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ORIGINAL ARTICLE

Calcineurin-inhibitor avoidance in elderly renal allograft recipients using ATG and basiliximab combined with mycophenolate mofetil

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Summary

In old recipients of renal allografts from old donors, benefits of calcineurin-inhibitors (CNI) are curtailed by nephrotoxicity. Intending to improve the outcome of these recipients, we analyzed a CNI-free immunosuppressive regimen consisting of anti-thymocyte globulin (ATG), basiliximab, mycophenolate mofetil (MMF) and steroids. Kidney allograft recipients with low immunological risk (panel reactive antibodies <30%) were eligible for this study. Immunosuppression induction included ATG (4 mg/kg, day 0), basiliximab (20 mg, day 0 + 4) and steroids, followed by MMF (TL 2–6 μg/ml) and steroid maintenance treatment. Patient and graft survival rates respectively were 89.3% and 85.4% (12 months), and 86.6% and 76.8% (24 months). Delayed graft function occurred in 44.6%. S-creatinine at 12 months was 1.85 ± 0.94 mg/dl. Thirty patients (53.6%) showed biopsy-proven rejections (6x Banff 3, 13x Banff 4I and 16x Banff 4II), 77% of which were steroid-sensitive, 23% required antibody treatment. After 12 months, 83% of the patients had an MMF-based immunosuppression, 43% were CNI-free. Cytomegalovirus (CMV) infections occurred in 28, tissue-invasive disease in three patients. Despite acceptable renal graft survival and function in some of patients with marginal organs, high incidences of rejections and CMV infections suggest the feasibility of CNI-avoidance using an MMF-based protocol only in carefully selected patients.

Introduction

Elderly patients are the fastest growing subpopulation requiring renal transplantation [1]. On the basis of a safe surgical procedure and increased survival following transplantation compared to dialysis even in older patients, age per se, should not be a barrier to transplantation [2]. However, success of transplantation in this group of patients is determined by their comorbidity with increased cardiovascular, infectious and neoplastic vulnerability, resulting in a shorter general life expectancy [2,3]. In addition, increased age at the time of transplantation has a significant impact on long-term graft survival, and death with functioning graft is a common event during follow-up [1]. With the intention to overcome the inevitably rising organ shortage, the allocation of kidneys from old cadaveric donors to older recipients has become a strategy in the Eurotransplant allocation policy (ET Senior Program [4]). However, the consequence may be rising incidences of delayed graft function (DGF), chronic allograft nephropathy (CAN) and graft loss. Currently, the reported one year allograft survival rate in patients older than 50 years ranges between 70% and 90% [1,5,6].

The aforementioned factors of co-morbidity in older patients may, in part, be intensified by adverse effects of immunosuppressive drugs [3]. As a consequence, optimal immunosuppression for these patients should combine avoidance of nephrotoxicity, preservation of limited morphological and functional reserves of older organs, and effective protection from acute allograft rejection [7,8]. In addition, cardiovascular risk factors such as hypertension, dyslipidemia and diabetes should not be aggravated [9,10]. Previous studies showed promising results after kidney transplantation using a calcineurin-inhibitor (CNI)-free regimen, combining immunosuppression with the desired reduction in its adverse effects [11,12]. Presuming the hypothesis of reduced immune reactivity to allografts in older patients and higher susceptibility to oxidative stress, amplified by acute CNI toxicity [8], we designed a CNI-free, MMF-based immunosuppression with the aim to improve the outcome in this challenging subgroup of kidney transplant recipients.

Methods

In this prospective, single center pilot study, 56 patients, above the age of 50 years with low immunological risk, were consecutively recruited from September 2002 to April 2005. Patients with panel reactive antibodies >30% and those receiving living-related transplants were excluded. The study was conducted according to the ethical standards laid down in an appropriate version of the 2000 Declaration of Helsinki and was approved by the ethics committee of the University of Munich.

All patients received an induction therapy with a single shot of anti-thymocyte globulin (4 mg/kg ATG Fresenius®, Bad Homburg, Germany) perioperatively combined with basiliximab (20 mg Simulect®, Novartis, Basel, Switzerland) on day 0 and 4 after transplantation. By using this approach, we attempted an optimized activity against ischemia-reperfusion related immune-processes [13] and a profound counteraction against IL-2 signaling without having to accept CNI toxicity. In addition, we expected an equal extent of anti-rejection activity than with a standard dose ATG as used in our preceding study [12] but with a better safety profile (e.g. opportunistic infections and cancer). Mycophenolate Mofetil (MMF, Cellcept®; Hoffmann-La Roche, Grenzach-Whylen, Germany) was started from day 0 with a dose of 1.5 g twice a day orally. Thereafter, MMF doses were adjusted to reach a MPA trough level of 2–6 μg/ml, according to recommendations in the current literature [14]. Methylprednisolone (MP, Urbason®; Aventis, Frankfurt, Germany) was administered according to our standard practice, starting with 250 mg intra-operatively and on day 1, subsequently tapered down to doses of 10 mg/day until day 21. Beyond month 3, steroids were progressively tapered. All patients received a concomitant anti-oxidative treatment consisting of vitamin E (1000 mg orally before surgery), vitamin C (3 × 500 mg/day i.v. from day 0–3) and acetylcysteine (ACC 3 × 300 mg i.v. from day 0–3).

All patients, regardless of their donor/recipient cytomegalovirus (CMV)-constellation, received a CMV prophylaxis with CMV hyperimmune globulin [Cytotect®; Biotest, Dreieich, Germany: day 0: 2 ml/kg body weight i.v.; day 7 (and in case of D+/R− constellation, additionally at day 14) pop: 1 ml/kg body weight i.v.]. In case of a CMV infection (>400 copies per ml in the CMV-PCR), patients received a pre-emptive treatment with intravenous ganciclovir (Cymeven®, Hoffmann-La Roche, Grenzach-Whylen, Germany) until three consecutive CMV-PCRs were negative.

Variables investigated were patient and graft survival, renal function, incidence of delayed graft, incidence of biopsy-proven acute rejections (BPARs), opportunistic infections, and the need for introduction of CNI treatment. Safety parameters were the incidence of opportunistic infections (e.g. CMV, herpes simplex virus, herpes zoster virus, *Pneumocystis carinii* and *Candida*).

The diagnosis of acute rejection was biopsy-proven and classified according to the Banff criteria. For safety reasons, renal allograft biopsies were rigorously performed beyond the standard criteria (elevation of baseline serum creatinine >20%), in all patients with graft dysfunction suggestive of an acute rejection episode. Acute rejection episodes were treated with three boluses of MP (250 mg), maintaining unchanged basal steroid doses. Patients with acute vascular rejection or steroid resistant rejection were treated with a course of anti-thymocyte globulin, anti-lymphocyte globulin or Muronomab (Orthoclone OKT 3®; Cilag, Sulzbach, Germany). Cyclosporine A (CsA) or tacrolimus were added according to the severity of the acute rejection episode.

Delayed graft function was diagnosed when patients required dialysis within the first week after transplantation, after ruling out accelerated or acute humoral rejection, vascular complications or urinary tract obstruction. Furthermore, the glomerular filtration rate (GFR) was calculated using the modified MDRD formula.

Statistical analyses were calculated in two populations: intention-to-treat analysis was performed to evaluate renal function, graft and patient survival and cumulative BPAR and included all patients who had received at least one dose of MMF, regardless of the drugs they were currently receiving at month 12. No patient was lost to follow-up for graft and patient survival, serum creatinine and acute rejection. Analysis of patients on protocol included all patients who remained on the assigned drugs as per protocol at 12 months. Comparisons between patient subgroups

were calculated by chi-squared test or *t*-test as appropriate. Actuarial patient and graft survival were calculated according to the Kaplan–Meier method, comparisons were performed by log-rank analysis. All calculations were performed with SPSS 14.0 (SPSS Inc. Chicago, IL, USA).

Results

Study population

Patients were consecutively recruited from September 25, 2002 to April 19, 2005. The median follow-up time was 23.5 months. Donor and recipient characteristics are summarized in Table 1. Delayed graft function occurred in 25 cases (44.6%). The mean interval until a spontaneous drop of serum creatinine after transplantation was 9.0 ± 6.7 (median 8.5) days.

Patient and graft survival and renal function

The CNI-free immunosuppressive therapy resulted in an acceptable patient and graft survival after renal transplantation. Patient survival was 89.3% after 12 months, 83.3% after 24 months and after 30 months, respectively. During the observation period, nine deaths occurred (3x cardiac deaths, 1x stroke, 1x hemorrhagic shock, 3x septic pneumonia, 1x esophageal cancer). At 12 months follow-up, graft survival was 85.4%, at 24 months 76.8% and at 30 months 73.2%. Graft loss occurred only in one case because of recurrent rejection, in two cases because of CAN, and in another one case because of an infectious complication (Fig. 1a).

Table 1. Patient demographics and donor characteristics, (n = 56).

Age (years)	63.1 ± 4.6 [54–74]
Gender (male/female)	44/12 (78.6/21.4)
Ethnicity	
Caucasian	56/56 (100)
BMI (kg/m^2)	25.4 ± 2.7 [19–32]
Primary cause of end-stage renal disease	
Glomerular disease	26/56 (46.4)
Polycystic disease	8/56 (14.3)
Hypertension/nephrosclerosis	9/56 (16.1)
Diabetes mellitus	8/56 (14.3)
Other	5/56 (8.9)
Cadaveric donor	56/56 (100)
Patients with delayed graft function	25/56 (44.6)
Mean HLA mismatches	2.8 ± 2.0
% Patients with panel reactive antibodies (10–30%)	1/56 (1.7)
Mean cold ischemia (hours)	14.0 ± 7.4 [4–47]
Mean donor age (years)	58.3 ± 16.1 [19–81]
Mean donor S-creatinine (mg/dl)	0.98 ± 0.43 [0.37-2.70]
CMV IgG	
(D+/R−)	16/56 (28.6)
(D+/R+)	16/56 (28.6)
(D−/R+)	16/56 (28.6)
(D−/R−)	8/56 (14.3)

Early avoidance of CNIs resulted in an excellent renal function in the first year as reflected by the calculated GFR and serum-creatinine values at 1, 3, 6 and 12 months after transplantation (Table 2). Patients maintained on a MMF based therapy showed a significantly better renal function already one month after transplantation. This beneficial effect of a CNI-free therapy was even more pronounced at month 12. However, only 21 of 48 patients (44%) with functioning grafts could be continuously kept on a CNI-free regimen with MMF and steroids throughout month 12. The reasons for an additional use of CNIs or Target of rapamycin (TOR) Inhibitors of all patients throughout the whole observation period are shown in Table 3.

Interestingly, among them, in one patient signs of CNI toxicity were noted, although the patient never had any exposure to CNIs.

Biopsy-proven acute rejections

A high frequency of late acute allograft rejection was a significant problem under CNI-free immunosuppression. Thirty of 56 (53.6%) patients experienced a BPAR (Banff 3: six patients; Banff 4I: 13 patients; Banff 4II: 16 patients). Five recurrent rejection episodes were observed in these patients. Twenty-seven rejections (77%) could be successfully treated by MP pulse therapy, only eight (23%) patients required antibody treatment. Within this protocol, acute allograft rejections occurred relatively late (mean 75.9 ± 85.3, median 54 days after transplantation) (Fig. 1b).

Impact of recipient/donor age and HLA mismatch on primary outcome parameters

The patients were analyzed in two subgroups (age 50–65 years vs. age above 65 years, the latter largely corresponding to the Eurotransplant Senior Program (ESP) population) to determine the impact of recipient and donor age on the outcome. Patient characteristics for both groups are shown in Table 4. As expected, donor and recipient age varied significantly between the two groups. Most evidently, because of the nature of the predominantly local allocation of organs within the ESP-program, the number of HLA mismatches was significantly higher among the older patient group (Table 4, $P < 0.0001$).

Thus, patients above 65 years of age, revealing a higher degree of mismatches showed a higher incidence and severity of BPAR (Table 4). A significant relation-

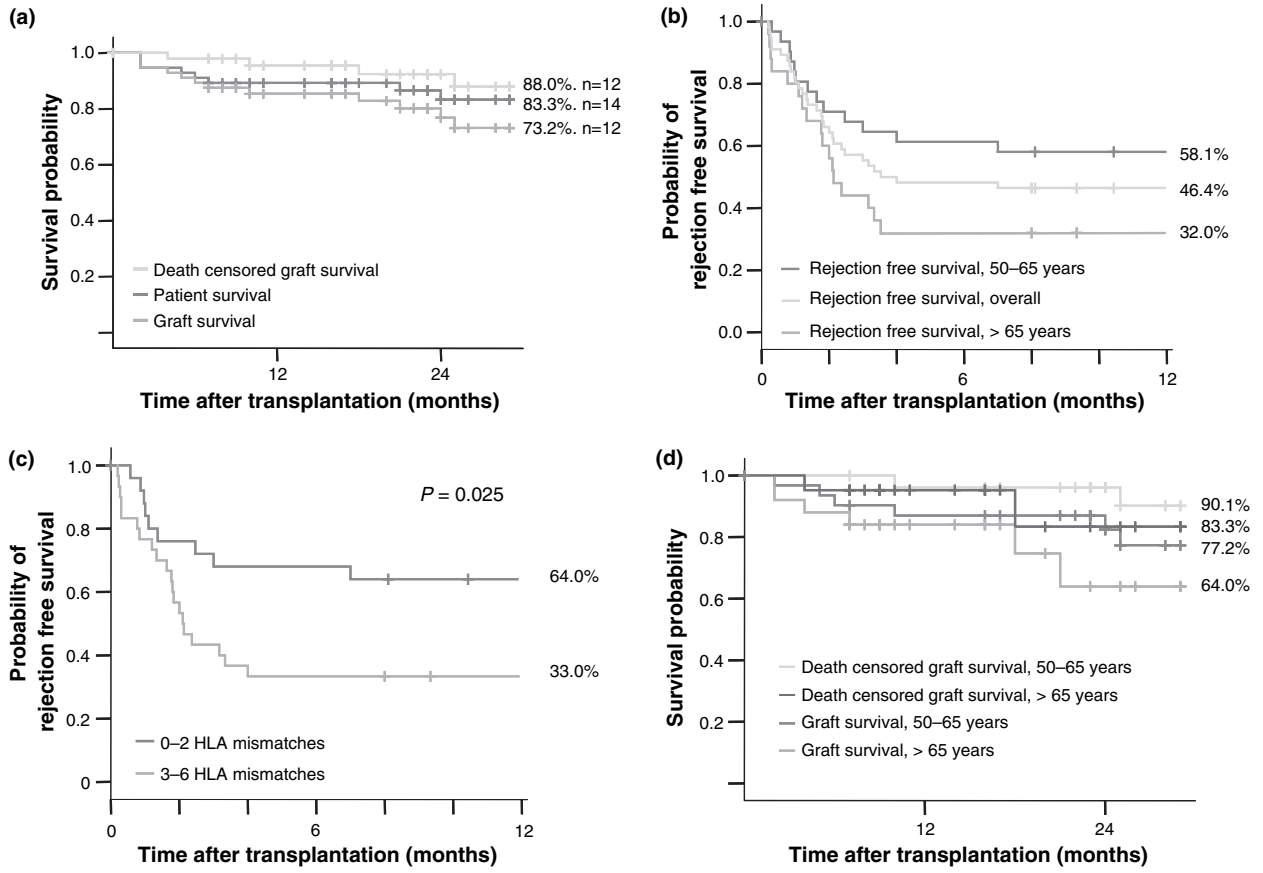

Figure 1 Kaplan–Meier estimates of patient survival. (a) Kaplan–Meier estimate after stratification for overall patient- and graft survival. The effect of immunosuppression on the graft survival independently from patient death in this elderly transplant population is demonstrated by simultaneous presentation of death-censored graft survival. (b) Kaplan–Meier estimate for the rejection-free survival is shown for all patients and age-stratified for patients between 50 and 65 years or patients above 65 (ET senior program). (c) The rejection free survival is shown in relation to the number of mismatches (0–2 vs. 3–6). Whereas log-rank analysis revealed only a slight difference in rejection free survival between the two age groups ($P = 0.05$), the significant impact of mismatches on rejections as calculated by chi-squared test (Table 4) and by the multiple logistic regression model was reconfirmed by significant differences in rejection free survival ($P = 0.025$) after stratification according to the number of mismatches. (d) To illustrate the effect of donor/recipient age on graft survival, Kaplan–Meier estimates are shown after stratification of the patient population into two age groups (50–65 and above 65 years). Log-rank analysis revealed no significant difference.

Table 2. Renal function: serum-creatinine and calculated glomerular filtration rate (GFR).

	Month 1	Month 3	Month 6	Month 12
Serum creatinine (mg/dl)				
Total	2.09 ± 1.37	1.81 ± 0.76	1.79 ± 0.75	1.85 ± 0.94
CNI-free	1.87 ± 1.47†	1.54 ± 0.58†	1.71 ± 0.89†	1.60 ± 0.78†
CNI ad-on	2.45 ± 1.64	2.14 ± 0.97	1.95 ± 0.61	2.13 ± 1.04
GFR (ml/min/1.73 m^2)				
Total*	42.4 ± 20.1	44.4 ± 18.0	44.5 ± 19.8	44.5 ± 21.8
CNI-free*	49.0 ± 20.9†	52.4 ± 19.6†	49.7 ± 21.0†	52.9 ± 23.5†
CNI ad-on*	35.8 ± 15.8	38.3 ± 16.6	37.1 ± 17.2	35.6 ± 15.9

*Calculated by the simplified MDRD formula: censored for death with functioning graft, a GFR value of 0 was set for graft loss.
†$P < 0.05$ CNI-free versus CNI ad-on.

ship between the number of HLA mismatches [low (0–2) versus high (3–6); $P = 0.025$] and the incidence of BPAR was observed. (see also Fig. 1c). In addition, patients above 65 showed a clear trend towards a higher incidence of BPAR as compared to patients between 50 and 65. ($P = 0.05$, Fig. 1b). However, graft

Table 3. Reasons for changes in the primary immunosuppression.

Patient	Time (days postTx)	Reason	New immunosuppression
A.P.	180	Recurrent CMV infection, diarrhea	CsA, MP
B.G.	9	Steroid resistant rejection Banff 4IA	CsA, MMF, MP
C.D.	217	Delayed wound healing, gastrointestinal adverse effects (diarrhea)	CsA, MP
D.F.	67	Severe CMV infection, history of renal cell carcinoma	SRL, MP
E.A.	86	Acute rejection Banff 4IA	CsA, MMF, MP
F.K.	19	Wound healing problems	CsA, Myfortic, MP
H.E.	68	CMV disease	CsA, MP
K.P.	159	Colitis	CsA, MP
Ki.M.	186	Steroid resistant rejection Banff 4IIA	Tac, MMF, MP
K.J.	24	Steroid resistant rejection Banff 4IA	CsA, MMF, MP
K.R.	169	Steroid resistant rejection Banff 4IA	CsA, MMF, MP
K.M.	33	Steroid sensitive rejection Banff 4IA	CsA, MMF, MP
L.M.	75	Leuco-/thrombocytopenia	CsA, MMF, MP
N.S.	102	Steroid resistant rejection Banff 4IA	Tac, MMF, MP
P.R.	51	Steroid sensitive rejection Banff 4IB, side effects of MMF (dose reduction required)	SRL, MMF; MP
R.A.	40	Steroid sensitive rejection Banff 3, side effects of MMF (dose reduction required)	SRL, MMF, MP
R.B.	74	Recurrent CMV infection	CsA, MP
M.R.	16	Steroid resistant rejection Banff4IIB (C4D pos.)	Tac, MMF, MP
S.-C.F.	26	Steroid resistant rejection Banff 4IIB	CsA, MMF, MP
S.J.	127	Steroid sensitive rejection Banff 4IA	Tac, MMF, MP
S.K.	89	Steroid sensitive rejection Banff 4IIB	Tac, MMF, MP
S.M.	29	Steroid sensitive rejection Banff 4IIA	CsA, MMF, MP
S.H.	74	CMV infection	CsA, MMF, MP
T.G.	62	Steroid sensitive rejection Banff 4IA	Tac, MMF, MP
W.K.	26	Steroid sensitive rejection Banff 4IA	CsA, MMF, MP
W.X.	70	Borderline rejection Banff III, CMV infection	CsA, MMF, MP
Z.G.	91	Steroid resistant rejection Banff 4IA, CMV infection	CsA, MMF, MP

CsA = cyclosporine A, MMF = mycophenolate mofetil, MP = methylprednisolone, Tac = tacrolimus, SRL = sirolimus.

Table 4. Impact of recipient and donor age on outcome parameters.

	Age (years)		
Patients	50–65	≥65 (ESP)	*P*-value
Recipient age (years)	59.9 ± 3.2	67.0 ± 2.4	<0.0001
Donor age (years)	48.0 ± 14.5	71.1 ± 5.0	<0.0001
Donor S-Cr mg/dl	1.00 ± 0.50	0.96 ± 0.31	0.77
Cold ischemia time (h)	16.3 ± 7.8	11.1 ± 5.8	0.009
Mismatches (*n*, median)	1.5 ± 1.5	4.3 ± 1.4	<0.0001
Deaths (*n*)	5/31 (16)	4/27 (15)	0.99
DGF (*n*)	11/31 (35)	14/27 (58)	<0.005
S-Cr (mg/dl) at 12 months	1.76 ± 0.78	2.06 ± 1.24	0.36
S-Urea (mg/dl) at 12 months	60.1 ± 28.9	73.1 ± 39.8	0.26
BPAR (*n*)	12/31 (39)	17/27 (63)	0.052
Grade 4 I BPAR (*n*)	6/12 (50)	4/17 (24)	0.95
Grade 4 II BPAR (*n*)	5/12 (42)	10/17 (59)	0.043

Data presented as mean ± SD, *P*-values as calculated by *t*-test or by chi-squared test as appropriate. ESP = Eurotransplant senior program, S-Cr = serum creatinine, S-urea = serum-urea, DGF = delayed graft function, BPAR = biopsy proven acute rejection.
Values in parentheses are percentages.

survival, as well as death censored graft survival did not differ between these two age-stratified patient categories. (Fig. 1d). Patients who experienced rejection episodes showed a significantly inferior serum creatinine at 3, 6, and 12 month after transplantation. However, GFR between 3 and 12 months showed no significant differences between rejectors and nonrejectors, suggesting that after the initial impairment of renal function,

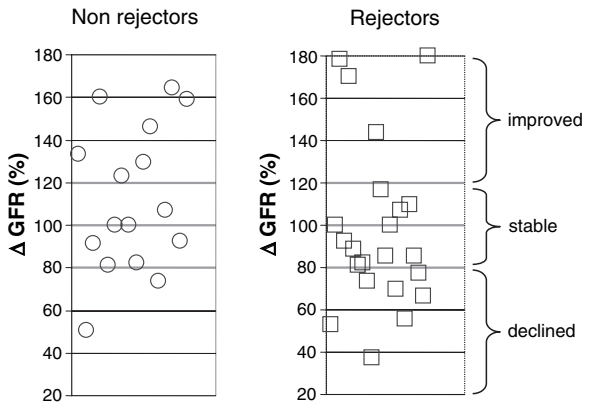

Figure 2 To identify a potential negative effect of acute rejection episodes on the development of renal function the ΔGFR (MDRD formula) between month 3 and 12 was calculated for patients experiencing acute allograft rejection and patients free of rejection. The results of this data show no significant difference between the two groups.

there was no further progression of renal function loss (Fig. 2).

Immunosuppressive therapy at the end of the first year

All patients recruited to this pilot trial were initially treated with a CNI-free, MMF-based immunosuppressive protocol. However, because of different handling with either acute rejection episodes, CMV infections or MMF-associated adverse events (diarrhea, leucopenia), the immunosuppressive therapy at the end of the first year - as a mirror of tolerability of the chosen treatment modality - was not homogenous. Forty of the 48 patients (83%) with a functioning graft could be kept under a MMF-based immunosuppressive protocol, 21 (44%) of them on a complete CNI-free immunosuppression with MMF. Patients maintained on MMF, reached a mean MMF trough level of 3.8 ± 2.0 μg/ml under a median dose of 2 g MMF/day. Our target trough MMF level (>2 μg/ml) was accomplished in 63% of all patients. At the end of the first year, methyprednisolone could be tapered to a mean dose of 3.7 ± 2.3 mg/day.

In 56% ($n = 27$) of the patients with graft function, the primary immunosuppression had to be changed within the first year after transplantation. In 19 patients, additional low-dose CNI treatment was initiated to manage acute rejection episodes. In two cases, sirolimus was used instead. In six cases, MMF had to be completely replaced by CNIs (5x) or sirolimus (1x) to manage recurrent CMV infection or severe gastrointestinal disorders. MMF doses had to be reduced in 18 patients predominantly because of CMV infections or leukopenia. Changes in the primary immunosuppression are shown in Table 3.

Infections and adverse effects

Cytomegalovirus infection occurred in 31 patients (55%), tissue invasive disease was evident in three cases (10%) during the whole observation period. Eleven patients (35%) experienced recurrent CMV infection episodes. All cases except one were resolved under a reduction of MMF with or without the additional use of CNI/TOR-Is, therapy with i.v. ganciclovir and/or valgancyclovir with or without additional CMV hyperimmune globulin. One patient died because of uncontrollable CMV pneumonia. The seroconversion rates for the different CMV donor/recipient constellations were: D+/R− 12/16 (75%), D+/R+ 12/16 (75%), D−/R+ 6/16 (38%) and D−/R− 1/8 (13%). Statistical analysis revealed no relationship between acute rejections and CMV infections.

Discussion

Despite the improvement in immunosuppressive CNI-based protocols, resulting in lower rates of acute rejection and improved early graft survival rates, long term renal allograft survival has not substantially improved over the last two decades. Even when using projected survival, the half-life for cadaveric donor kidney transplants is only around 10 years [15]. CAN remains the major cause of late graft loss in surviving patients [16]. While the causes and progression of CAN are multifactorial, the nephrotoxic effects of CNI drugs have emerged as important contributors to this process [17]. Therefore, current trends in immunosuppression have focused on minimizing or elimination of CNIs with the expectation of an improved long term allograft survival [11,12].

Especially, older patients receiving suboptimal organs, show an enhanced vulnerability to the acute and chronic toxicity of CNI drugs. It was shown that in this patient category the incidence of DGF can rise up to more than 50%, associated with a permanent impairment of renal function [3,5]. Moreover, marginal grafts also seem to be more susceptible to chronic CNI nephrotoxicity [3]. Consequently, avoiding the use of nephrotoxic immunosuppressive drugs, i.e. CNI might particularly serve elderly patients receiving renal allografts from aged or marginal donors. Based on the hypothesis, that the risk of acute rejection decreases with recipient age and elderly patients have a higher susceptibility to oxidative stress after ischemia and reperfusion, amplified by acute CNI toxicity [18,19], we attempted to establish an MMF-based CNI-free regimen in renal transplant patients with low immunological risk and aged older than 50 years. A preceding study revealed the feasibility of a CNI free regimen [12]. However, the commonly used 10-day course of ATG, as used in that study, is associated with a high incidence of

tumor induction and viral infections. This led us to the concept of double induction, combining the positive effects on ischemia and reperfusion by ATG [20] with simultaneously counteracting against the IL-2 signal. Following a combined ATG and basiliximab induction, it was our expectation that a dual therapy with MMF and steroids would keep acute rejections in a reasonable range, while minimizing global CNI toxicity for renal transplant recipients.

However, DGF varied between 35% and 58% depending on the age group analyzed in this study. This may be within the usual range for elderly patients as shown by several groups recently [21,22]. Notably, we observed in the majority of patients an immediate recovery from DGF, with most of the patients requiring <1 week of postoperative dialysis. No permanent impairment of renal function occurred. The early avoidance of CNIs in all patients may have contributed to this rapid improvement in renal function. Supplementary CNI treatment – as far as necessary – could be postponed to 10 weeks after transplantation on average, and after complete recovery of renal function.

Taking our patient population into account, graft and adjusted graft survival rates at 12 and 30 months after transplantation were better than the latest UNOS and CTS data. Nevertheless, the expected striking breakthrough of improved survival was not achieved. One predominant reason for graft loss in the first 3 years was death with functioning graft; only four grafts were lost for reasons of chronic immunological or infectious conditions (2x CAN, 1x chronic rejection, 1x chronic urinary tract infection). Therefore, we attribute the low rate of death-censored graft loss in part to a successful avoidance of CNIs, especially in the early postoperative phase. This conclusion is further favored by the mean serum creatinine values remaining stable during the 12-month follow-up period after transplantation. Patients seemed to benefit from a CNI-free medication resulting in significantly better serum creatinine and GFR at 12 months after transplantation when compared with patients with additional CNI medication.

Our data may suggest that freedom from acute rejection and CNI avoidance have a beneficial effect on renal graft function. However, one has to admit that those patients, who ultimately switched to a CNI treatment, were the ones who experienced problems with the original protocol and/or acute allograft rejections. In contrast to the graft survival rates and one year renal functional data, the frequency of acute rejection episodes was unexpectedly and unacceptably high. Especially in the older patient category, associated with a high degree of HLA mismatches allograft, rejection episodes were found in 63% of the patients. Although, this does not allow the conclusion of a generally higher immunological response of the older patients, because of the different allocation procedures (ESP), there was an association with a high degree of HLA mismatches in this group. On the other hand, we do not have any evidence to support the hypothesis of an immunosenecence in older allograft recipients. In fact, the immunological response in the setting, using aged donor organs for elderly recipients remains to be determined. Tesi *et al.* [19] found a lower number of immunologically triggered graft losses in old recipients and Ciciarelli *et al.* [23] postulated that the HLA matching effect in kidney transplantation is lost in donors above 40 years of age. In contrast, we found that the number of mismatches does have an influence on the incidence of rejections in this elderly patient category. These findings at least suggest a sufficiently remaining immunological capacity in aged recipients, which is in accordance with recent studies reporting rejection episodes in the range of 40–70% by using conventional CsA based triple therapy [2,24,25].

One of the most remarkable predictors of CAN is acute rejection, which is associated with a worse prognosis, if there are multiple episodes, or when late onset occurs. Therefore, one might expect that the observed high incidence of acute rejection episodes in our study might be associated with an inferior graft survival or graft function. Yet, this was not the case: we observed an even better graft survival compared with conventional CNI based therapy [4,12,24]. Whereas renal function was significantly reduced in patients who experienced a BPAR, $\Delta GFR^{M12\text{-}M3}$, which is supposed to be a good predictor for long-term graft survival, showed no clear evidence for a progressive deterioration of graft function after allograft rejection.

Interestingly, we observed a relatively late onset of rejection episodes in our patients with a median time of rejection 54 days after transplantation. This time pattern suggests that the induction with a combined single-shot of low dose ATG and basiliximab does allow CNI avoidance during the early postoperative phase, but may postpone rejection episodes to later time points. In contrast, the low rejection rate of 23.6% in a previous study [12] using ATG induction for 4–10 days suggests that an extended ATG protocol might be capable of inducing a more sustained immunomodulatory effect. Apparently, depletion of CD25 positive cells by two doses of IL-2 receptor antibodies does not have the capacity to abandon CNIs in the first months after transplantation in all patients [26]. This notion is supported by our own observation that CD25 positive cells were virtually absent at least in the first month after transplantation (data not shown) and by a recent work by Vincenti *et al.*, which also observed a high rejection rate with an MMF-based

protocol in the presence of a complete IL-2 receptor blockade [27].

The overall tolerability of this regimen was good, but a number of problems could be identified. Foremost, the relatively high rate of CMV infections frequently resulted in a reduction in MMF and/or conversion to cyclosporine. The role of high doses of MMF for the development of CMV infections is controversial, but may have contributed to this phenomenon [28,29]. In fact, increased incidences of CMV infections under MMF-based therapy have been observed in many centers and are believed to be because of a weaker antiviral effect of MMF compared with that of CNI. However, in our patients, most of the CMV infections were diagnosed on the basis of a positive PCR, without signs of CMV syndrome or disease. Only 3 cases of tissue-invasive disease overall underline this fact.

In summary, the results of this pilot study in immunologically low-risk allograft recipients provided only partial success in our patient population. Although a sizeable amount of patients allowed the limitation of chronic CNI treatment, the rate of 53.6% of acute rejections and complicated CMV infections in 35% of the patients seems to be unacceptably high. Because of a higher mismatch, especially older patients were at risk of an acute rejection episode. Together, our immunosuppressive concept with 'double' induction, followed by MMF plus steroids long term immunosuppression might not be sufficient for a sustained improvement in long-term outcome in renal transplantation and may thus not be recommended as ideal CNI free protocol. A lower rate of acute rejection may be required before wide acceptance of this CNI avoidance strategy, even in consideration of the acceptable patient and graft survival. To improve further the idea of CNI avoidance, modifications such as the temporary use of additional immunosuppression in a selected subpopulation at higher risk of acute rejection should be considered.

Authorship

M.G. and M.R. wrote the paper and analyzed the data, H.A. and W.G.L. designed the study, W.D.I., U.S. and K.-W. J. contributed important reagents, C.D.W. and A.U. collected the data.

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