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Use of different immunosuppressive strategies in recipients of kidneys from nonheart-beating donors

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Summary

In nonheart-beating donor (NHBD) kidney transplants, immunosuppressive management is difficult mainly because of the high incidence of acute tubular necrosis. This has meant that since the start of our NHBD transplant program, several immunosuppression regimes have been used. The aim of this retrospective study was to evaluate the results obtained over 7 years using different treatment protocols. A total of 172 consecutive NHBD transplants performed between April 1996 and December 2002 were treated as follows: G-I ($n = 21$), cyclosporine (8 mg/kg/day) plus azathioprine plus steroids; G-II ($n = 65$), low-dose cyclosporine (5 mg/kg/day) plus mycophenolate plus steroids; G-III ($n = 17$), low-dose tacrolimus (0.1 mg/kg/day) plus mycophenolate plus steroids; and G-IV ($n = 69$), daclizumab plus low-dose tacrolimus plus mycophenolate plus steroids. Delayed graft function rates were 76.2%, 72.3%, 76.5%, and 42%, respectively, for the four groups ($P = 0.000$). Rejection-free patient rates were 76.2%, 46.2%, 35.3%, and 71% ($P < 0.001$). Vascular rejection rates were 19%, 30.8%, 52.9%, and 18.8%, ($P = 0.025$). Two-year graft survival was 71.4% in group I, 95.4% in group II, 94.1 in group III, and 93.8% in group IV ($P = 0.004$). Patient survival was worse in group I (75.2% in group I, 100% in group II, 100% in group III, and 96.7% in group IV at 2 years; $P < 0.001$). The use of daclizumab and low-dose tacrolimus could be effective at lowering the incidence of delayed graft function in NHBDT, with no negative repercussions on acute rejection.

Introduction

Less restrictive transplant criteria and an ever-increasing number of patients on dialysis have led to a shortage of kidneys for transplant. Although there is a need to extend the use of brain-dead donors with heartbeats, there is still room for additional sources of organs, prompting us to use the nonheart-beating donor (NHBD). Kidneys from NHBD, nevertheless, show a high incidence of delayed graft function [1–3]. The time of delayed graft function (DGF) can be shortened by avoiding the use, or reducing the dose, of calcineurin inhibitors, but this may put the patient at an increased risk of acute rejection [4], with possible effects on long-term outcome. This has led to the use in most regimes of low dose calcineurin inhibitors coupled with potent immunosuppressants such as monoclonal or polyclonal antilymphocyte antibodies. However, these agents can be considered excessive immunosuppression as they increase the risk of subsequent opportunistic infection and cancer [5,6]. Continuous developments in the field of immunosuppression have led to the appearance of new drugs that are able to reduce the risk of rejection without producing overimmunosuppression. It is because of developments such as these that we have been constantly changing our treatment protocol for the NHBD transplant.

The aim of this retrospective study was to compare the efficacy of several immunosuppressive protocols in terms of preventing acute rejection in a series of patients undergoing kidney transplant from NHBD at our center.

Materials and Methods

Patients

This retrospective study was performed on 172 patients who were consecutively transplanted with a kidney from an NHBD at our center over the period April 1996 to December 2002. Hyperimmunized patients (PRA >50%) were not included ($n = 24$). The immunosuppressive protocols were as given below:

1 April 1996 to July 1997: triple therapy with cyclosporine (4 mg/kg pretransplant and 8 mg/kg/day starting on the first day post-transplant to achieve levels between 200 and 250 ng/ml), azathioprine (1 mg/kg/day) and steroids (pretransplant methylprednisolone 250 mg i.v., 2 mg/kg/day on the first day post-transplant reduced to 1 mg/kg/day on day 10 and 0.5 mg/kg/day on day 30 reduced to 10 mg/day between months 4–6 (group I; $n = 21$).

2 July 1997 to September 2000: low-dose cyclosporine (2.5 mg/kg pretransplant and 5 mg/kg/day starting on the first day post-transplant to achieve levels between 130 and 175 ng/ml), mycophenolate mofetil (1 g pretransplant and then 2 g/day) and steroid (pretransplant methyl prednisolone 250 mg i.v., 2 mg/kg/day on the first day post-transplant reduced to 1 mg/kg/day on day 10, and 0.5 mg/kg/day on day 20 tapered to 10 mg/day by the third month (group II; $n = 65$).

3 September 2000 to March 2001: low-dose tacrolimus (0.05 mg/kg pretransplant and 0.1 mg/kg/day starting on the first day to achieve levels of 5–8 ng/ml), mycophenolate mofetil (1 g pretransplant, 2 g/day during the first month, then 1 g/day) and steroid (pretransplant methyl prednisolone 250 mg i.v., 125 mg i.v. on the first day post-transplant, 1 mg/kg/day on day 2 tapered to 0.5 mg/kg/day by day 10 and tapered again to 10 mg/day by month 3 (group III; $n = 17$).

4 March 2001 onwards: daclizumab (Zenapax®; Hoffmann-La Roche, Grenzach-Wyhlen, Germany) administered i.v. over a period of 15 min at 1 mg/kg on the day of surgery, and a second dose 7 days later plus low-dose tacrolimus (same regime as for group III), mycophenolate mofetil (same regime as for group III) and steroids (pretransplant methyl prednisolone 250 mg i.v., 125 mg i.v. on the first day post-transplant, 20 mg/day on day 2 reduced to 10 mg/day by the end of the first month (Group IV; $n = 69$). In groups III and IV, the dose of tacrolimus was increased to keep levels in the range 10–15 ng/ml as from post-transplant day 10.

All the NHBD were Maastricht classification type I or II. The criteria used to select this type of donor and their management have been described in a previous publication [1]. In 45 patients, 41 of whom were in groups II or III, the donors had been subjected to normothermal perfusion during bypass before hypothermia and subsequent transplant.

All other (nonimmunosuppressive) therapeutic protocols remained unaltered during the 6 years of patient entry. Cytomegalovirus (CMV) infection prophylaxis was only given to seronegative patients who received a kidney from a seropositive donor. These patients were treated with preemptive intravenous gancyclovir for 3 months followed by oral gancyclovir for 3 months (the dose was modulated according to renal function) and CMV immune globulin (Cytotect Biotest®; Madaus, Dreieich, Germany) (three consecutive doses followed by weekly doses of 2 ml/kg/day to complete 10 doses). Patients seropositive for CMV did not receive preemptive treatment. CMV infection was diagnosed by the pp65 antigenemia assay or PCR. This determination was made weekly during the first 3 months post-transplant, every 2 weeks for the subsequent 3 months and then once a month. Patients with CMV infection were treated with intravenous gancyclovir. When there were clinical signs of CMV disease, intravenous gancyclovir and anti-CMV specific immunoglobulin were given. No patient was subjected to anti-pneumocystis carini prophylaxis.

The CD25 subset of T lymphocytes was quantified by flow cytometry in blood samples obtained at weeks 2, 4, and 8 and months 3, 6, 9 and 12 post-transplant from the group II, III and IV patients. Fluorescent labeling was performed using FITC monoclonal anti-human CD3 antibodies (Caltag Laboratories, Burlingame, CA, USA) and anti IL2R1-RD1 antibodies (cytostat/coulter clone) and their respective isotype controls according to the standard procedure. The analysis was conducted in a Coulter Epics XL flow cytometer (Beckman, Fullerton, CA, USA) after selecting the lymphocyte population by forward (FSC) and side scatter (SSC).

Delayed graft function was defined as the need for dialysis during the immediate post-transplant period. Patients who showed DGF were biopsied every 7 days until renal function started to improve. Acute rejection episodes were suspected in patients showing a rise in serum creatinine levels and confirmed by biopsy. Acute rejection was graded according to Banff '97 classification [7]. Grade I rejection (interstitial infiltration and tubulitis) was treated with three doses of 250 mg methyl prednisolone, and cyclosporine or tacrolimus doses were increased in patients in groups II, III and IV. Grade II (intimal arteritis) or grade III (transmural arteritis) rejec-

tion was treated with muromonab-CD3. Graft loss was defined as graft nephrectomy, retransplantation, permanent return to dialysis or death.

Statistical analysis

Continuous variables (expressed as mean ± SD) were compared using the Student's *t*-test, while categorical variables were compared by the chi-squared test. Delayed graft function was predicted by logistic regression. The following variables were included in the analysis: graft cold ischemia time, donor and recipient age and sex, human leukocyte antigen (HLA) compatibility, months on dialysis, type of dialysis, immunosuppression treatment, transplant number, peak preformed reactive antibodies, corticoresistant or corticosensitive rejection and year of transplant. The times of donor cardiac arrest, extra- and intrahospital CPR and bypass were also considered. We also took into account whether the donor had been subjected to normothermal perfusion during bypass before hypothermia. Variables showing significant effects on DGF ($P < 0.15$) in the univariate analysis and variables which, based on prior knowledge, could also affect this outcome measure, were then included in a multivariate logistic regression analysis. Adjusted odds ratios (adjOR) and their 95% confidence intervals (95%CI) were calculated using estimated regression coefficients and their standard errors in the logistic regression analysis. Acute rejection and patient and graft survival rates were estimated by the Kaplan–Meier method. The Breslow exact and log rank tests were used to evaluate differences in the survival curves. The null hypothesis was rejected in each statistical test when $P < 0.05$. Analysis was performed using windows SPSS version 11.0 software.

Results

Donor and recipient characteristics

Table 1 shows the demographic characteristics of the donors and recipients. Donor age was higher in group IV than in the remaining groups. There were no marked differences among the groups in terms of the degree of HLA, A, B, or DR mismatches between donors and recipients. The cold ischemia time to which the grafts had been subjected was shorter in the daclizumab group (IV) although the warm ischemia time was longer for these patients.

The median follow-up time was 86 months (range 83–97 months) for group I, 65 months (range 44–82 months) for group II, 39 months (range 38–44 months) for group III and 25 months (range 15–37 months) for group IV.

Efficacy of treatment

The incidence of DGF was 76.2% in group I, 72.3% in group II, 76.5% in group III, and 42% in group IV ($P < 0.001$). In the subgroups of patients with DGF, the median time of DGF was 16 days (P_{25-75} 12–21 days) in group I, 11 days (P_{25-75} 9–17 days) in group II, 13 days (P_{25-75} 10–18 days) in group III, and 12 days (P_{25-75} 9–15 days) in group IV ($P = 0.04$). A lower incidence of DGF was observed in 41 patients whose grafts had been subjected to normothermal perfusion during bypass before hypothermia (29 group II and 12 group III), compared with patients in the same groups who received a graft subjected to by-pass in conditions of hypothermia (61% vs. 85.5%; $P = 0.013$). Table 2 provides the risk factors associated with DGF.

Table 1. General characteristics of the patients.

	Group I	Group II	Group III	Group IV	*P*-value
Donor age (years)	33.5 ± 2.4	35.6 ± 1.4	32.9 ± 2.7	39.4 ± 1.4	0.041
Male donor (%)	90.5	93.8	76.5	85.5	0.20
Recipient age (years)	50.8 ± 2.8	46.6 ± 1.8	43.3 ± 2.7	48.8 ± 1.7	0.31
Male recipient (%)	47.6	72.3	58.8	63.8	0.18
HLA DR mismatch (mean number)	1.33 ± 0.14	1.15 ± 0.08	1.18 ± 0.10	1.10 ± 0.09	0.72
HLA B mismatch (mean number)	1.10 ± 0.14	1.37 ± 0.08	1.53 ± 0.15	1.43 ± 0.08	0.15
HLA A mismatch (mean number)	0.95 ± 1.61	1.32 ± 0.08	1.18 ± 0.15	1.33 ± 0.08	0.10
Current PRA (%)	3.00 ± 1.76	2.10 ± 0.72	1.94 ± 1.12	1.11 ± 0.48	0.49
Regraft (%)	4.8	6.2	11.8	8.7	0.81
Cytomegalovirus IgG positive	95.2	87.7	94.1	78.3	0.12
Warm ischemia time (min)	91.3 ± 8.1	108.9 ± 2.7	110.5 ± 5.8	116.6 ± 3.0	0.002
Cold ischemia time (h)	19.6 ± 0.8	19.1 ± 0.5	18.2 ± 0.7	17.2 ± 0.4	0.004
Donor by-pass perfusion					
Hypothermia	95.2	55.4	29.4	95.7	0.001
Normothermia plus hypothermia	4.8	44.6	70.6	4.3	

Table 2. Adjusted odds ratios for delayed graft function in nonheart-beating donor transplants according to logistic regression analysis.

Variable	AdjOR (95%CI)	*P*-value
Treatment group		
Group I	5.54 (1.71–17.88)	0.001
Group II	4.32 (1.75–10.69)	
Group III	5.90 (1.43–24.35)	
Group IV	1	
Cold ischemia time (h)		
<16	1	0.06
≥16	2.06 (0.96–4.43)	
Corticosensitive acute rejection		
Yes	2.4 (1.0–5.7)	0.01
No	1	
Corticoresistant acute rejection		
Yes	3.74 (1.30–10.74)	0.03
No	1	
Warm ischemia time (min)		
≤150	1	0.024
>150	2.39 (1.11–5.17)	
Donor by-pass perfusion		
Normothermia plus hypothermia	1	
Hypothermia	3.91 (1.40–10.92)	0.008

*adjOR, adjusted odds ratio; CI, confidence interval.

Table 3. Incidence of acute rejection in nonheart-beating donor transplants (first year).

	Rejection-free	Grade I	Grade II	Grade III
Group I	16 (76.2%)	1 (4.8%)	4 (19%)	0
Group II	30 (46.2%)	15 (23.1%)	16 (24.6%)	4 (6.2%)
Group III	6 (35.3%)	2 (11.8%)	5 (29.4%)	4 (23.5%)
Group IV	49 (71%)	7 (10.1%)	12 (17.4%)	1 (1.4%)

$P = 0.001$. All except one of the grade I rejections were grade I-A.

As indicated in Table 3, the number of rejection episodes in the first year was lower in group I and the daclizumab group (IV) compared with the other groups. The rejection rate was higher in group II versus group IV [OR 2.9 (95%CI) 1.4–5.8] and group III versus group IV [OR 4.5 (1.5–13.8)].

The proportions of patients who underwent vascular rejection (Banff grade II or III) were 19% in group I, 30.8% in group II, 52.9% in group III and 18.8% in group IV ($P = 0.025$). Figure 1 shows the Kaplan–Meier analysis of the likelihood of being vascular rejection-free. In 19 group II subjects, cyclosporine was replaced with full-dose tacrolimus because of acute rejection.

In group IV patients, CD25 expression on T lymphocytes decreased during the study period, as described in Table 4 (this variable was not determined in the group I patients).

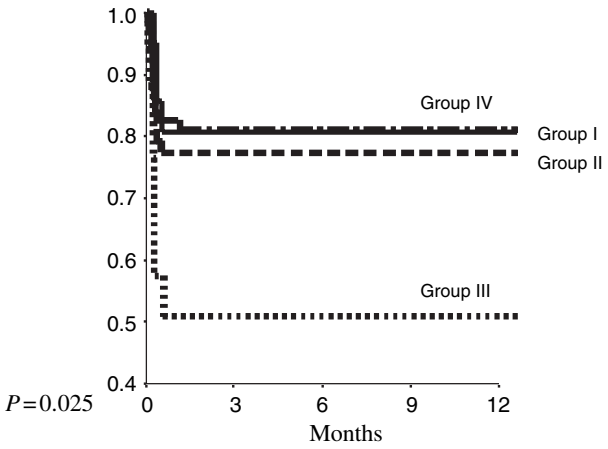

Figure 1 Probability of freedom from acute vascular rejection (Banff classification grade II or III) according to the immunosuppressive treatment regime in nonheart-beating donor kidney transplants: Group I (dash-dot line), group II (dotted line) group III (broken line) and group IV (solid line). The log-rank test was used to calculate the *P*-values.

Renal function and lipid metabolism

No differences in 1-year renal function, determined as serum creatinine, were noted among the groups (1.52 ± 0.10 mg/dl in group I, 1.42 ± 0.07 in group II, 1.65 ± 0.09 in group III, and 1.50 ± 0.07 in group IV; $P = 0.33$). One year after transplant, patients in group IV showed the lowest total cholesterol (215.0 ± 9.1 in group I, 210.6 ± 5.7 in group II, 190.3 ± 9.2 in group III and 185.2 ± 5.9 in group IV; $P = 0.006$) and triglyceride levels (174.6 ± 18.7 in group I, 157.0 ± 13.77 in group II, 136.6 ± 9.9 in group III, 115.5 ± 7.8 in group IV; $P = 0.02$).

Adverse events

No differences were observed in the proportions of patients with CMV infection among the seronegative patients. However, among the seropositive patients, a lower incidence of infection was observed in group IV (70% in group I, 73.7% in group II, 56.3% in group III, and 29.6% in group IV; $P < 0.001$). The incidence of CMV tissue invasive disease was 9.5% in group I, 4.6% in group II, 0% in group III and 1.4% in group IV ($P = 0.26$).

The mean number of re-admissions during the first post-transplant year was 1.2 ± 0.3 in group I, 0.6 ± 0.1 in group 2, 0.5 ± 0.2 in group III, and 0.6 ± 0.1 in group IV ($P = 0.067$). Re-admission was required in 66.7% of the patients in group I, 41.5% in group II, 41.2% in group III, and 32.8% in group IV ($P = 0.06$). The most common motive for admission was sepsis mostly of urinary origin (28.6% of the group I patients, 23.1% group II, 17.6% group III, and 20.3% group IV; $P = 0.83$).

Table 4. Changes in T lymphocyte CD25 expression during the study period.

	Group II	Group III	Group IV	*P*-value
%CD25 2 weeks	20.6 (2.9–37.8) ($n = 26$)	10.1 (5.2–43.3) ($n = 9$)	1.8 (0–30.4) ($n = 59$)	<0.001
%CD25 4 weeks	23.5 (2.2–67.5) ($n = 16$)	17.1 (0.9–100) ($n = 9$)	1.3 (0–43.8) ($n = 52$)	<0.001
%CD25 8 weeks	13.5 (1.1–71.3) ($n = 12$)	7.5 (2.8–83.4) ($n = 14$)	1.3 (0.1–27.5) ($n = 53$)	<0.001
%CD25 3 months	13.3 (2.6–41.6) ($n = 13$)	9.2 (1.2–69.5) ($n = 15$)	4.0 (0.1–58.1) ($n = 64$)	0.04
%CD25 6 months	16.4 (2.7–44.3) ($n = 21$)	3.2 (1.1–26.8) ($n = 15$)	7.0 (1.1–56.0) ($n = 67$)	0.02
%CD25 9 months	9.7 (1.2–41) ($n = 19$)	2.6 (1.4–9.5) ($n = 15$)	5.1 (0.5–19.8) ($n = 58$)	0.13
%CD25 12 months	5.8 (0.4–50.7) ($n = 23$)	5.7 (1.3–16.1) ($n = 15$)	5 (0.5–28.3) ($n = 56$)	0.69

Values are given as median (range).

The incidence of post-transplant diabetes was three patients (15.8%) in group I, six (9.5%) in group II, 4 (23.5%) in group III, and nine (13%) in group IV ($P = 0.48$). One year after transplantation, diabetes was resolved in three group I, three group II, two group III, and five group IV patients.

Tumors developed in two of the group II patients (a lung carcinoma 6 months after transplant and a colon carcinoma 14 months after transplant), three of the patients in group III (a kaposi tumor at 39 months post-transplant, a breast carcinoma at 14-month post-transplant and a kidney tumor at 20-month post-transplant) and one patient in group IV (a prostate tumor detected in the immediate transplant postoperative period).

In 46 of the group IV patients who underwent their first renal transplant and showed stable graft function and serum creatinine <2.5 mg/dl, treatment with steroids was discontinued at a median time of 9 months (P_{25-75} 5–11 months) with no rejection episodes. Steroids were also discontinued in 3 of the group I, 3 of the group II and 2 of the group III patients because of post-transplant diabetes.

Graft and patient survival

One and two years graft survival was 85.7% and 71.4% in group I, 98.5% and 95.4% in group II, 94.1% and 94.1% in group III, and 94.2 and 92.7% in group IV ($P = 0.006$) (Fig. 2). Table 5 provides a summary of the causes of graft failure during the first 2 years post-transplantation. It is remarkable that the main cause of graft failure in the group I was death with functioning graft. Patient survival was worse in group I (75.2% in group I, 100% in group II, 100% in group III, and 96.7% in group IV at 2 years; $P < 0.001$). Causes of death during the first two post-transplant years were in the group I hepatitis C virus infection ($n = 1$), cardiovascular disease ($n = 2$) and tumors ($n = 2$), and in the group IV nocardiosis ($n = 1$), acute pancreatitis ($n = 1$), and hepatitis B ($n = 1$).

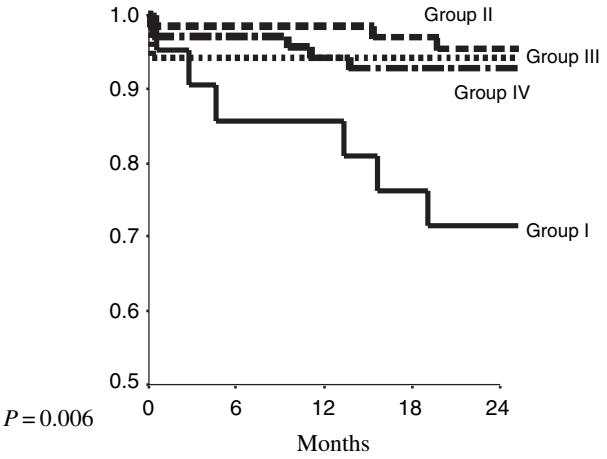

Figure 2 Actuarial graft survival according to the immunosuppressive treatment in non-heart beating donor kidney transplants. Group I (dash-dot line), group II (dotted line), group III (broken line) and group IV (solid line). The log-rank test was used to calculate the *P*-values.

Table 5. Causes of graft failure in the first 24 months after renal transplant.

Cause	Group I	Group II	Group III	Group IV
Total no. of patients	6 (28.6%)	3 (4.6%)	1 (5.9%)	4 (5.8%)
Acute rejection	0	1 (1.5%)	1 (5.9%)	1 (1.4%)
Chronic nephropathy	0	1 (1.5%)	0	0
Surgical complications	0	1 (1.5%)	0	0
Death with functioning graft	5 (23.8%)	0	0	3 (4.9%)
Other causes	1 (4.8%)	0	0	1 (1.4%)

Discussion

Immunosuppressive management in NHBD transplants is difficult mainly because of the high incidence of DGF which makes this type of renal transplant especially susceptible to calcineurin inhibitor mediated vasoconstriction and nephrotoxicity. Moreover, the prompt use of calcineurin inhibitors in cases of DGF may exacerbate ischemic injury and delay recovery from DGF or impair

long-term graft function. Hence, a good immunosuppressive strategy is to reduce the dose of the calcineurin inhibitor or avoid its use altogether. The consequence of this action, however, is an increased risk of acute rejection, which requires that the rest of the immunosuppressive medication be increased. This, in turn, increases the incidence of infections and malignancies [6,8]. In our experience, NHBD transplant recipients treated with quadruple sequential therapy –antithymocyte globulin, azathioprine, steroids, and cyclosporine started between days 5 and 7 post-transplant – show a higher incidence of CMV disease and a worse patient survival rate [8]. These poor results prompted us to change our treatment regime for NHBD transplants in the middle of the 1990s to the conventional triple therapy. The emergence of clinical studies that showed that mycophenolate mofetil in combination with cyclosporine and steroids could decrease the acute rejection rate from about 40% to 20% when azathioprine was replaced with mycophenolate mofetil [9–11], led us to consider that the immunosuppressive benefits of this drug would allow us to reduce the dose of cyclosporine. However, we found it increased the percentage of rejections from 23.8% to 53.8%, probably because of the reduced cyclosporine doses. Subsequently, when tacrolimus was approved in our country, we used this drug (at half the usual dose) to replace cyclosporine, but this still did not reduce the rate of acute rejection. The appearance of daclizumab, a humanized anti-IL-2R antibody, which in phase III clinical trials led to a significant decrease in acute rejection episodes at 6 months and 12 months post-transplant [12–14], prompted our idea that the combined use of this drug with mycophenolate mofetil, corticosteroids and low-dose tacrolimus could be an effective immunosuppressive regimen of low nephrotoxicity. If effective, this regimen could simplify the management of NHBD transplant patients by diminishing the incidence of DGF and the need for dialysis. Our present findings indicate that by using this regime, we were able to reduce the rate of delayed graft function with no consequent increase in acute rejection.

The protocol at our center for grafts showing DGF is to perform a biopsy every 7 days. In these biopsies, tubulitis and an interstitial infiltrate are relatively common findings. Indeed, the Banff grade I rejection episodes were grade I-A in all but one of the present patients. Considering that tubulitis is sometimes seen in stable grafts, and their presence has been demonstrated in routine biopsies undertaken 3–18 days postoperatively in patients with well-functioning kidney transplants [15], we believe, as do others [16], that tubulitis lesions are 'sensitive' yet not 'specific' criteria for rejection. We would therefore say that for an immunosuppressive strategy for NHBD transplants to be considered successful, it should reduce the incidence of Banff grade II or III acute rejection. This was achieved here with daclizumab. Curiously, the biopsy specimens of our patients in the daclizumab treatment group with Banff grade II-A rejection showed isolated endothelialitis not accompanied by tubulitis. Besides reducing the acute rejection rate, daclizumab treatment (group IV) led to a lower proportion of patients with DGF. Thus, in groups II and III, the incidence of DGF was higher than in group IV because, according to our protocol, the dose of cyclosporine or tacrolimus is increased when there are signs of acute rejection, and in 19 of the group II patients, cyclosporine was replaced with full-dose tacrolimus for this reason. Although the mean time of cold ischemia for the grafts was shorter in group IV, which would probably contribute towards lessening the incidence of DGF, logistic regression analysis demonstrated that the type of treatment received also affected the appearance or not of DGF. A further factor known to help avoid DGF is the initial period of graft perfusion under conditions of normothermia. In marginal donor kidney transplants or transplants with DGF, all from heart-beating donors, a low rejection rate was achieved using a calcineurin inhibitor-free protocol with antibody induction (basiliximab or thymoglobulin) [17] or the delayed introduction of low-dose tacrolimus [18]. In a low immunologic risk renal transplant population from heart-beating donors, Kuypers *et al.* [19] used a regimen of five doses of 1 mg/kg daclizumab, low-dose steroids, low-dose tacrolimus and mycophenolate to achieve a low incidence of acute rejection, and excellent graft and patient survival. Like us, these authors were also able to discontinue the use of steroids in a high proportion of their patients. The incidence of DGF was also lowered, although not significantly.

In phase III trials, the use of daclizumab in a five-dose regimen of 1 mg/kg at 2-weekly intervals led to the saturation of IL-2Rα receptors on circulating lymphocytes for up to 120 days after renal transplantation [12,13]. The daclizumab regimen used in our study (two doses) was thus slightly shorter than that described to produce this saturation. However, the latest episode of rejection observed in group IV occurred 35 days after transplant. No subsequent episodes were recorded despite discontinuing steroid treatment. The percentage of CD25 T lymphocytes was significantly lower in the daclizumab group until the sixth post-transplant month, although it seems that the time of CD25 saturation can predict the occurrence of acute cellular rejection [20]. Recently, Vicenti *et al.* [21] reported that one dose (2 mg/kg) or two doses (second dose 1 mg/kg) of daclizumab in addition to maintenance immunosuppression therapy consisting of either tacrolimus or cyclosporine, mycophenolate mofetil and prednisone, were sufficient to

saturate the IL-2Rα receptors on circulating lymphocytes for a little over 40 days. The successful use of lower doses of daclizumab than initially recommended has also been described [22,23].

The adverse events observed in our patients were no greater in the daclizumab group. The high incidence of CMV infection in our transplants could be the result of our protocol of systematic weekly testing for the virus during the first post-transplant term. Although no prophylaxis is given during the immediate post-transplant period, once we detect viral replication we start treatment even if the patient is asymptomatic. The incidence of CMV infection was lower in the daclizumab treatment group, probably because of the lower number of rejections and consequently less immunosuppression. The incidence of tissue CMV disease was low for the entire patient series. Steroids were discontinued in 67% of the patients in group IV, with no consequent episodes of acute rejection. This measure might have a beneficial effect on the risk of cardiovascular disease and perhaps on other factors such as osteoporosis, cataracts, etc. Also, it is probable that the use of different steroid regimens could have had a positive impact on group IV morbidity.

The main limitation of our study was that the treatment protocols varied according to the era. Thus an era effect rather than immunosuppressive effect cannot be ruled out.

In conclusion, our findings suggest that anti-CD25 monoclonal antibody induction with initial low-dose tacrolimus plus mycophenolate mofetil and steroids could be effective at reducing the risk of delayed graft function, without increasing the incidence of acute rejection and with acceptable safety in NHBD transplants.

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