Transpl Int (2003) 16: 820–827
DOI 10.1007/s00147-003-0638-7

ORIGINAL ARTICLE

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Calcineurin inhibitor-free immunosuppression based on antithymocyte globulin and mycophenolate mofetil in cadaveric kidney transplantation: results after 5 years

Received: 21 November 2002
Revised: 6 March 2003
Accepted: 20 March 2003
Published online: 22 July 2003

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Abstract Kidney grafts from suboptimal donors are more likely to suffer the nephrotoxic side-effects of cyclosporine than kidneys from standard donors. In an attempt to avoid the use of cyclosporine, we carried out a prospective study in low-immunological risk recipients of suboptimal kidneys, using an immunosuppressive protocol combining Thymoglobuline in induction with a bi-therapy of mycophenolate mofetil (MMF) and steroids. Patients with panel reactive antibodies (PRA) < 50% receiving a first renal transplant from a suboptimal donor (age ≥50, non heart beating, arterial hypertension, or acute renal failure) or a kidney at risk of delayed graft function (DGF) because of a prolonged cold ischaemia time (CIT) of 24 h or more, were eligible for this trial. Between September 1996 and December 1999, 30 patients were enrolled for the trial and treated with MMF 2 g orally, pre-operatively, and 3 g daily, post-operatively; Thymoglobuline 2 mg/kg IV pre-operatively, 1.5 mg/kg IV the next day, and for doses of 1 mg/kg IV given on alternate days; and prednisolone 0.25 mg/kg per day, reduced progressively from the end of the first month to 0.1 mg/kg per day by 3 months post-transplant. Cyclosporine was added only if rejection grade II or higher, or a reduction in MMF below 1 g daily, occurred. Ten patients (30%) suffered from DGF, and one kidney suffered primary non function. Seven patients (24%) suffered acute rejection (six were biopsy proven, 3 grade I and 3 grade II). MMF dosage was reduced in 28 patients because of adverse events, and calcineurin inhibitors were introduced in 16 patients. There were 14 episodes of opportunistic infection (cytomegalovirus (CMV 10), Herpes zoster 2, Listeria monocytogenes 1, Pseudomonas aeuruginosa 1), and 7 malignancies (skin 2, thyroid 1, lung 1, Kaposi's sarcoma 2, post-transplantation lymphoproliferative disorder 1). Mean serum creatinine was 178, 199, 213, and 218 μmol/l at 1, 2, 3 and 5 years after transplantation, respectively. Actuarial patient and graft (after censoring for death) survival was 94% and 83% after 1 year and 79% and 65% after 5 years, respectively. These results show that with the combination of MMF, Thymoglobuline and steroids the use of cyclosporine can be delayed, and in a few cases completely avoided, with good efficacy in terms of prevention of rejection and recovery of renal function. Regardless of acceptable patient and graft survival, side-effects of MMF at the doses used in this protocol were common and led to overimmunosuppression in the long-term. Starting MMF at low dose, MPA monitoring and probably CMV prophylaxis may improve the results of this regimen.

Keywords Thymoglobuline · Cyclosporine · Mycophenolic acid Suboptimal grafts · Renal transplantation

Introduction

Following its launch on the market in 1984, cyclosporine has been routinely included in most immunosuppressive regimens in the transplantation field because of its demonstrated efficacy in reducing acute rejection rates and improving patient survival. Unfortunately cyclosporine, like later calcineurin inhibitors, has a number of undesirable adverse effects, in particular nephrotoxicity [1]. In renal transplantation, acute cyclosporine nephrotoxicity in ischaemic post-transplant renal failure may prolong oliguria and reduce graft survival rates [2, 3]. Early introduction of a known nephrotoxic agent, such as an anticalcineurin agent, probably adds to existing post-preservation ischaemic injuries by two mechanisms; acute vasoconstriction of the glomerular capillaries and direct tubular toxicity, slowing the rate of tubular regeneration [1, 4, 5]. At the histological level, published clinical studies have reported contradictory results, some describing renal allograft interstitial fibrosis [6], others reporting chronic nephropathy [7], and others describing no abnormal histology related to long term use of cyclosporine [8]. In heart transplantation, chronic nephropathy leading to chronic renal failure has been described in association with long-term treatment with cyclosporine [9].

With the increasing use of sub-optimal donors (the elderly, kidneys with pre-existing lesions, prolonged cold ischaemia time) because of the increasing donor shortage, it is becoming even more important to avoid these nephrotoxic effects. Suboptimal kidneys are associated with a higher incidence of delayed graft function (DGF) and, in addition, are more susceptible to calcineurin inhibitor nephrotoxicity than "optimal" kidneys [10, 11]. We thus decided to examine the possibility of using an immunosuppressive regimen which avoided the use of calcineurin inhibitors in patients receiving kidneys from suboptimal donors.

Mycophenolate mofetil (MMF)(Cellcept, Roche, Spain) and Thymoglobuline (rabbit anti-human thymocyte globulin, Imtix-SangStat, France) have both been shown to be effective immunosuppressants in renal transplantation [12, 13, 14, 15]. We therefore chose an immunosuppressive regimen using these two agents (MMF long-term and Thymoglobuline in induction), together with steroids. In a previous work we reported on a 6-month follow-up of the first 17 patients included in the protocol [16]. The results after 6 months were promising, showing a 12% incidence of DGF and a 24% incidence of acute rejection. To gain insight into the safety of using this protocol, we present here a 5-year follow-up of the 30 patients included in this prospective study.

Materials and methods

Patients

All consecutive patients between September 1996 and December 1999 given first renal transplants from sub-optimal donors, and who had given their informed consent were enrolled in this trial, provided that their peak panel reactive antibodies (PRA) were less than 50%. Donors were defined as sub-optimal if they fulfilled one or more of the following criteria: age ≥50; non heart beating; arterial hypertension with proteinuria within 0.3–1 g/day ; acute renal failure before organ harvesting. Kidneys were considered to be at high risk of delayed graft function (DGF) if the cold ischaemia time (CIT) ≥24 h.

Immunosuppression

The immunosuppressive protocol consisted in administering MMF 2 g orally pre-operatively, and then at a dose of 3 g daily, given in two divided doses, starting within 72 h of surgery (as soon as the patient could tolerate oral medication); methylprednisolone 1 mg/kg IV intra-operatively, and post-operatively at a dose of 0.25 mg/kg per day, converting to oral prednisolone at the same dose when possible, and tapering after the end of the first month to achieve a dose of 0.1 mg/kg per day by the end of month 3; and an induction course of Thymoglobuline 2 mg/kg pre-operatively (day 0), 1.5 mg/kg on day 1, and then 4 doses of 1 mg/kg per day on days 3, 5 and 7, and 9 (equal to a cumulative dose of 7.5 mg/kg). Before each infusion of Thymoglobuline, an additional bolus dose of steroids (25 mg) was administered.

Calcineurin inhibitors (cyclosporine or tacrolimus) were only introduced under the following circumstances: acute rejection ≥grade II; grade I acute rejection if the clinician considered it necessary; insufficiently immunosuppressive dose of MMF (< 1 g/day).

Treatment of acute rejection was achieved with 3 methylprednisolone boluses of 500 mg IV while maintaining the dose of oral steroids unchanged, with cyclosporine added if acute rejection was grade II or higher. Biopsy was carried out in clinically suspected cases of acute rejection before treatment was initiated.

Clinical management and definitions; efficacy and safety parameters

No routine prophylaxis or monitoring of cytomegalovirus (CMV) infection was carried out. If there was clinical suspicion of CMV infection, it was confirmed by the measurement of antigenaemia levels before treatment with ganciclovir. Routine antibacterial prophylaxis of aztreonam 1 g/day and amoxicillin-clavulanic acid 1 g was administered 3 times daily pre-transplant and for 2 days

Table 1 Donor characteristics in each case enrolled in the study. *M* male, *F* female; *NHBD* non-heart-beating-donor; *CIT* cold ischemia time; *HTA* arterial hypertension; *CVA* cerebro-vascular accident; *CT* cranial trauma; *CH* cerebral haemorrhage; *ARF* acute renal failure; *UK* unknown

Case	Age (years)	Sex	NHBD	CIT (h)	HTA	Cause of death	ARF
1	60	M	No	27	Yes	CVA	No
2	74	M	No	18,1	Yes	CVA	No
3	60	M	No	17,5	UK	CVA	No
4	48	M	No	21,2	Yes	CVA	Yes
5	62	M	No	23	UK	CT	No
6	74	M	No	15,2	Yes	CVA	No
7	41	F	No	18	No	Methanol	Yes
8	21	F	Yes	16	No	Cerebral tumour	No
9	71	F	No	14,5	UK	CT	No
10	60	M	No	14,5	Yes	CVA	No
11	41	F	No	23	No	Methanol	Yes
12	71	F	No	18	UK	CT	No
13	44	F	No	19	Yes	CVA	No
14	76	M	No	24	Yes	CT	No
15	62	M	No	24,2	Yes	CVA	No
16	44	M	No	16	Yes	CVA	Yes
17	59	M	No	12,1	Yes	CVA	No
18	63	M	No	18,4	UK	CVA	No
19	60	M	No	23	Yes	CVA	No
20	35	M	No	24,1	No	CT	No
21	40	M	No	18,2	Yes	CVA	No
22	62	M	No	19	UK	CT	No
23	67	M	No	34	UK	CT	No
24	62	M	No	20,2	Yes	CVA	No
25	21	F	Yes	22	No	CH	No
26	21	F	Yes	28	No	CH	No
27	68	F	No	22	UK	CT	No
28	77	M	No	17	Yes	CVA	No
29	77	M	No	21	Yes	CVA	No
30	31	M	No	20	Yes	CVA-Cirrhosis	Yes

following transplantation. No anti-fungal prophylaxis was given. Delayed graft function (DGF) was defined as the need for dialysis in the week following transplantation caused by rising serum creatinine levels, but not in consequence of accelerated or hyperacute rejection, vascular rejection or urinary tract obstruction. The following efficacy parameters were collected: incidence of biopsy-proven acute rejection with Banff grading [17]; patients needing the introduction of cyclosporine; graft loss; recipient death; incidence of DGF; incidence of oliguria (defined as a urinary output of < 400 ml/day); and renal function (measured by serum creatinine levels). Graft loss was defined as the institution of long-term dialysis, re-transplantation or transplant nephrectomy. Safety parameters were the incidence of opportunistic infections (in particular cytomegalovirus [CMV]); adverse events, malignancies and deaths. Patient and graft survival after 1 and 5 years was estimated with the Kaplan–Meier survival analysis.

Results

Baseline characteristics

Patients were followed up for a median of 45 months post-transplant (range 20–60). No patients dropped out or were lost to follow up. Baseline donor and recipient characteristics are shown in Tables 1 and 2. In particular, it can be seen that the average donor age was 55, ranging from 21 to 77, and the average recipient age was 56, ranging from 24 to 75 years. Kidneys fell into the following "suboptimal" categories, defined by four donor characteristics: non heart beating ($n=3$); high blood pressure ($n=16$); >50 years ($n=19$); acute renal failure pre-harvesting ($n=5$); CIT ≥ 24 h ($n=5$). As shown in Table 1 some cases had more than one criterion for classification as suboptimal.

Delayed graft function and acute rejection

Of the 30 patients, 10 (33%) suffered from DGF, 5 additional patients sufferend from oliguria without the

Table 2 Baseline recipient data ($n=30$)

Characteristic	Mean ± SD (range) or n
Recipients ($n=30$)	
Age	56 ± 13
Gender M:F	18:12
HLA mismatch (number of loci)	3.4 ± 1.27
Peak PRA > 20%	3 (10%)
Primary pathology (ERSD)	
Chronic glomerulonephritis	6
Chronic interstitial nephritis	3
Nephroangiosclerosis	5
Polycystic kidneys	6
Haemolytic uraemic syndrome	1
unknown	9

need for dialysis. Of these 15 patients, renal function recovered in 14; the kidney in the remaining case never functioned. Thus, 29/30 patients recovered renal function. Six episodes of biopsy-proven acute rejection occurred over the whole follow-up period in 6 patients (20%). All episodes occurred in first 6 months. An additional case of acute rejection was clinically diagnosed (this case was diagnosed at the weekend and responded to treatment, so no biopsy was taken). In total, there were 7 cases of diagnosed and treated acute rejection (24%). Of the six biopsy-proven acute rejections, three episodes were grade I, and 3 were grade II (Table 3). All cases responded to methylprednisolone boluses (cyclosporine was introduced concomitantantly in 4 cases). There were no recurrent rejection episodes.

MMF dose adjustments

MMF dose was reduced at least once in 28 out of 29 patients because of side-effects (14 episodes of diarrhoea and abdominal discomfort, 9 leucopenia, 5 anaemia, 13 opportunistic infections and 1 neoplasia). Reductions were mainly done in the first 3 months (in 23 out of 29 patients). Late MMF reductions took place in the 11th month due to CMV infection, the 14th month due to disseminated VZV, and in the 34th, 51st and 54th month due to gastrointestinal adverse events. In 8 patients (27%) the dose of MMF had to be reduced to below 1 g/day because of MMF-related side-effects (7 cases of gastro-intestinal effects and one case of leucopenia). In addition, MMF was permanently stopped in 7 patients (3 due to neoplasia, 4 to adverse events related to MMF and not reversed by MMF dose reduction).

Calcineurin inhibitor introduction

A total of 16 patients were started on calcineurin inhibitors, 12 received cyclosporine (5 for acute rejection, 6 for MMF adverse reactions requiring a reduction of MMF to less than 1 g/day and 1 because of proteinuria) and 4 received tacrolimus (1 for proteinuria, 1 for biopsy proven chronic rejection and 2 because of

Table 3 Acute rejection. *KT* kidney transplantation; *Dx* diagnosis; *sCrea* serum creatinine; *CNI* calcineurin-inhibitor introduction; *CsA* cyclosporine

Patient	Time after KT	Histological Dx (Banff)	sCrea at Dx	sCrea (post)	CNI
6	Day 5	Grade I	280	78	no
10	Day 12	ND	210	129	CsA
11	Day 121	Grade I	446	335	no
12	Day 29	Grade I	252	163	no
14	Day 18	Grade II	273	150	CsA
24	Day 34	Grade II	241	144	CsA
29	Day 75	Grade II	327	234	CsA

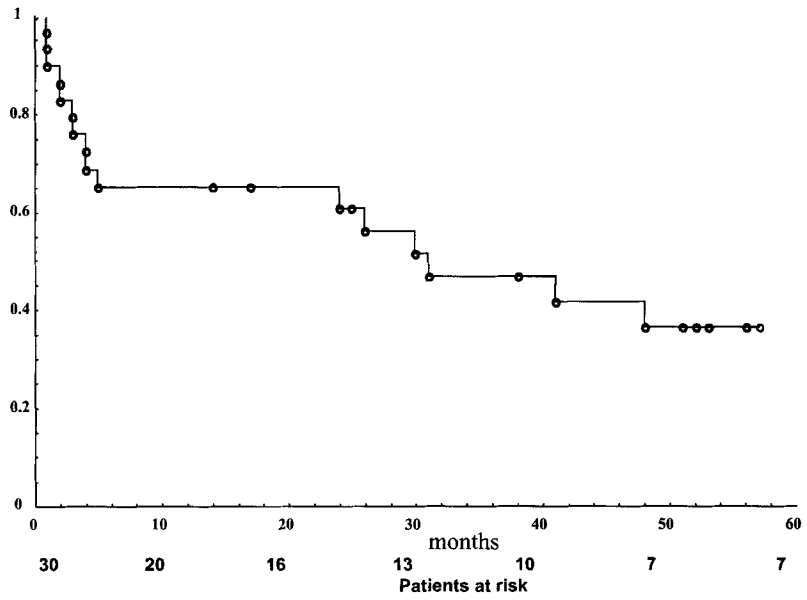

Fig. 1 Cumulative percent Kaplan–Meier analysis of calcineurin-free patients throughout the follow-up period. Product limit estimates for calcineurin-free was 79%, 65%, 60%, 46% and 36% after 3, 12, 24, 36 and 60 months, respectively

MMF adverse reactions; requiring a reduction of MMF to less than 1 g/day). Figure 1 illustrates the evolution of the percentage of calcineurin-free patients during the 5-year follow-up. It was 65% at 1 year, but at 5 years it decreased below 36%.

Opportunistic infection and malignancy

There were 14 episodes of opportunistic infection in 14 patients (46.6%), which consisted of 10 CMV (3 tissue invasive); 2 herpes zoster, 1 sepsis by listeria monocytogenes and 1 sepsis by pseudomonas aeruginosa. All cases were resolved with specific antimicrobial treatment with the exception of the patient with sepsis by pseudomonas aeruginosa who died. In 7 patients, 7 malignancies were diagnosed over the period of follow up (cutaneous squamous carcinoma 2; thyroid 1; lung 1; Kaposi's sarcoma 2; PTLD 1). The squamous carcinomas of the skin appeared 9 and 21 months after transplantation and were treated successfully by excision. The case of thyroid carcinoma appeared 4 months after transplantation and was treated successfully with total thyroidectomy and cervical lymphadenectomy. The case of squamous lung cancer occurred in a smoker, was diagnosed 1 month after transplantation and treated by lower left pulmonary segmentectomy. This patient was the only patient with a malignancy who died of the malignancy over the period of follow up, 13 months after transplantation. The case of PTLD was diagnosed unexpectedly on a routine kidney biopsy (B cell lymphoma) performed 14 months after transplantation, so MMF was stopped and the patient had a full recovery following treatment with chemotherapy, but died 1 year later due to congestive heart failure. The Kaposi's sarcomas were diagnosed 5 and 7 months after transplantation, respectively. Both were treated with MMF discontinuation with good response.

Patient and graft survival

There were 5 deaths (1 lung cancer, 1 congestive heart failure, 1 Pseudomonas aeruginosa sepsis, 1 cerebral haemorrhage, 1 acute myocardial infarction). Serum creatinine at the time of these events was 130, 180, 178, 122 and 140 μmol/l, respectively. By Kaplan–Meier analysis, product limit estimates for patient survival were 94% after 1 year and 79% after 5 years. The Kaplan–Meier patient survival curve is given as Fig. 2A.

There were 14 graft losses. The main cause of graft loss was chronic transplant nephropathy ($n=6$), followed by death with a functioning kidney ($n=5$). There was one case of primary non function (mentioned above). Other causes of graft loss were: renal biopsy induced bleeding, at 6 months ($n=1$), Kaposi sarcoma with concomitant MMF discontinuation at 5 months ($n=1$). Those cases of death with a functioning kidney were censored in the graft survival analysis. By Kaplan–Meier analysis product limit estimates for graft survival were 83% at 1 year and 65% at 5 years (Fig. 2B).

Serum creatinine was 178 ± 96 (range 77–496), 199 ± 130 (range 84–698), 213 ± 114 (range 88–526) and 218 ± 155 (range 88–623) at 1, 2, 3 and 5 years after transplantation, respectively.

Discussion

The patients reported here were given "at risk" kidneys, meaning kidneys from sub-optimal donors or kidneys exposed to a long cold ischaemia time. This population of patients has previously been described as having an incidence of DGF of greater than 50% [18, 19, 20]. Our percentage (30%) of patients suffering from DGF compares favourably with the published results. The classic approach to prevent nephrotoxicity and to facilitate recovery from DGF is sequential immunosuppressive therapy in which calcineurin inhibitors are only introduced when renal function is at least partially recovered. In a recent paper it was found that a sirolimus/chimeric-IL-2R monoclonal antibody (basilximab)/prednisolone combination with late introduction of cyclosporine provided excellent acute rejection prophylaxis, although long-term results are needed to assess the effectiveness of these new sequential strategies [21, 22].

There was a low incidence of acute rejection in the patients in this study (24%), in spite of the fact that initially they were not treated with calcineurin inhibitors. This relatively low incidence of acute rejection combined with a relatively high incidence of CMV infection (30% of patients suffered a CMV infection) suggests that the combination of Thymoglobuline and MMF is highly immunosuppressive, at least in the doses used in this study. Similar rates of acute rejection with a high rate of CMV antigenaemia are reported by Zanker et al [23], when a combination of antithymocyte globulin and MMF was used. In contrast, in calcineurin inhibitor-free regimens based on anti-IL2R, the incidence of acute rejection appears to be higher and the CMV infection rate lower [24]. These data suggest that a less selective polyclonal preparation may induce a more potent immunosuppression than a highly selective anti-IL2R mAb in the absence of cyclosporine or tacrolimus. In addition, three of the cases of malignancy we observed were classically associated with immunosuppression (2 Kaposi's sarcoma, 1 PTLD). Concluding from the high incidence of CMV and malignancy we can assume that our treatment caused over-immunosupression. In this study, patients did not receive CMV prophylaxis. Thus, in the light of the results, CMV

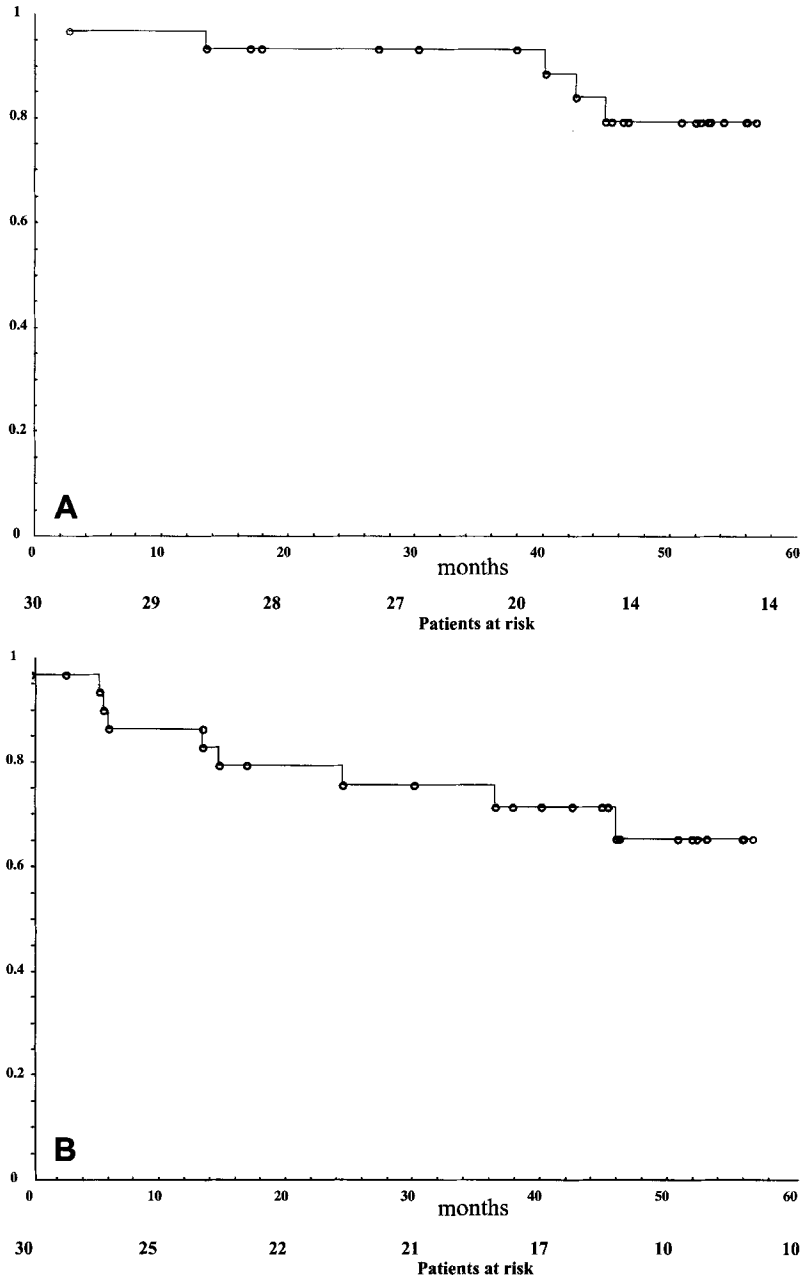

Fig. 2 Kaplan–Meier analysis of patient **A** and graft survival **B**. Product limit estimates for patient survival was 94% after 1 year and 79% after 5 years and for graft survival (after censoring for death) were 83% after 1 year and 65% after 5 years

prophylaxis may be beneficial in recipients of suboptimal renal grafts treated with an immunosuppressive protocol based on antithymocyte globulin and MMF.

It should be noted that the initial doses of MMF we used were high for a protocol which lacked cyclosporine. At the time when this protocol was designed we did not know that MPA exposure is reduced by cyclosporine because of interference at the pharmacokinetic level (MPAG excretion into bile being inhibited by cyclosporine). [25]. Moreover, Mourad et at [26] showed that there was a progressive MPA-AUC increase over time as a result of a decrease of MPA clearance and metabolism. Thus, the major limitation of our study was the lack of MPA monitoring. Thus, there was a high incidence of MMF-related side effects, with the consequence that a high proportion of patients discontinued MMF or were on an insufficiently immunosuppressant dose, so that calcineurin inhibitors had to be introduced. On the other hand, MMF reduction beyond the therapeutic range may favour, in some cases, acute and chronic rejection. Therefore, in the future initial doses of MMF should be reduced, and dose adjustment by monitoring of MPA trough levels should be mandatory. Despite all these concerns, 5-year patient (79%) and graft (65%) survival

was acceptable, mainly considering the "at risk" nature of these grafts.

The cumulative percentage of calcineurin-free patients was 79% after 1 year and only 36% after 5 years. So, nearly 2/3 of the patients in the end were given calcineurin inhibitors, but at least the early post-transplant period was without calcineurin inhibitors, allowing poor quality kidneys some time for recovery of adequate function, as previously reported by other groups which introduce calcineurin-inhibitor several weeks after transplantation [21, 23]. Our protocol failed to achieve long-term calcineurin-inhibitor-free immunosuppresion, which we believe to be desireable in the scenario of suboptimal grafts from older donors. In the future, MPA monitoring with MMF dose adjustment can help to attain this objective.

We conclude that the combination of MMF, Thymoglobuline and steroids are an effective immunosuppressive regimen, allowing the avoidance of calcineurin inhibitors early after transplantation and providing acceptable rates of 5-year patient and graft survival. However, there is a high incidence of opportunistic infections (mainly CMV) and malignancies, suggesting that this therapeutic approach induces overimmunosuppression, in the long term. Furthermore, there is a high incidence of MMF-related adverse events, necessitating the introduction of a calcineurin-inhibitor. Therefore, in the future, initial doses of MMF should be reduced, MMF dose adjustment by monitoring of MPA trough levels should be mandatory and CMV prophylaxis should be considered.

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