachem, Weil am Rhein, Germany) and inulin (Inutest 25%; Fresenius, Linz, Austria). Briefly, the following procedure was used: after a bolus infusion of 6.5 g of PAH and 2.5 g of inulin, a constant dose of PAH (4 g/h) and inulin (dose dependent on plasma creatinine concentration and individual body surface, i.e. between 1.5 and 0.1 g/h) was infused for 200 mins. After an equilibration period of 30 min, five exactly timed serum samples were drawn (after 30, 60, 80, 100, 120, 140 and 160 min), mixed with EDTA. Samples were centrifuged and frozen at −20 °C and preserved for analysis.

Inulin was measured by the colorimetric determination of fructose (resorcin method) after hydrolytic cleavage of inulin in plasma and urine. PAH was determined by a diazo reaction of the PAH amino group with sodium nitrite yielding a red colour complex (colorimetric determination [ΔE] at 546 nm, method according to Smith *et al.* [19]).

For the evaluation of the clearances without urine sampling, it is assumed that after equilibration of the extracellular fluid volume (EFV) after constant infusion of PAH rsp. inulin, a constant extinction value occurs and a steady state is established. Only the extinction values of the steady state are referred to for the clearance calculation. At the steady state, the measured serum concentration of PAH rsp. inulin equals the assumed urine concentration of PAH rsp. inulin because as much serum is cleared by PAH rsp. inulin as has been given by the constant infusion. This method allows clearance determination of PAH rsp. inulin without urine sampling and was first described in 1980 [20]. Finally, the filtration fraction (ff) was calculated as:

$$\text{ff}(\%) = \frac{\text{GFR}}{\text{ERBF}} \times 100.$$

Statistical analysis

The statistical analysis was performed using the software SPSS (Statistical Package for Social Sciences, SPSS GmbH, München, Germany) and Excel 2003 (Microsoft). Data

were expressed as mean ± standard deviation or as absolute numbers and percentage. Variables for groups A and B were tested with the Kolmogorov–Smirnov test for normal population of the patient cohort and statistical significance was calculated using the Student's *t*-test for independent random probes or for matched probes, where appropriate. All tests were two-sided and statistical significance was defined by $P < 0.05$. Using the Wilcoxon test, mean serum creatinine values of groups A and B were additionally compared at single time points throughout the whole observation period of 30 months. This analysis could reaffirm the significances that were evaluated with the Student's *t*-test. The *P*-values of the Wilcoxon analysis were adjusted two-sided and by the method of Bonferroni–Holm. The progression in mean serum creatinine values in each group was finally expressed as regression analysis for 30 months. Assuming a linear progression in transplant function, a mixed model of ANOVA was performed and serum creatinine values were expressed in a logarithmic scale with base 2. The regression coefficient equalled the slope of the regression line and served as a measure for the progression of transplant function over time.

Ethics

This study fulfilled all criteria of the declaration of Helsinki 1990 for clinical studies and was approved by the local ethics committee (study number 1832, February 2001).

Results

Transplant function

Before switching to tacrolimus, group A ($n = 31$) showed a significantly worse transplant function with a serum creatinine of 2.7 ± 0.16 mg/dl compared with group B ($n = 31$) with 1.7 ± 0.09 mg/dl ($P < 0.001$, Fig. 1). After 24 months, serum creatinine values in both groups were similar. In group A ($n = 21$ at that time), serum creatinine was significantly lower than before switching (2.0 ± 0.14 mg/dl, $P < 0.001$), whereas serum creatinine slightly but not significantly worsened to 1.8 ± 0.16 mg/dl in group B ($n = 24$; Fig. 1). At 30 months after switching, group A ($n = 15$) still presented with a significantly decreased mean serum creatinine ($P < 0.01$) of 2.0 ± 0.18 mg/dl, whereas group B ($n = 10$) showed a significantly worse mean serum creatinine of 1.9 ± 0.13 mg/dl compared with the beginning of the study ($P < 0.05$; Fig. 1). Four patients in group A and two patients in group B lost transplant function throughout observation time. This was mainly not caused by immunologic processes: In group A, the reasons were a severe infectious disease with acute transplant failure in one patient, acute renal failure caused by MPA induced diarrhea and volume depletion in one patient, noncompliance with insufficient immunosuppressive therapy in one patient, and an irreversible chronic transplant rejection with additional hypertensive damaging of the allo-

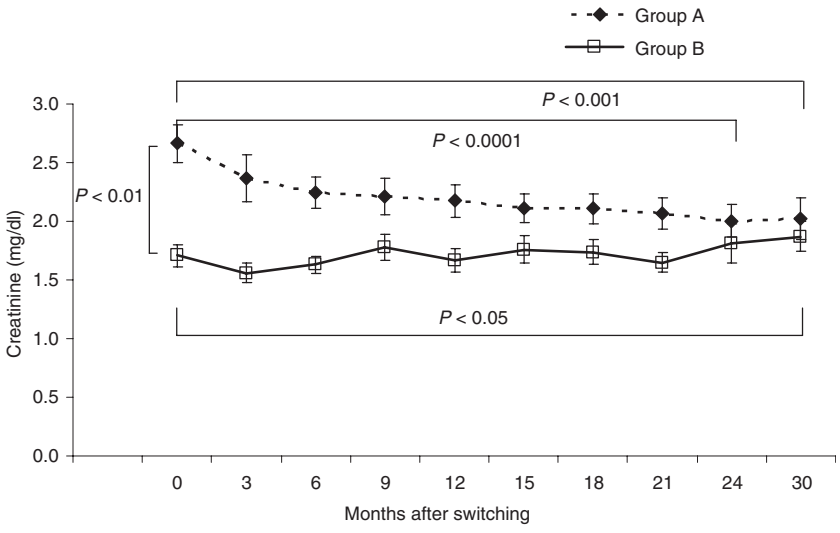

Figure 1 The mean transplant function of groups A and B is shown for 30 months according to serum creatinine values (mean ± SEM). Statistical significance was assessed according to Student's *t*-test for matched pairs comparing group A and group B at the beginning of the study and 24 and 30 months after switching to tacrolimus: group A versus group B at switching ($P < 0.01$, $n = 31$ ± SEM), group A versus group B at 24 months (NS, $n = 19$ in group A ± SEM, $n = 24$ ± SEM in group B), group A versus group B at 30 months (NS, $n = 15$ in group A ± SEM, $n = 10$ ± SEM in group B). Group A and group B mean values were also compared at different time points as indicated: group A at switching versus group A at 24 months ($P < 0.0001$, $n = 19$ ± SEM), group A at switching versus group A at 30 months ($P < 0.001$, $n = 15$ ± SEM), group B at switching versus group B at 30 months ($P < 0.05$, $n = 10$ ± SEM).

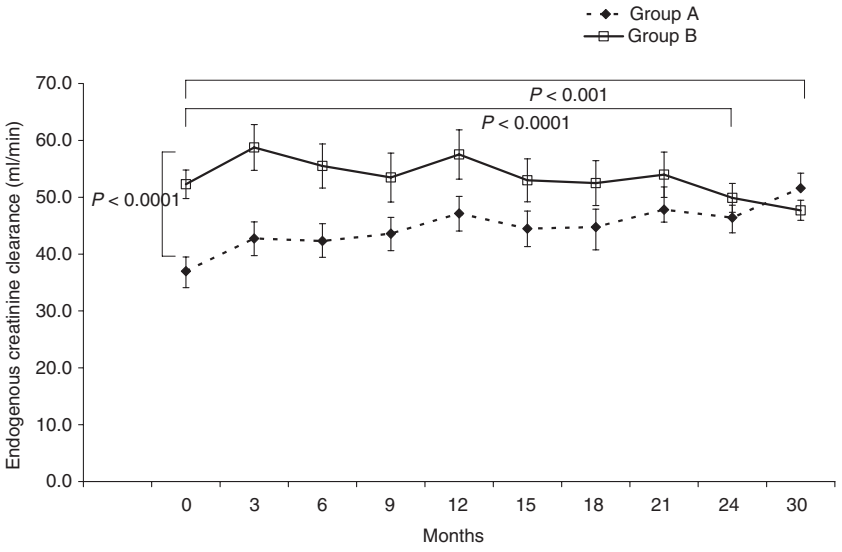

Figure 2 Transplant function as assessed by the Cockroft–Gault formula (*n* as indicated in Fig. 1 ± SEM), mean values at switching 24 and 30 months later (*t*-test for matched pairs, group A with $P < 0.0001$ versus group A at 24 months and group A with $P < 0.001$ versus group A at 30 months, group B versus group B at 24 rsp. 30 months, NS). Student's *t*-test: group A with $P < 0.0001$ versus group B at switching.

graft in another patient. In group B, a biopsy proven cyclosporine toxicity occurred in one patient, an acute renal failure because of diarrhea and volume depletion in another patient.

The significance of serum creatinine values as a measure for transplant function was confirmed using the Cockroft–Gault estimates (Fig. 2): group A showed a significantly lower GFR ($P < 0.0001$) than group B before switching (37.7 ± 2.7 ml/min vs. 52.3 ± 2.5 ml/min), but transplant function significantly ameliorated during 24 months up to 46.4 ± 3.3 ml/min ($P < 0.0001$), whereas it was stable (48.3 ± 3.0 ml/min) in group B. At 30 months after switching, both groups had a comparable GFR (group A: 51.6 ± 4.9 ml/min vs. group B: 47.7 ± 3.9 ml/min). Again, transplant function was significantly better in group A with $P < 0.001$ and slightly but not significantly decreased in group B compared to the time before switching (Fig. 2).

Analysing the differences in the mean transplant function in both groups month per month throughout the whole observation period using the Wilcoxon test, transplant function in group A was significantly worse than in group B during the first 6 months of the study (data not shown). Mean transplant function was not significantly different in both groups from month 7 on and up to the end of the study. Assuming a linear progression of serum creatinine values using a mixed covariance model of ANOVA, regression coefficients were calculated with a logarithmic scale for the serum creatinine values in group A and group B (Fig. 3) as a measure of the transplant function trend. The regression slope in group A was significant with −0.01 and this equals an amelioration of transplant function by 23.3% for 30 months (this equals approximately 8.6 ml/min ± SD). In contrast, the regression slope

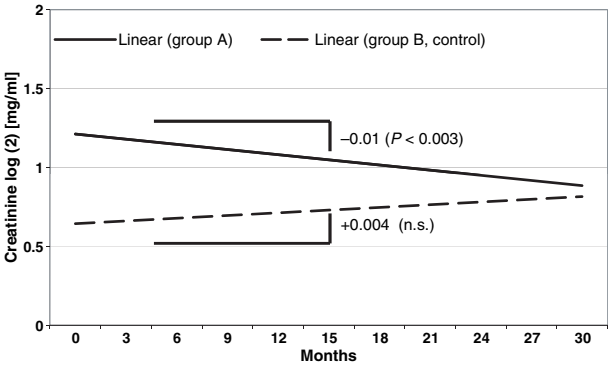

Figure 3 Progression of transplant function (*n* as indicated ± SD) in group A and B patients within the observation time of 30 months according to group-specific regression lines. The regression coefficients are given and are equivalent with the slope of the regression lines.

was stable, but not significantly altered throughout the observation time with 0.004 in group B.

Mean arterial blood pressure and number of antihypertensive drugs

The mean arterial blood pressure (MAP) was not significantly different in both groups at the beginning of the study (96.4 ± 21.6 mmHg in group A vs. 98.4 ± 20.1 mmHg in group B). At the end of the study, MAP values were still equal (99.7 ± 11.3; 99.8 ± 10.3 mmHg). Before switching to tacrolimus, the number of antihypertensive drugs applied to group A was significantly higher than that applied to group B (3.1 ± 1.6 vs. 2.3 ± 1.4; $P < 0.05$). At the end of observation time of 30 months,

no difference between the groups was apparent any more (group A: 2.3 ± 1.42 vs. group B: 2.4 ± 1.4), but the portion of ACE-inhibitors and Ang II-antagonists in group A was significantly increased (0.3 ± 0.5 vs. 0.7 ± 0.7; $P < 0.01$) whereas it remained stable in group B (0.5 ± 0.5; 0.5 ± 0.6).

Serum lipids

In both groups, serum cholesterol was significantly decreased up to the end of observation time of 30 months: in group A, cholesterol decreased from 243.2 ± 54.6 to 199.1 ± 42.4 mg/dl ($P < 0.005$) and from 237.3 ± 55.2 to 205.6 ± 45.2 mg/dl in group B ($P < 0.001$), but the use of statins in both groups remained unchanged throughout the study. Triglycerides, LDL and HDL stayed at the same level in both groups.

Adverse side effects

Throughout the study, 14 patients in group A presented with gastrointestinal disorders as vomiting, diarrhea, dyspepsia, all of them being co-medicated with mycophenolate. In group B, only one patient suffered from diarrhea. Twelve patients in group A presented neurologic disturbances as tremor, itchiness or paraesthesia, none in group B. Furthermore, 11 group A patients showed oedema of the lower legs or ankles compared to one in group B; eight group A patients complained of adynamia, fatigue or lassitude compared to one patient in group B. Two group A patients acquired a CMV disease, there was none in group B. One group A patient presented a newly diagnosed diabetes mellitus, again there was no patient in group B. Two patients in group A developed carcinoma (patient 1: malignant melanoma and renal cell carcinoma of the native kidney, patient 2: urothel carcinoma). One patient in group B was diagnosed with gingiva hyperplasia, but none in group A (Table 1).

Table 1. Adverse side effects among patients of group A and group B throughout the study.

Adverse side effects	Frequency of occurrence in group A patients (%)	Frequency of occurrence in group B patients (%)
Gastrointestinal disorders	14/31 (45.2)	1/31 (3.2)
Neurologic disorders	12/31 (37.7)	None
oedema (lower legs, ankles)	11/31 (35.5)	1/31 (3.2)
Adynamia, fatigue or lassitude	8/31 (25.8)	1/31 (3.2)
CMV-disease	2/31 (6.5)	None
Malignoma	2/31 (6.4)	None
Diabetes mellitus	1/31 (3.2)	None
Gingiva hyperplasia	None	1/31 (3.2)

Inulin and PAH clearances

Analysis of transplant function during the first 3 months of the study revealed no significant changes in the filtration rate according to the ratio of inulin/PAH-clearance in subgroups C and D. In group C (tacrolimus), this ratio was 0.14 ± 0.03 before switching and only marginally rose to 0.16 ± 0.06 3 months after switching. The filtration rate remained unchanged with 0.15 ± 0.04 at the beginning and 3 months later in group B (data not shown).

8 h kinetic of MPA and its metabolites

The pharmacokinetic of MPA and its metabolites acyl-MPA-glucuronide and MPA-glucuronide were investigated for 8 h. In group C ($n = 6$), the mean 8 h concentration of MPA/g MMF increased compared to before switching to tacrolimus (MPA: 29.7 ± 6.4 μg/ml/g vs. 18.9 ± 5.2 μg/ml/g MMF, $P < 0.01$ using Student's *t*-test, Fig. 4), whereas the concentrations of the metabolites decreased (mycophenolate glucuronide (MPAG) 885.5 ± 339.7 μg/ml/g vs. 1423.3 ± 472.1 μg/ml/g MMF; acyl-MPAG 3.9 ± 2.2 μg/ml/g vs. 11.2 ± 6.9 μg/ml/g MMF; NS using Student's *t*-test). In group D, MPA, acyl-MPAG and MPAG remained unchanged (MPA 19.6 ± 4.9 per g MMF vs. 21.8 ± 5.3 per g MMF, Fig. 4; MPAG: 1430.2 ± 423.8 per g MMF vs. 1511.9 ± 418.9 per g MMF; acyl-MPAG 8.48 ± 3.80 per g MMF vs. 8.13 ± 5.68 per g MMF).

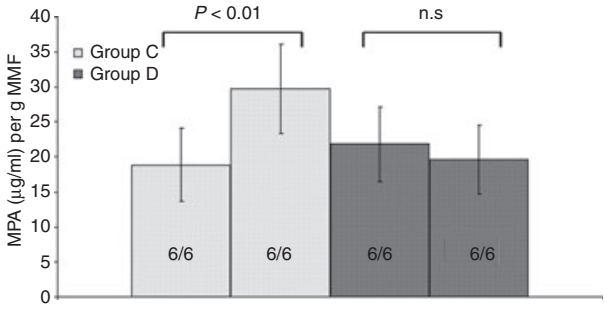

Figure 4 Group A was divided into three subgroups according to the BANFF stages of the renal transplant biopsy findings (grade I, II and not BANFF-related = 'BANFF X'). Upper figure: GFR (*n* as indicated ± SD) according to Cockroft–Gault of each BANFF-group was compared using the *t*-test for matched pairs (*n* as indicated ± SD) before and 24 months after switching to tacrolimus (BANFF I versus BANFF I at 24 months: $P < 0.001$; BANFF II versus BANFF II at 24 months: $P < 0.05$; BANFF X versus BANFF X at 24 months NS). Lower Figure: GFR (*n* as indicated ± SD) of group A patients with BANFF grade I and II was followed up for 30 months (*t*-test for matched pairs, BANFF I versus BANFF I at 30 months: $P < 0.01$; BANFF II versus BANFF II at 30 months: $P < 0.05$). At this time, only one patient with BANFF X was left (data not shown).

Table 2. Renal allograft biopsy findings of group A patients ($n = 31$), according to the BANFF classification [3].

Biopsy findings	No. patients	Additional findings
Interstitial fibrosis and tubular atrophy		
Grade 1 (BANFF1)	14	Acute tubular necrosis in one patient
Grade 2 (BANFF 2)	10	Mild acute rejection type I a in one patient
Other biopsy findings (BANFF X)		
Signs of CNI toxicity	3	
Acute tubular necrosis	2	
T-cell-mediated rejection type I a	None	
T-cell-mediated rejection type I b	2	Acute tubular necrosis in one patient
	$\sum$ = 31 patients of group A	

Classification according to BANFF and transplant function

Group A patients ($n = 14$) scored with 'mild chronic allograft nephropathy' according to BANFF classification I showed a significant increase in mean creatinine value ($P < 0.001$, Student's *t*-test) at 24 months after switching as well as 30 months after switching ($P < 0.01$, Student's *t*-test). Mean transplant function according to the serum creatinine in group A patients ($n = 10$) scored with BANFF grade II ('moderate CAN') also increased significantly at 24 and 30 months ($P < 0.05$, Student's *t*-test). Group A patients with divergent findings ($n = 7$, BANFF X, Table 2) did no show an improvement in transplant function at 24 months and could not be followed up for 30 months (lost to follow up) (Fig. 5).

Discussion

The results of this prospective study suggest that early switching from cyclosporine to tacrolimus in patients with CAN, a chronic rejection according to BANFF grade I and II after kidney transplantation led to amelioration and final stabilization in transplant function. The mean transplant function decreased after switching to tacrolimus and transplant function progression was positive.

Patients with histologic findings of other source (indicated here as 'BANFF X') as signs of CNI toxicity, acute tubular necrosis, T-cell-mediated rejection type I a and I b did not benefit from the switching to tacrolimus. This underlines the importance of an adequate histologic diagnosis among the divergent findings in patients with CAN. Other therapeutic options such as CNI-free immunosuppressive therapy or others have to be found for these patients.

Throughout the observation period, the mean serum creatinine value of the control group B without a biopsy-proven rejection and resting on cyclosporine significantly worsened. This was already shown in healthy volunteers [21] and in patients without CAN after kidney transplantation, in transplanted patients without CAN or chronic rejection [22] and in patients with an elevated risk for chronic rejection [23]. In the study of Weid *et al.* [23], transplanted patients with comparable transplant function were followed up comparably under tacrolimus and under cyclosporine. The tacrolimus group presented with a slightly decreasing serum creatinine from 2.5 to 2.3 mg/dl after 24 months, whereas transplant function in the cyclosporine group increased from 2.5 to 2.6 mg/dl ($P = 0.01$). Artz *et al.* [22] showed a stabilization in transplant function after switching to tacrolimus and an increase in serum creatinine values under resting cyclosporine therapy from 142 ± 48 to 157 ± 62 μmol/l ($P < 0.05$). Furthermore, Meier *et al.* [24] showed a clear improvement in allograft function in CAN patients induced by switching from cyclosporine to tacrolimus with a prolonged long-term graft survival.

In the work presented here, the Cockroft–Gault estimates of transplant function mainly confirmed the results for the serum creatinine values. In the beginning of the study, there was a significant difference in the mean transplant function between the groups. Although transplant function of group A significantly ameliorated up to 24 and 30 months, transplant function of group B worsened in a significant way according to serum creatinine values up to 30 months. This latter result was not confirmed by assessment of the Cockroft–Gault formula on transplant function in group B.

An increasing trend in the mean serum creatinine level in group A was also confirmed by linear regression analysis according to the negative regression slope of −0.01 that is equivalent to a transplant function of 23.3 % for 30 months. This result is concordant with other studies: Artz *et al.* [22] showed a stabilization of transplant function in the tacrolimus group (60 ± 22 ml/min vs. 64 ± 33 ml/min after 2 years), whereas the cyclosporine

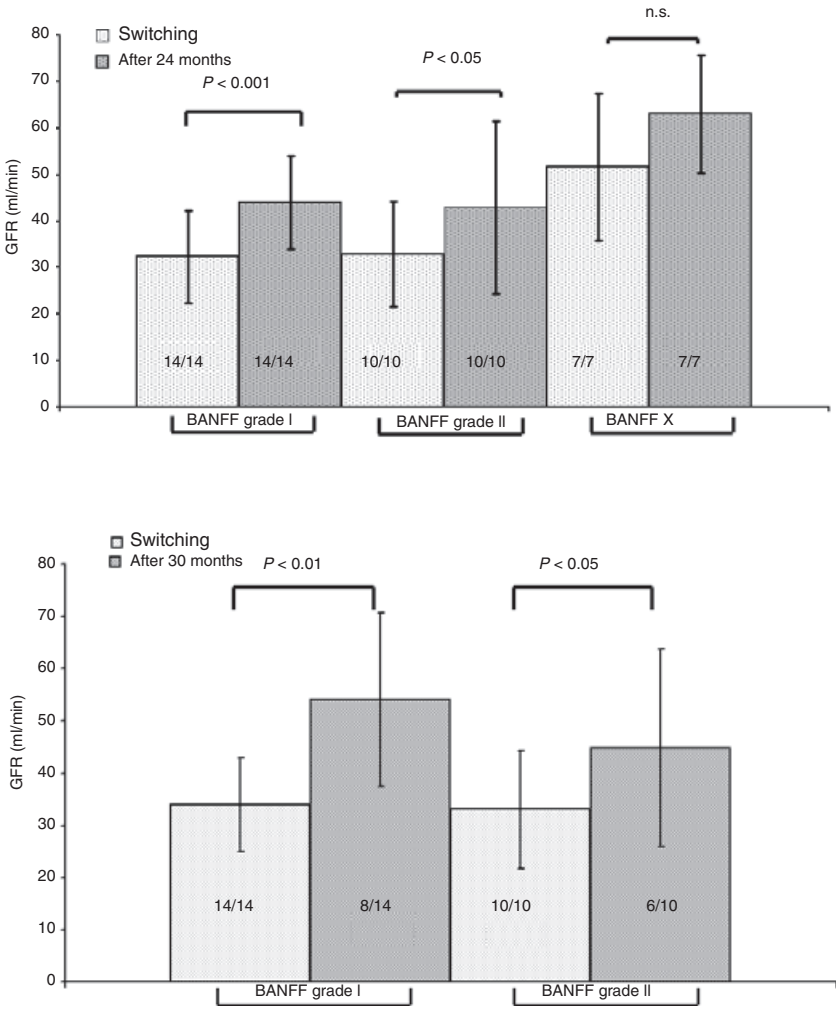

Figure 5 Group A was divided into three subgroups according to the BANFF stages of the renal transplant biopsy findings (grade I, II and not BANFF-related = 'BANFF X'). Upper figure: GFR (*n* as indicated ± SD) according to Cockroft-Gault of each BANFF-group was compared using the *t*-test for matched pairs (*n* as indicated ± SD) before and 24 months after conversion to tacrolimus (BANFF 1 versus BANFF 1 at 24 months: $P < 0.001$; BANFF II versus BANFF II at 24 months: $P < 0.05$; BANFF X versus BANFF X at 24 months n.s.). Lower figure: GFR (*n* as indicated ± SD) of group A patients with BANFF grade I and II was followed over 30 months (*t*-test for matched pairs, BANFF I at 30 months: $P < 0.01$; BANFF II versus BANFF II at 30 months: $P < 0.05$). At this time, only one patient with BANFF X was left (data not shown).

group lost transplant function (59 ± 26 ml/min vs. 49 ± 22 ml/min after 2 years); GFRs here were calculated as endogenous creatinine clearances.

One possible cause for these results could be a minor nephrotoxicity of tacrolimus compared with cyclosporine. Koefeld-Nielsen *et al.* [25] found a greater calcineurin inhibition of cyclosporine than of tacrolimus using phosphorylated peptides and liquid scintillation. In his study including patients with well-functioning grafts, a minimal calcineurin phosphatase activity in tacrolimus-treated patients was sufficient to maintain a stable graft function [25]. To see if these significantly different calcineurin activity profiles have an impact on graft function, we monitored inulin and PAH clearances of a subgroup of groups A and B for 3 months. Yet, inulin and PAH clearances, representing the GFR and ERBF of the transplanted patients, did not yield significant differences before and after switching or between the two groups. This indicates that a different grade of nephrotoxicity of both calcineurin inhibitors may not be the major reason for the increase in transplant function in group A with CAN. Cyclosporine in group B showed no significant effect on transplant function, at least not within 3 months.

Hypertonia and hyperlipidemia also contribute to the deterioration in transplant function in a CAN. For that reason, a therapy of CAN always has to involve an optimization of those factors. In our groups A and B, there was no significant change in blood pressure parameters (MAP or number of antihypertensive drugs) over the 30 months of observation time. Yet, we were able to show that CAN patients need clearly more antihypertensive drugs to achieve comparable blood pressure values than patients without CAN. For the reason that among patients of the CAN group A, there were significantly more patients medicated with ACE inhibitors and Ang II antagonists at the end of the study than in group B, we have to discuss a certain impact of these kinds of

antihypertensive drugs on the increasing transplant function in this patient cohort. In contrast, clinical studies dealing with the subject of possible enhancement of transplant function under the influence of drugs affecting the renin angiotensin system show divergent results and it is not clear up to now whether those substances are beneficial for the transplant function over time [26]. It has been shown earlier that tacrolimus therapy is associated with a low negative influence on post-transplantation hypertension than cyclosporine [27]. We observed no significant changes in serum lipids after switching to tacrolimus. Both groups A and B showed a similar and significant decrease in serum cholesterol until the end of the observation period. In contrast, recently published studies show a clear decrease in LDL cholesterol and triglycerides under tacrolimus [24,28].

The subgroup analysis of groups C and D yielded an increase in MPA after switching to tacrolimus, in contrast to cyclosporine that was associated with a relative decrease in MPA metabolites as acyl-MPA-glucuronide and MPA-glucuronide. This was shown earlier by Zucker *et al.* [29], Filler *et al.* [30] and Van Gelder *et al.* [31] and was explained by omission of the inhibiting effect of cyclosporine on the glucuronidation of MPA. Studies of Naito *et al.* [32] and Mandla *et al.* [33] showed an increased metabolic ratio of MPAG/MPA in patients under CsA, which is caused by an inhibition of the biliary excretion of MPAG under CsA. Therefore, patients taking tacrolimus might have a bigger exposure of their gut toward MPA and this could explain the higher rate of gastrointestinal side effects under therapy with tacrolimus and MPA [34,35].

Taken together, we would suggest an early switching to tacrolimus in patients with CAN especially at stages according to the BANFF classifications I and II. We do not think that tacrolimus is less nephrotoxic than CsA because this has not been confirmed by our PAH and inulin clearances 3 months after switching. The beneficial and rejection reducing effect of tacrolimus may be associated with a concomitant elevation of the effecting MPA concentration, which allows moderate tacrolimus trough levels. Anyhow, tacrolimus therapy was accompanied by a much higher rate of adverse side effects than cyclosporine therapy that led in single patients of our study cohort to transplant loss. Tacrolimus and MMF therapy therefore afford intensive care for the patients after switching.

Authorship

MT: performed research/study. IK: designed research/study. GB: designed research/study. WR: analysed data. RLC: designed research/study. HU: analysed data. BC: designed research/study and wrote the paper.

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