

Randomized clinical trial of daclizumab induction and delayed introduction of tacrolimus for recipients of non-heart-beating kidney transplants

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Background: Kidneys from non-heart-beating donors (NHBDs) have high rates of delayed graft function (DGF). Use of calcineurin inhibitors is associated with a reduction in renal blood flow, which may delay graft recovery from ischaemic acute tubular necrosis.

Methods: To assess whether daclizumab (DZB) could safely replace tacrolimus in the immediate postoperative period, patients were randomized to receive DZB induction and daily mycophenolate mofetil with steroids (DZB group) or standard tacrolimus-based triple therapy (control group). Tacrolimus was given to patients in the DZB group when the serum creatinine level dropped below 350 µmol/l.

Results: Fifty-one patients were recruited at two centres over a 2-year interval between 2000 and 2003. The overall rate of immediate function was 28 per cent (13 of 46 grafts), with the highest rate in recipients of machine-perfused kidneys treated with DZB (eight of 15 patients).

Conclusion: Induction with DZB and delayed introduction of tacrolimus reduced the incidence of DGF in recipients of machine-perfused NHBD kidneys.

Presented to the British Transplantation Society, Birmingham, UK, April 2004, and the International Society of Organ Donation and Procurement, Warsaw, Poland, December 2003; published in abstract form as *Ann Transplant* 2004; 9: 29

Paper accepted 21 January 2005

Published online 26 April 2005 in Wiley InterScience (www.bjcs.co.uk). DOI: 10.1002/bjs.4976

Introduction

The gulf between the demand and supply of kidneys for transplantation continues to grow. One solution to this problem has been to return to the practice of using grafts from non-heart-beating donors (NHBDs). The challenge of NHBD transplantation is to minimize the first period of warm ischaemia and the consequent reperfusion injury. Ischaemia–reperfusion injury of the kidney is a spectrum ranging from mild immediate function, through moderate reversible delayed graft function (DGF) to severe irreversible primary non-function (*Table 1*). In 1995 the Maastricht group produced a classification of NHBD scenarios to structure discussion and aid comparison of results¹.

Primary non-function and DGF were previously the almost universal outcome for recipients of category 2 grafts, compared with a DGF rate of less than 40 per cent for grafts from brainstem-dead donors². Rates

of primary non-function are now acceptably low at around 10 per cent of grafts implanted, but DGF continued to affect 90–95 per cent of successful NHBD transplants in 2000³. In the short term, the consequences of acute tubular necrosis are economic^{4,5}, as recipients with DGF use more hospital resources. In the long term, graft function may be impaired as a result of reduced nephron mass, higher rates of chronic allograft nephropathy and increased atherosclerosis^{6,7}.

A small number of NHBD kidney transplant recipients have immediate function, and factors other than just the duration of warm and cold ischaemia appear to be important². The key to graft recovery seems to be the recipient's ability effectively to perfuse the graft with oxygenated, pressurized blood. Coronary artery disease, peripheral vascular disease, hypotension, infection, acute rejection and recipient age have all been shown significantly to influence the rate and duration of DGF⁸. The situation is

Table 1 Spectrum of kidney reperfusion injury

Classification	Clinical	Pathology	Dialysis requirements after transplantation
Mild	Immediate function	None	≤ 1
Moderate	Delayed graft function	Acute tubular necrosis	> 1
Severe	Primary non-function	Cortical necrosis and infarction	Continuous

further complicated by the use of nephrotoxic drugs such as calcineurin inhibitors, which are the mainstay of modern immunosuppressive regimens. These agents are thought to compromise renal perfusion by increasing intrarenal resistance^{9,10}.

This study evaluated an immunosuppressive regimen based on an anti-interleukin 2 receptor (IL-2R) monoclonal antibody, daclizumab (DZB), to minimize the early use of calcineurin inhibitors.

Patients and methods

Minor differences exist in the techniques used by the centres in Leicester and Newcastle to retrieve kidneys from both controlled (categories 3 and 4) and uncontrolled (category 2) NHBDs. Outcomes have been described elsewhere^{3,11}. Both centres use aortic double-balloon catheters, inserted across the common femoral artery, in uncontrolled donors¹². During theatre transfer, chilled preservation solution and heparin are administered via the cannula to cool the abdominal organs and clear the microcirculation of thrombus. In Newcastle, a dose of the thrombolytic agent streptokinase is given as a preflush¹³. At both centres, controlled donors may have treatment withdrawn in either the intensive care department or the operating theatre. In Newcastle, category 2 and 3 grafts are placed on hypothermic machine perfusion for 4 h to assess viability. In Leicester, viability at the time of retrieval is assessed by means of subjective measures alone, minimizing the cold ischaemia time. Both centres have reported rates of primary non-function of around 10 per cent^{11,14}.

All recipients in this study were evaluated by the Newcastle cardiovascular risk score (Table 2). The scoring system was designed to delineate a detailed pathway of further coronary and left ventricular imaging, aiding patient assessment and communication of operative risk between nephrologists, transplant surgeons and anaesthetists based in different hospitals around the Northern region. In the context of this study, the scoring system also characterizes certain recipient risk factors for DGF⁷.

This was a two-centre randomized clinical trial evaluating induction with DZB as part of a calcineurin-sparing

Table 2 Newcastle renal transplant anaesthetic cardiovascular risk score

	Score
Age (years)	
< 45	0
45–65	2
> 65	4
Diabetes	3
Blood pressure	
Systolic blood pressure < 110 mmHg	4
< 150/90 but < 110 mmHg systolic	2
Systolic > 110 or > 150/90 mmHg with or without medication	3
Stable maximal angina pectoris	3
Angina pectoris on minimal exertion (40–50 m)	4
One MI or one coronary artery bypass graft; two-vessel disease or angioplasty	3
Two MIs or coronary bypass grafting with triple-vessel disease, or two surgical procedures with coronary artery bypass grafting	4
Mitral, tricuspid or pulmonary disease, or valvular surgery	2
Aortic valve surgery or disease	3
Exercising distance	
50–200 m or slows on stairs	3
< 50 m or stops on stairs	4
Previous stroke or transient ischaemic attack	3
Peripheral vascular disease	3
Body mass index < 18 or > 30	3
Total score	0–36

MI, myocardial infarction.

regimen. Approval for the study was obtained from both Leicester and Newcastle regional ethics committees. All patients were supplied with a detailed information sheet in plain English describing the study, and invited to participate on the basis of informed consent.

The aim of the study was to demonstrate an improvement in graft immediate function rates from 5 to 40 per cent, a figure attained by the Maastricht group in Holland¹². Patients in the two trial groups received immunosuppressants according to the following schedule. The DZB group received an induction dose of 2 mg/kg DZB (Zenepax®; Roche, Basle, Switzerland) and methylprednisolone 500 mg intravenously before graft implantation. Subsequent doses of 1 mg/kg DZB were given at 14-day intervals up to a maximum of five doses. Tacrolimus (Prograf®; Fujisawa, Munich, Germany) at a

dose of 0.1 mg/kg twice daily was instituted (and further DZB doses withheld) when either the recipient's serum creatinine concentration had fallen below 350 $\mu\text{mol/l}$ or there was evidence of acute rejection on core biopsy. The control group received a single induction dose of methylprednisolone, and tacrolimus was started the following morning. Background immunosuppression in both groups was with mycophenolate mofetil (Cellcept®; Roche) 2 g per day and prednisolone 20 mg daily. Dosing of tacrolimus in both groups was aimed at maintaining a trough level of 8–12 ng/l.

The primary endpoint of the study was graft function. The incidence of DGF was defined by the requirement for more than one dialysis session in the first week after transplantation, with subsequent evidence of graft function (Table 1). The duration of DGF was taken as the interval between transplantation and the last haemodialysis session or peritoneal dialysate exchange. For patients who were predialysis, grafts were deemed to be functioning when the daily serum creatinine levels showed sustained improvement. Transplant recipients who required one or no dialysis session after surgery were deemed to have immediate function. Grafts with primary non-function had no demonstrable function after transplantation, and evidence of cortical infarction and necrosis on core biopsy, and were censored from detailed analyses.

The total warm ischaemia time was calculated as the sum of the first and second warm ischaemia times during graft implantation. The first warm period was the interval from cardiac arrest or cardiac asystole to the start of cold preservation perfusion; a period of cardiopulmonary resuscitation from trained medical professionals was considered outwith this time.

Indices of graft function and acute rejection were evaluated to compare safety and efficacy as secondary endpoints. Creatinine clearance at 3 months was estimated from the serum creatinine concentration using the Cockcroft–Gault estimation. Graft core biopsy under ultrasonographic guidance was used to diagnose rejection and evaluated according to the Banff classification¹⁵; both centres have a policy of biopsying allografts that have no evidence of function or deteriorating function 5 days after implantation. Mild allograft rejection (Banff IA, IB or IIA) was treated with three boluses of methylprednisolone 500 mg administered intravenously on consecutive days. Persistent (steroid-resistant) rejection and severe rejection (Banff IIB or III) were treated with a course of antithymocyte globulin (Thymoglobuline®; Sangstat, Lyons, France). All participants had detailed follow-up for a period of 3 months after transplantation.

Statistical analysis

Initial patient selection for transplantation was based on pre-existing local protocols. A power calculation (immediate function 5–40 per cent, power 80 per cent, $\alpha = 0.02$) suggested that 24 patients would be required in each group. Randomization was performed using a balanced block-of-four scheme. This randomization method ensured that, for every four patients entered into the trial, two were allocated to the control and two to the treatment arm (i.e. DCCD or CCDD or CDDC or DDCC). The trial was unblinded and the predesignated sequence overseen by the investigators in Newcastle. Bias was avoided by strict enrolment of all eligible patients into the trial. A two-sample *t* test (for normally distributed data) or Mann–Whitney *U* test was used to compare continuous variables. Probability values for categorical variables were calculated using Pearson's χ^2 or Fisher's exact test (expected cell size less than 5). $P < 0.050$ was considered significant and two-sided values are reported.

Results

All 51 recipients of NHBD grafts at the two centres between November 2000 and March 2003 consented to

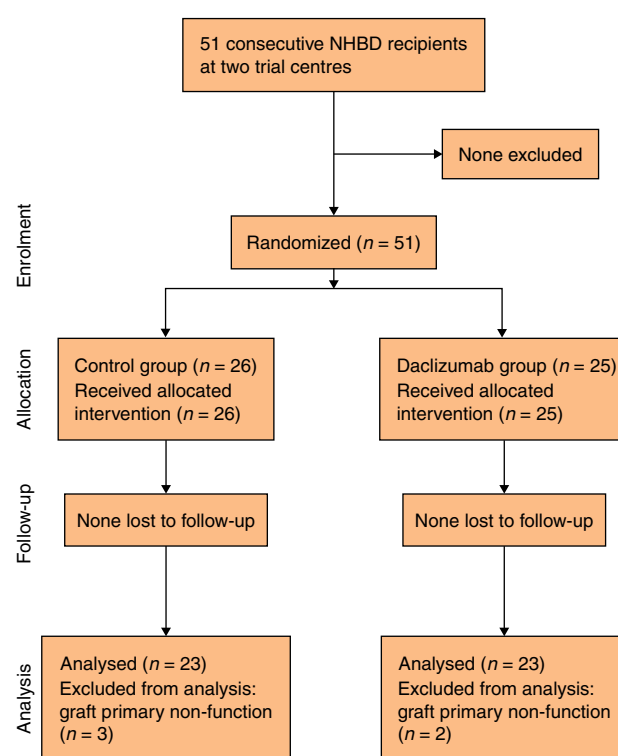


Fig. 1 CONSORT flow diagram. NHBD, non-heart-beating donor

randomization; 25 patients received DZB induction and 26 the control treatment (*Fig. 1*). Data relating to donors and recipients are shown in *Tables 3* and *4* respectively.

One patient in the DZB group died during the study period from an acute coronary syndrome after a non-functioning graft had been removed. The primary non-function rate was comparable between the two groups (two in the DZB group and three in the control group); these recipients were censored from further analysis (*Fig. 1*).

Donor characteristics were similar in the two groups (*Table 3*). Recipients in the DZB group had a tendency to be older and had a significantly higher cardiovascular risk score (*Table 4*). Only one patient required tacrolimus on the basis of biopsy findings of rejection. The rates of immediate function (eight of 23 in the DZB group and five of 23 control patients) and acute rejection (measured in terms of courses of methylprednisolone) were similar in the two groups (*Table 4*); the overall rate of immediate function was 28 per cent (13 of 46 grafts). In the DZB

Table 3 Donor characteristics

	Daclizumab (n = 25)	Control (n = 26)
Donor age (years)	47(15)	44(15)
Maastricht category		
2	12	12
3	11	13
4	2	1
Total warm ischaemia time (min)	60(16)	61(14)
Cold ischaemia time (h)	23(8)	21(6)

Values are mean(s.d.).

group, the median number of DZB doses required was 2 (range 1–5).

Analysis of the subgroup of machine-perfused grafts (Newcastle only) showed that the combination of machine perfusion and DZB induction produced the highest rate of immediate function, with eight of 15 patients requiring

Table 4 Recipient variables

	Daclizumab (n = 25)	Control (n = 26)	P
Age (years)*	53(14)	47(12)	0.064¶
Sex ratio (M:F)	17:8	14:12	0.301**
Predialysis	1	1	1.000††
HLA-DR mismatch			
0	9	10	
1	12	16	
2	4	0	0.112**
Cardiovascular risk score‡	5 (0–9)	3 (0–8)	0.027‡‡
Cytomegalovirus status‡	4	7	0.343**
Primary non-function	2	3	1.000††
Immediate function	8	5	0.326**
Delayed graft function (days)†	7 (0–82)	7 (0–44)	0.885‡‡
Courses of methylprednisolone*	0.4(0.8)	0.4(0.6)	0.845¶
No. of patients requiring antithymocyte globulin	1	1	1.000††
Creatinine clearance at 3 months*§	50(24)	55(25)	0.478¶

Values are *mean(s.d.) or †median (range). ‡Positive donor to negative recipient. §Cockcroft–Gault estimation. ¶*t* test; **Pearson's χ^2 test; ††Fisher's exact test (two tailed); ‡‡Mann–Whitney *U* test. HLA-DR, human leukocyte antigen.

Table 5 Data for machine-perfused subgroup (Maastricht category 2 and 3 grafts in Newcastle only)

	Daclizumab (n = 17)	Control (n = 19)	P
Primary non-function	2	3	1.000§
Immediate function	8	2	0.015§
Delayed graft function (days)*	0 (0–28)	8 (0–16)	0.198¶
Time to serum creatinine < 350 μ mol/l (days)*	12 (4–67)	17 (2–31)	0.250¶
Courses of methylprednisolone†	0.5(0.9)	0.5(0.7)	0.911**
No. of patients requiring antithymocyte globulin	1	1	1.000§
Transplant biopsies*‡	0 (0–6)	1 (0–3)	0.626¶
Hospital stay (days)*	23(13–98)	21(14–50)	0.740¶
Creatinine clearance at 3 months†‡	50(23)	57(25)	0.406**

Values are *median (range) or †mean(s.d.). ‡Cockcroft–Gault estimation. §Fisher's exact test (two tailed); ¶Mann–Whitney *U* test; ***t* test.

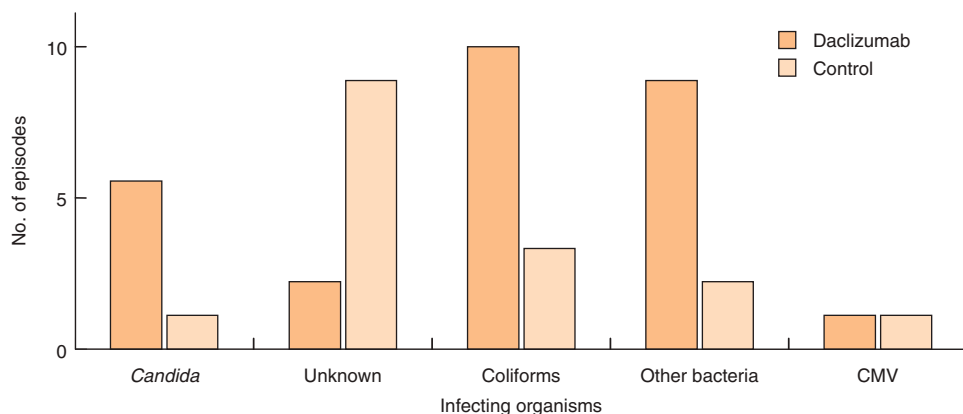


Fig. 2 Infective complications in the machine-perfused subgroup. Coliforms were *Escherichia coli*, *Klebsiella* species and *Enterococcus*. Other bacteria were *Pseudomonas aeruginosa*, *Clostridium difficile*, *Campylobacter* species, *Staphylococcus aureus* and *Epidermidis*. CMV, cytomegalovirus

only one or no dialysis session (Table 5). This reduced the median DGF time from 8 days in controls to zero in the DZB group; one 74-year-old recipient had an extended period of DGF (28 days), reducing the statistical significance of this finding. The serum creatinine level tended to drop below 350 $\mu\text{mol/l}$ more quickly in the DZB group (Table 5).

Despite the improvement in the rate of immediate function in the DZB group, the hospital stay and number of transplant core biopsies were similar in the two groups. This may be explained by the higher number of infective episodes in the DZB group (25 versus 15; $P = 0.087$) (Fig. 2). All of these infections were mild or moderate; no patient required readmission to hospital or critical care intervention.

Discussion

The use of induction polyclonal antibodies has shown considerable promise as a strategy for ameliorating reperfusion injury and reducing the deleterious effects of DGF^{16–18}. These agents reduce the early requirement for calcineurin inhibitors and also contain antibodies specific for endothelial adhesion molecules, so reducing the ability of innate immune effectors (neutrophils and macrophages) to enter and damage the graft¹⁹. Unfortunately, polyclonal antibodies have also been associated with significant side-effects and even death^{20,21}. The newer monoclonal antibodies, basiliximab and DZB, are specific for the CD25 component of the IL-2R and reduce the incidence of acute rejection when used with calcineurin-based triple therapy^{22,23}. Unlike their predecessors, they do not appear to be associated with either infective complications or

lymphomas^{22,23}. It was slightly surprising, therefore, to find more infections in the machine-perfused subgroup.

Since the start of this trial, other groups have reported the effects of similar delayed calcineurin induction regimens on DGF and acute rejection. DZB induction with delayed introduction of tacrolimus was reported to be successful in a retrospective review of more than 180 NHBD kidneys²⁴, and a trial aimed primarily at reducing acute rejection rates for all types of kidney graft reported a DGF rate of only 2.6 per cent²⁵. Hong and Kahan²⁶ compared DGF rates in recipients of marginal organs immunosuppressed with three separate regimens. The DZB regimen combined with delayed cyclosporin was the most effective (3 per cent DGF) and there was no excess of acute rejection²⁶. The overall immediate function rate of 28 per cent in the present study is comparable to that in other high-volume NHBD centres²⁴; however, with a combination of machine perfusion and DZB induction, the rate was substantially higher (eight of 15 patients). There is evidence that machine perfusion alone reduces the rate of DGF compared with cold storage^{27,28}. This is thought to be the result of maintenance of nitric oxide synthase activity²⁹ and reduction in intrarenal resistance at reperfusion.

Monoclonal antibodies are considerably more expensive than calcineurin inhibitors. In the present study the approximate extra cost for each recipient in the DZB group was £2000, although the UK National Institute for Clinical Excellence has assessed anti-IL-2R monoclonal antibodies as cost-effective and recommended their use in all renal transplant recipients³⁰. In the light of the present findings, it may be that NHBD renal transplant recipients may also benefit from the use of anti-IL-2R monoclonal

antibodies as part of a specific calcineurin-sparing regimen to reduce the incidence of DGF. Further trials are needed to clarify whether these agents are cost-effective in this context.

Acknowledgements

The authors thank Dr Mike Ward for the Newcastle cardiovascular risk score and Karin Green for help in collating the data. The study was funded jointly by Fujisawa and Roche; neither organization contributed to the preparation of this manuscript.

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